Detection of Intracellular Gold Nanoparticles

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Abstract – Latest advances in functionalization chemistry have generated a class of versatile bio-nanomaterials (such as gold nanoparticles) with applications in the field of Medicine. The current review focuses on the detection of intracellular gold nanoparticles with therapeutic applications.

Keywords – gold nanoparticles, intracellular detection, fluorescence

I. INTRODUCTION

Latest advances in functionalization chemistry have generated a class of versatile bio-nanomaterials with applications in the field of oncology.[1-4] There are several pieces of evidence that gold nanoparticles (GNPs) bound to various active biomolecules are currently used in clinical trials and may prolong the survival in advanced cases of cancers in humans. In this context it is very important at the current time to fully understand the interaction between gold nanoparticles and cellular environment.[5] The current review focuses on the detection of intracellular gold nanoparticles with therapeutic applications.

II. FLUORESCENCE

There are two ways of detecting gold nanoparticles by fluorescence: a fluorescent label bound to the gold core or a fluorescent gold core.[6]

One of the most popular methods of detecting GNPs is through the use of fluorescent molecules attached to the gold core. Nevertheless, one should bear in mind the destructive effect of the gold core. Based on the distance between the fluorescent label and the gold core[7], nanoparticle size and the fluorescent dye load[7] energy transfers may inhibit photon emission (or enhance their emission). Thus, there should be a considerable distance between the molecule and the gold core and when the fluorescent signal reports particle localization, independent proof that the conjugate is intact ought to be provided. It has been demonstrated that a ligand can be removed during or after cell uptake, either by ligand exchange[8] or by proteolysis[6, 9]. The fluorescence can be assessed by wild field microscopy[10] or, for enhanced resolution, by laser scanning confocal microscopy. The latter offers an image of a thin section of the sample, thereby providing better information on localization. For example, the absorption of 3.5 nm lysine and FITC functionalized gold nanoparticles was assessed in RAW264.7 macrophage cells by means of laser confocal scanning microscopy. Gold clusters have also been employed to display intrinsic fluorescence which depends on cluster size[6, 11]. The research conducted by Chang et al. noted the synthesis of naturally fluorescent gold nanoclusters (GNC@DHLA) of less than 4 nm in size. These particles exhibited less photobleaching than organic fluorophores, achieving successful conjugations with PEG, PEG-biotin and streptavidin[12]. Endogenous biotin of HepG2 fixed cells was specifically label employing these particles. In a preliminary study, unconjugated GNC@DHLA have been detected inside live HAEC cells as a ‘proof of principle’ of their potential imaging in live cells[12]. Endocytosis of fluorescent gold clusters in living cells was also reported in other studies[11]. However, there is need for controls in the absence of nanoparticles[13], and in both cases, fluorescence microscopy images indicate endosomal localization, even though Lin et al. assert nuclear localization.

III. TRANSMISSION ELECTRON MICROSCOPY (TEM)

TEM is a widely used microscopy technique for the intracellular detection of metal nanoparticles, their high electron density enabling their direct visualization. In conventional TEM (in contrast to high resolution TEM), nanoparticles bigger than 5 nm can easily be imaged inside cells due to the good contrast provided by the high electron density of metal nanoparticles.[6] It is a powerful technique that allows the imaging of single nanoparticles in cellular compartments and organelles. Nevertheless, it has rather low throughput since it requires time-consuming sample processing (cell fixation and resin embedding) and multiple images taken from a large number of sliced cells in order to acquire good results on localization. Many TEM experiments exhibit a single representative image, but lack important statistical information, such as the number of

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nanoparticles observed, of images analyzed or of different cells imaged.

The number of citrate-coated gold nanoparticles in vesicles after absorption in mammalian cells has been assessed by TEM [14]. In this study, TEM results have been further validated by inductively coupled plasma atomic emission spectroscopy (ICP-AES). This technique allows the quantification of the number of gold atoms, thereby offering a more precise assessment of the number of gold spheres, still similar to TEM estimation. As for ICP-AES, inductively coupled plasma mass spectrometry (ICP-MS) provides an elemental analysis and helps estimate the number of nanoparticles in the sample. It has been successfully used to study the absorption of GdIII-enriched polyvalent Cy3-DNA-gold nanoparticles conjugates (Cy3-DNA-GdIII@Au-NP) inside NIH/3T3 and HeLa cells[15].

These techniques require extensive cell lysis. They determine the average number of population absorption at a given time, but do not show cell-to-cell variations, dynamics or intracellular localization.[6]

IV. ENDOCYTOSIS OF GOLD NANOPARTICLES

The mechanism by which GNP s are absorbed inside cells has been widely disputed. One of the most likely ones is non-specific receptor mediated endocytosis (RME)[16]. In vivo, even without functionalization, nanoparticles passively accumulate at tumour sites having punctured, immature vasculature with wider openings in the walls than normal mature blood vessels [17]. This phenomenon is the enhanced permeability and retention (EPR) effect. It is difficult to use the EPR effect for tumour drug delivery due to tumour vasculature heterogeneity, especially in the middle of poorly differentiated cancers, as well as to particle detection and absorption by the reticuloendothelial system (RES) [17]. The most common processes of decreasing RES absorption is PEGylation, producing a hydrated barrier causing steric obstructions in the attachment of phagocytes [18]. The association between the EPR effect and longer circulation times, often provided by PEGylation, can result in a 10–100-fold increase in drug concentrations in tumours, compared to free drug use [19].

Tumour targeting can be further attained by actively attaching tumour-specific recognition molecules, such as epidermal growth factor, folate, transferrin or monoclonal antibodies, to nanoparticles [20]. GNP toxicity studies are contradictory, with little understanding of the cellular, intracellular and molecular GNPs - tissue interactions [21]. Some studies have reported no cellular toxicity, while other in vitro and in vivo studies have indicated the production of cellular reactive oxygen species, cytokine release, mitochondrial toxicity, apoptosis and necrosis [22].

V. SCATTERING AND ABSORPTION

There have been recent reviews regarding the optical properties of metal nanoparticles and their biosensing applications [6, 23]. The aim of this study is to select those reports that employ these procedures in live cells.

In a study conducted by Curry et al., EGFR was assessed by dark-field microspectrometry and anti-EGFR antibody conjugated gold nanoparticles (60 nm) [24]. Reflected darkfield microscopy allowed the imaging of the receptor-mediated internalization of gold nanoparticles in live cells and spectrometry helped recording the scattering spectra of gold nanoparticles. However, there is need for further proof on the fact that individual nanoparticles of this size can be differentiated in a cell-like scattering environment. Anti-EGFR antibody conjugated and unconjugated gold nanoparticles were compared and the scattering data indicated different distributions of peak wavelengths. Plain gold nanoparticles exhibited a red-shift and broader distribution than conjugated GNPs. The red-shift can be explained by nanoparticle aggregation. TEM images managed to detect unconjugated gold nanoparticles in endosomes, in close proximity one to another, when anti-EGFR antibody conjugated particles were isolated. Kumar et al. have used 20 nm gold nanoparticles functionalized with an anti-actin antibody to attach actin in live cells [25]. The study does not note single particle detection (and TEM indicates a very high density of labels), but clearly proves the potential of dark field microscopy to improve the understanding of nanoparticle interaction with cells. Louit et al. have reported single particle imaging, tracking and spectroscopy using dark field microscopy on live cells [26].

They used large 80 nm nanoparticles, which could overcome the scattering background of the cells. In order to get significant information from single particle or single molecule studies, it is usually necessary to look at hundreds of traces and to carry out rigorous statistical analyses. Louit et al. did not report such analysis, but their research is a clear evidence that this technique might assess the interaction and internalization of single large gold nanoparticles in live cells. They suggested that information on particle environment could be figured out from a detected modulation in the scattering spectrum.

VI. PLASMON COUPLING

Distance-dependent scattering properties of GNPs have been employed in biosensing for the detection of biological events in single molecules. These properties can help achieve the optical contrast between isolated and neighboring gold nanoparticles (differences in peak intensity and wavelength) [27]. A sensor to explore molecular interactions might be provided by the inducing
the close proximity of plasmonic particles in the presence of a specific target[28]. Plasmon resonance coupling of nanoparticles enabled dynamic imaging of growth factor in living cells [29]. A recent application of plasmonic coupling in living cells has been the detection of protease activation in single molecules. The signal provided by plasmon rulers based on the optical imaging of crown gold nanoparticles enabled the continuous monitoring of caspase-3 activity in live cells [30]. Sensor detection in cells can also be achieved by exploring the fluorescence suppressive properties of gold nanoparticles. This helps track mRNA in living cells using nanoflares [31]. Ion metal sensing in living HeLa cells can be assessed by plasmonic resonance energy transfer (PRET)[32].

VII. RAMAN SPECTROSCOPY
Surface-enhanced Raman spectroscopy (SERS) has also been employed for live cell investigations. SERS can meet the requirements of dynamic in vivo systems by using low laser powers and short data acquisition times. Raman scattering takes place during the collision between photons and molecules. Throughout this process, photons gain or lose energy and this energy variation results in scattered photons. A Raman spectral image is produced by various molecular vibrations and offers a vibrational fingerprint of a molecule. The magnitude of Raman scattering signals can be increased for the molecules which are next to metal nanostructures. Gold nanoparticles are able to enhance the performances of internalized molecules by 1014- to 1015-fold, allowing spectroscopic detection of single molecules. There are many applications ranging from chemical investigations in intracellular compartments (pH) [33], to subcellular detection gold nanoparticles (20 nm) functionalized with large T-antigen nuclear localization signal (NLS)[34], or in vivo tumor detection by means of PEGylated SERS nanotags [35]. Time-resolved spectroscopy has also enabled the detection of gold nanoparticle transport inside a living cell [36].

VIII. DIFFERENTIAL INTERFERENCE CONTRAST (DIC)
In their study, Tkachenko et al. assessed the endocytosis of 20 nm NLS-functionalized gold nanoparticles using VEC-DIC. The technique seems attractive, as it provides a single image where gold nanoparticles should show up as colored areas together with the cells shape and topography provided by DIC, recorded at video rate. Nevertheless, the imaging of individual nanoparticles still needs evidence and the amount of absorption required to obtain a significant signal is still not clear. Dual-wavelength differential interference contrast microscopy has been recently developed to image 20–80 nm silver or/and gold nanoparticles, based on the wavelength-dependent contrast of metal nanoparticles [36]. The technique has also been employed to detect the endocytosis of Tat-functionalized gold nanoparticles (40 nm) in live HeLa cells at video rates[37]. It is based on the comparison between two DIC images taken simultaneously at different wavelengths, one at 540 nm, corresponding to the maximum contrast wavelength of gold nanoparticles, meant to detect nanoparticles and cells, and one at 720 nm as a control image for the sole detection of cells. Differential interference contrast is not only dependent on nanoparticle size, but on the multiple refractive indices of metal nanoparticles, too [37].

IX. PHOTOTHERMAL MICROSCOPY
The photothermal imaging of gold nanoparticles was introduced by Boyer et al. in 2002 [38]. The technique is based on absorption, enabling the direct imaging of metal nanoparticles[39], nanotubes[40], or quantum dots [41]. The technique relies on two overlapping laser beams, one that triggers a small localized variation in temperature (∼1–2°C) in the presence of a nano-absorber, the other detects possible changes in temperature. It has been employed to image membrane proteins by means of gold nanoparticle labels [42]. It imaged single small nanoparticles (10 nm) for a long period of time, with no blinking or photobleaching. The sensitivity of the technique has been enhanced in order to image 5 nm gold nanoparticles in living cells and it was renamed Laser Induced Scattering around a NanoAbsorber (LISNA). Single nanoparticle photothermal tracking (SNaPT) has been developed by the same group. The technique provides lengthy video-rate imaging of metal nanoparticles. The authors observed the lateral movement of a membrane protein AMPA receptor in live neurons by tracking 5 nm AMPA receptor-conjugated gold nanoparticles for more than 5 minutes [43]. Photothermal absorption correlation spectroscopy (PhACS), the equivalent of fluorescence correlation spectroscopy (FCS), where detection is based on metal nanoparticle endocytosis, would also allow the measurement of diffusion constants of metal nanoparticles and the hydrodynamic radii of functionalized nanoparticles, but for the moment, there have been no such applications on live cells.

References


25