Imaging Agents

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Metal Carbonyl–Gold Nanoparticle Conjugates for Live-Cell SERS Imaging**

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The field of bioorganometallic chemistry, the junction of organometallic chemistry and biology, is coming of age. In particular, applications of metal carbonyl compounds in biological immunoassays (metallo-immunoassays), pharmaceuticals, CO therapy (CO-releasing molecules), and bioimaging have emerged.[1–4] One of the useful characteristics of metal carbonyl compounds is the strong CO stretching vibrations in the mid-IR region (1800–2200 cm^{-1}); a region that is relatively free of interference from absorbance of biomolecules. This has been utilized in the development of carbonyl immunoassays,[1f] and more recently, in cell imaging, with detection techniques that include FTIR, Raman, and PTIR spectroscopy.[4]

Although the first two detection methods can be carried out with readily available instrumentation, a major hindrance to IR detection for live-cell imaging is the interference from a strong absorption band of water at approximately 1600 cm^{-1}. Although Raman spectroscopy provides a far better spatial resolution with minimal interference from water,[5] it suffers from a low scattering cross section.[6] This necessitates a high concentration of the metal carbonyl based biotag, which poses the problem of undesired cytotoxicity and hence limits its potential in clinical applications. Furthermore, most metal carbonyl compounds have low solubility in water, thus requiring complicated bioconjugation to render them more water soluble or dispersible, and biocompatible. Therefore, there is a need to develop a highly sensitive, targeted, and low-toxicity metal carbonyl based biotag toward eventual biological applications.

One promising approach to signal enhancement is surface-enhanced Raman spectroscopy (SERS); the Raman signals of molecules on colloidal gold or silver nanoparticles can be enhanced by several orders of magnitude (typically 10^6 to 10^{14}) as a result of the strong surface plasmon resonance of the nanostructured surface. This spectroscopic method has been successfully adapted to chemical sensing applications, at lower concentrations but with better detection limits,[7] and is exemplified by its use in DNA detection,[8] cancer diagnosis,[9] and detection of cellular molecules.[10] The possibility that SERS may also be used to enhance the CO stretching vibration signal of metal carbonyl compounds is attractive. Furthermore, detection by Raman microscopy will afford spatial resolution into the submicron range.

The use of organometallic osmium carbonyl clusters (OM) has been demonstrated earlier;[4a] they have the advantages of being oxygen- and moisture-stable, and are very robust in comparison with other organometallic compounds. The preparation and mode of interaction of osmium carbonyl clusters with the surface of gold nanoparticles (NPs) have also been reported recently.[11] We thus envisage that the binding of an organometallic osmium carbonyl cluster, $Os_3(CO)_{10}(\mu\text{-}H)_2$, onto the surface of gold nanoparticles (henceforth designated as "OM-NP") may significantly enhance the inherently weak Raman signal of the metal carbonyl CO stretching vibrations (Figure 1).

Indeed, the Raman spectrum of the osmium carbonyl cluster showed no detectable CO signal in ethanol/water (1:4, *v*/*v*) at 10 μM concentration; it could only be detected at a much higher concentration (50 mM). In contrast, the CO signals in the OM-NP conjugates were significantly enhanced (Figure 2); the CO intensity was increased by over four orders of magnitude (a factor of ≈15000). This enhancement was estimated as the ratio of the signal intensity of a suspension of the OM-NP conjugate to that of a 50 mM solution of the osmium carbonyl cluster in ethanol/water (1:4, *v*/*v*); the concentration of clusters in the conjugates was found to be approximately 34 μM by both IR spectroscopy and inductively coupled plasma mass spectrometry (ICP-MS; see the Supporting Information for details).

These OM-NP conjugates also exhibit remarkably good storage stability. The CO signals in aqueous solution remain consistent for up to 28 days (Figure 3a and b); any decomposition of the metal carbonyl on the gold nanoparticle

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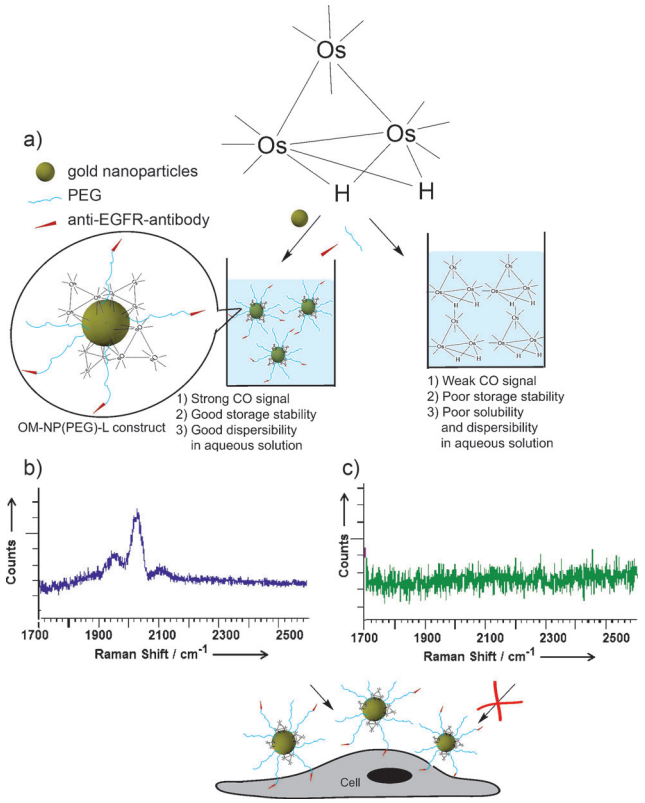

Figure 1. a) Preparation of OM-NP conjugates using the strong interactions of $Os_3(CO)_{10}(\mu\text{-}H)_2$ with gold nanoparticles. OM-NP conjugates are then functionalized with antibodies and PEG. b) CO signal of OM-NP(PEG)-L conjugates (34 μM of OM on 4.31×10^{-5} μM NP) in aqueous solution at an excitation wavelength of 633 nm. c) Absence of detectable CO signal of $Os_3(CO)_{10}(\mu\text{-}H)_2$ (50 mM) in ethanol/water (1:4, *v*/*v*) at excitation wavelenght of 633 nm.

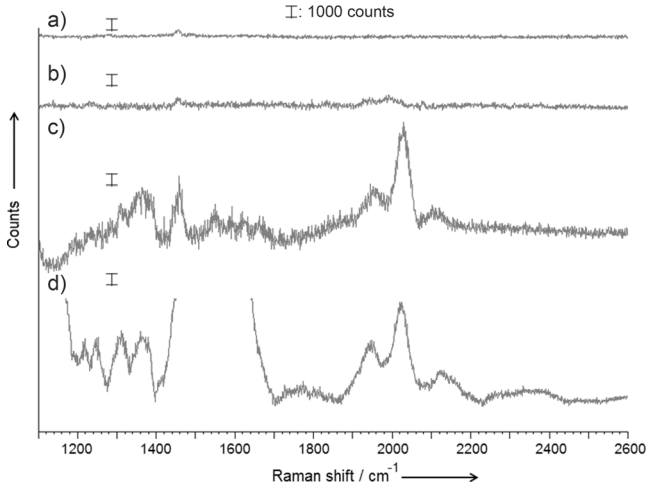

Figure 2. Raman spectra of a) 10 μM and b) 50 mM $Os_3(CO)_{10}(\mu\text{-}H)_2$ in ethanol/water (1:4, *v*/*v*), and of c) OM-NP and d) OM-NP(PEG)-L conjugates (34 μM of OM on 4.31×10^{-5} μM NP) in aqueous solutions.

surface would have shown up as a change in the CO signal intensity. According to the TEM images (Figure 3c and d), the conjugates are well dispersed, and show no aggregation

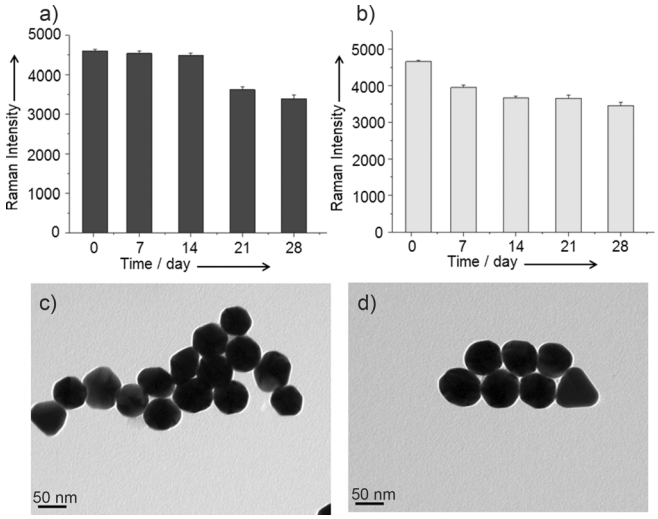

Figure 3. Time–course study of a) OM-NP conjugates and b) OM-NP(PEG)-L conjugates over 28 days in water, and TEM images of the OM-NP conjugates at c) day 1 and d) day 30.

even after 30 days. The conjugates are spherical, with an average size of 60 nm.

The stability of the OM-NP conjugates in the cell culture medium (with excess GSH) and OSCC cell extract was also tested. No precipitation was observed. There was no change in the characteristic CO vibrational bands, which remained sharp, even in the presence of excess GSH.

For the live-cell imaging, we conjugated the OM-NP conjugates with an antibody against epidermal growth factor receptors (anti-EGFR), as it is highly expressed in diverse cancer cells and hence has been used in many biological studies.[12] Successful conjugation was confirmed by the observation of an absorbance maximum at 280 nm (see Figure S1 in the Supporting Information).[13] PEG was also added in the bioconjugation process to improve the stability and biocompatibility of the conjugates.[14] These OM-NP-(PEG)-L conjugates (in which L represents a binding ligand, anti-EGFR here) also exhibit good storage stability, with minimal detriment (< 10%) in the CO signal over 28 days (Figure 3b).

The cytotoxicity of these OM-NP(PEG)-L conjugates have been assessed with the OSCC cell line (epidermoid carcinoma), in which EGFR is overexpressed. Interestingly, while the cluster $Os_3(CO)_{10}(\mu\text{-}H)_2$ is clearly cytotoxic, the

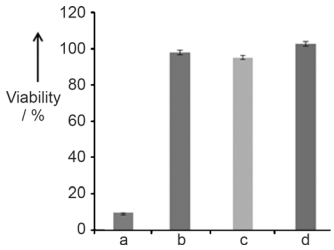

Figure 4. Cell viability of OSCC cells after 24 h incubation with a) $Os_3(CO)_{10}(\mu\text{-}H)_2$ (34 μM), b) gold nanoparticles (4.31×10^{-5} μM), c) OM-NP conjugates (34 μM of OM on 4.31×10^{-5} μM NP), and d) OM-NP(PEG)-L conjugates (34 μM of OM on 4.31×10^{-5} μM NP).

conjugates are not; the cells remained nearly 100% viable with respect to the control (Figure 4).

OSCC cells that had been treated with the OM-NP(PEG)-L conjugates were imaged using the SERS-enhanced CO absorption signal at 2030 cm^{-1}, and the image was closely correlated with the bright-field and dark-field microscope images (Figure 5a–d); the latter were contrast-enhanced microscope images. The cells still afforded strong images

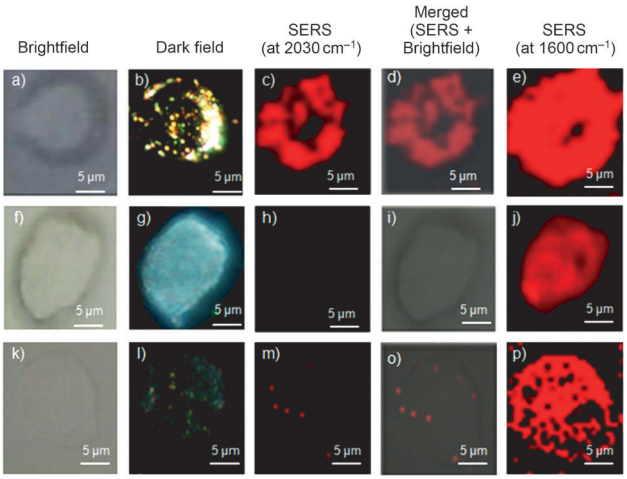

Figure 5. Bright-field, dark-field, and SERS mapping images of a–e) OSCC cells, f–j) SKOV cells treated with OM-NP(PEG)-L conjugates, and k–p) OSCC cells treated with anti-EGFR prior to incubation with OM-NP-(PEG)-L conjugate. All SERS mapping images of CO (2030 cm^{-1}) and protein (1600 cm^{-1}) were taken at an interval of 1 mm (633 nm excitation).

after having been stored for three days; the observed spectrum for the CO signals remained unchanged (see Figure S4 in the Supporting Information). A similar set of images (Figure 5e–h), obtained with an EGFR-negative cell line, SKOV3 (ovarian carcinoma), clearly shows the absence of the conjugates. Magnified dark-field images of these two treated cell lines clearly show that there is much stronger light scattering from the OSCC cells compared to the SKOV3 cells (Figure 5a and f). This result clearly shows the specificity and efficient targeting of OM-NP(PEG)-L conjugates to EGFR-positive cells; the scattering bright spots are clearly correlated with the locations at which the CO vibration signals can be found (Figure 6a and b). Specificity of the targeting was also further verified by blocking with unlabeled anti-EGFR prior to incubation with conjugates, which showed much reduced SERS signals (Figure 5k–p).

The EGFR receptor is one of the key membrane proteins located on the cell surface and hence it is expected that the OM-NP-(PEG)-L conjugates would be mainly distributed in the cell membrane. This is clearly seen in a depth scan which shows that there are no CO signals associated with the cellular cytoplasm; the bright ring observed is characteristic of labelling of the membrane and is in agreement with prior work (Figure S5).

In summary, we have prepared a novel metal carbonyl based biotag by combining osmium carbonyl clusters and gold

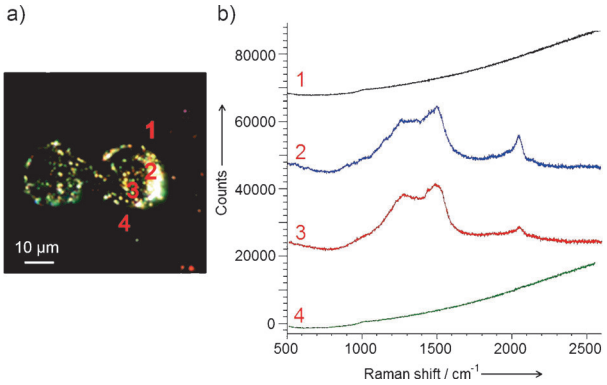

Figure 6. a) Dark-field image of OSCC cells after incubation with OM-NP(PEG)-L conjugates. b) SERS spectra of OSCC cells at the four different locations indicated.

nanoparticles, as an example of an organometallic-nanoparticle (OM-NP) conjugate. These OM-NP conjugates display excellent aqueous dispersibility and storage stability. Furthermore, they can be readily functionalized with suitable binding ligands to produce biologically functional OM-NP-L conjugates, which we have demonstrated in live-cell imaging. It shows clearly the advantage of transition-metal carbonyl compounds, for which the CO stretching vibration signal is well-separated from other molecular vibrational modes of the cell, in live-cell imaging. In addition, in comparison to the other detection modes, our approach offers both greater sensitivity (signal strength and signal-to-noise) and good spatial resolution.

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