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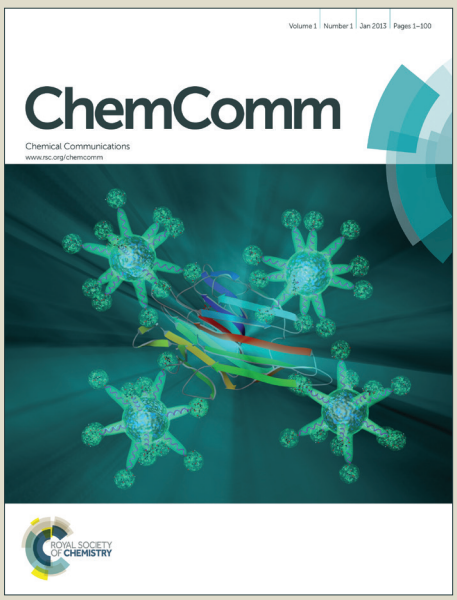

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Organometallic Carbonyl Clusters: A New Class of Contrast Agents for Photoacoustic Cerebral Vascular Imaging†

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We report, for the first time, the use of a water-soluble organometallic compound as a stable, reliable and high-contrast photoacoustic contrast agent. This gives a significantly higher contrast than is achievable with alternatives such as single-wall carbon nanotubes. Image enhancement of the rat cerebral cortex vasculature was observed with in vivo dark-field photoacoustic microscopy.

There is an increasing interest in photoacoustic microscopy as an imaging technique because it can provide higher spatial resolution and deeper tissue penetration, together with high contrast, compared to most optical imaging techniques.[1-4] Very often, however, the use of an exogenous contrast agent may be desirable.[5, 6] While many organic-based contrast agents, notably the cyanine dyes, nanoparticles, polyhydroxy-fullerene and carbon nanotubes, have become available the use of organometallic compounds as a contrast agent for photoacoustic imaging has not been explored.[7-11] Organometallic compounds have been used in the labeling of peptides and enzymes, and in immunoassays.[12-19] Many organometallic compounds have also been found to have potential applications in cancer therapy; their potential use as pharmaceuticals has also been reviewed.[20-23] As an optical contrast agent, their use in surface enhanced Raman spectroscopy (SERS)-based imaging was recently demonstrated.[24] Herein, we wish to report our study on the feasibility of organometallic carbonyl clusters as a reliable photoacoustic contrast agent, and its performance is demonstrated with an *in vivo* brain microvascular imaging of the rat.

The trinuclear carbonyl clusters of the group 8 metals, *viz.*, $Fe_3(CO)_{12}$, $Ru_3(CO)_{12}$, and $Os_3(CO)_{12}$ are highly opaque compounds which absorb a large fraction of the incident light (Fig. 1a).The absorption spectra of these clusters in the visible region are fairly distinct (Fig. 1b), and the contrast quality of the photoacoustic images generated by these clusters is also notably high (Fig. 1c). Among the three clusters, $Os_3(CO)_{12}$ exhibits the highest photoacoustic signal; about 1.5 and 1.2 times higher than that for $Fe_3(CO)_{12}$ and $Ru_3(CO)_{12}$, respectively, and 2.4 times higher than that for single-walled carbon nanotubes (SWNT). In fact, the photoacoustic signal of $Os_3(CO)_{12}$ can be detected even down to 0.1 μM (Fig. SI-1).

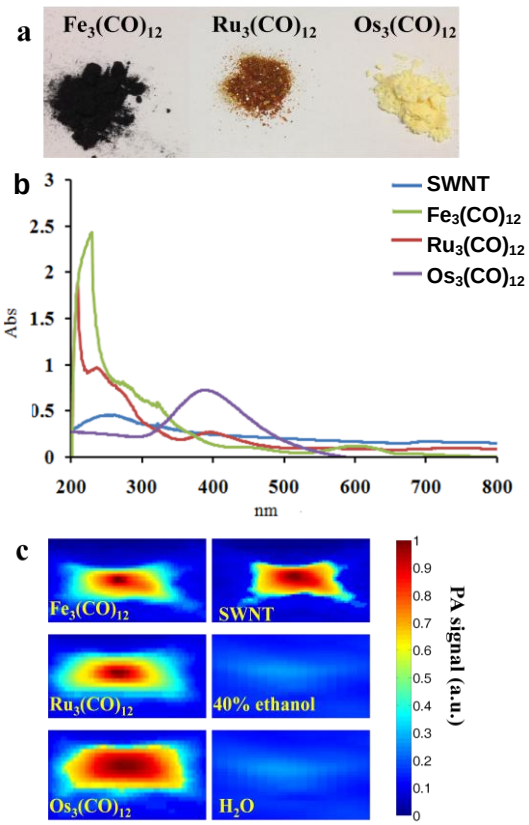

Fig. 1 (a) Photograph of trinuclear carbonyl clusters of the group 8. (b) UV-vis spectra. (c) False color images representing the relative photoacoustic B-scan signal strengths of SWNT and the organometallic carbonyl clusters at 410 nm incident wavelength.

The absorption maxima at longer wavelengths for all three clusters have been assigned to $\sigma \rightarrow \sigma^*$ and $\sigma^{*\prime} \rightarrow \sigma^*$ transitions involving the metal-metal bonds.[25, 26] For instance, these two transitions are located at 437 nm and 600 nm, respectively, for $Fe_3(CO)_{12}$. The higher absorptivity of $Os_3(CO)_{12}$ at the incident

wavelength of 410 nm accounts for its stronger photoacoustic signal.[26, 27] The osmium compound is also the most suitable candidate for this study because it has been shown to possess excellent chemical stability and negligible cytotoxicity.[28-30] The former can be attributed to the strong metal-metal bond which, in general, increases down the periodic group, with Fe-Fe being the weakest and Os-Os the strongest; the low cytotoxicity of the osmium carbonyl cluster reflects its chemical inertness.[28-31]

To improve biocompatibility, a water soluble analogue of the osmium carbonyl cluster, *viz.*, $[Os_3(CO)_{10}(\mu\text{-}H)(\mu\text{-}S(CH_2)_2COO)]^-Na^+$ (Os salt), has been prepared (Fig. 2). Its cytotoxicity has been evaluated by MTS assay against the oral squamous cell carcinoma (OSCC) cell line and shows that it poses little effect on cell viability (Fig. SI-3). This water soluble cluster also gives high contrast photoacoustic images, even down to 2.5 μM (Fig. SI-2), suggesting that the photoacoustic property has not been affected by incorporation of the water soluble moiety.

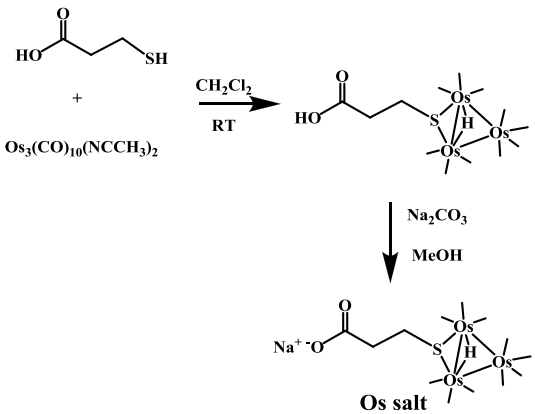

Fig. 2 Synthetic scheme for the water-soluble cluster $[Os_3(CO)_{10}(\mu\text{-}H)(\mu\text{-}S(CH_2)_2COO)]^-Na^+$ (Os salt). Short lines represent CO ligands.

To assess the performance of the Os salt as an exogenous photoacoustic contrast agent, it was injected into the rat for *in vivo* photoacoustic imaging of its cerebral vasculature (Fig. 3a-b). Much attention has been paid to developing imaging techniques and contrast agents for brain vascular imaging,[32-38] as these provide important information for understanding the cerebral angio-architecture and in the assessment of acute ischaemic stroke and regional hemodynamic activities. The photoacoustic microscopy (PAM) imaging system used has been built in-house and customized for imaging of the rat brain at a high spatial resolution.[35-37] The superior sagittal sinus (SSS) and other vessels on the cortical surface could be observed visually. The SSS is the largest vein in the rodent brain cortex.[39] The large volumetric size of the SSS, however, weakens the signal collection in photoacoustic B-scan imaging so that although it can be seen in B-scan images, the photoacoustic signals are weaker than those from other cortical vessels,[35-37] resulting in poor image quality. Similar difficulties have also been reported by others.[40]

Compared to the B-scan image obtained from the intrinsic optical contrast, that acquired 20 min after the administration of a solution of Os salt showed the brain vascular with greater clarity, especially the SSS (Fig. 3c). Notably, the absorption enhancement remained high, even after 60 min, indicating a sufficient amount of Os salt circulating in the cerebral vasculature. The image enhancement and contrast by the Os salt are significantly greater than that of SWNT (Fig. 3c-d), and the C-scan image shows the brain vasculature with great clarity (Fig. 3e). The Os salt also exhibited remarkably good stability in a blood sample; this was determined by analysing the sample on a gold substrate with surface enhanced Raman spectroscopy (SERS). The characteristic CO vibrations of the Os salt remained sharp for up till 40 hours and did not show any sign of decomposition which would otherwise be observed as changes in the CO vibrations (Fig. SI-9).[24, 30] This is important as a contrast agent with few side effects and toxicity is highly desirable. Indeed, the rats remained alive after the imaging session.

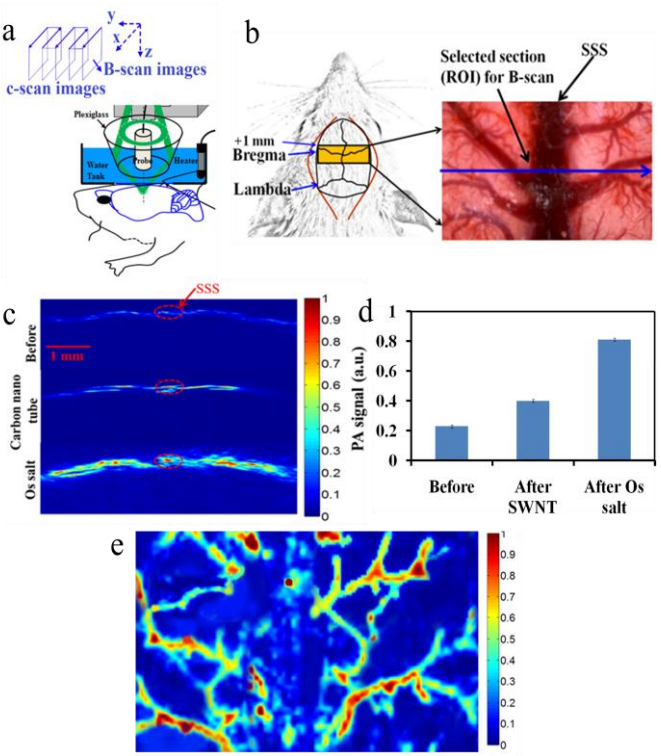

Fig. 3 *In vivo* photoacoustic imaging with Os salt. (a) Customized photoacoustic microscopy for high-resolution B and C-scan imaging on rat brain. (b) Photograph of the rat cortical blood vessels, with the superior sagittal sinus (SSS) indicated. The ROI cross-section location is indicated by the blue arrow. (c) Photoacoustic B-scan images acquired before, and 20 min after, the administration of a 30 μM aqueous solution of Os salt or carbon naotube. The SSS is highlighted with a red oval. (d) Photoacoustic signal strength in SSS after injection of SWNT and Os salt. (e) The brain vasculature shows up clearly in the photoacoustic C-scan.

In summary, we have shown in this study that organometallic carbonyl clusters such as that reported here can be safe contrast agents for use in photoacoustic imaging. This has been demonstrated here with a water-soluble osmium carbonyl cluster for *in vivo* imaging of the rat cerebral vasculature, affording high spatial resolution and good sensitivity. The use of an exogenous contrast agent can potentially provide a method to monitor intra- or extravascular hemodynamics. This is important for accurate assessment of conditions such as acute ischaemic stroke, lesion development, thermal injury, neovascularization, tumor angiogenesis, tumor necrosis, hepatic function and regional hemodynamic activity. Osmium carbonyl clusters offer high photoacoustic activity, chemical stability and low toxicity, and they

offer multiple sites for the attachment of various ligands as property modifiers. Through conjugation to bioactive materials such as proteins, antibodies, and drugs, they can also enable molecular imaging and therapeutic monitoring.

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Notes and references

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