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Locating and Characterizing Self-Assembled Gadolinium Chelate Nanoparticles in Stem Cells Using TEM

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Gadolinium (Gd) based contrast agents are commonly used in magnetic resonance imaging (MRI) diagnosis. These agents possess relatively low contrast efficiency, requiring the administration of large doses of the contrast agent leading to concerns over safety. Self-forming gadolinium chelate nanoparticles (Gd NPs) provide a unique means to create higher efficiency contrast agents by bringing the gadolinium atoms in close proximity to each other, resulting in enhanced relaxivity. The self-assembly process can be activated through a number of mechanisms including changes in pH, the presence of light or any number of enzymatic reactions. These nanoparticles can be used to target and identify specific molecular events and even diseases such as cancer.

The Gd NPs are comprised of an organic matrix that both chelates the Gd ions and allows for the self-assembly reaction to occur. As a result, the Gd NPs form as amorphous nanoparticles with a low overall concentration of Gd atoms. This makes it very difficult to identify and characterize the Gd NPs using transmission electron microscopy (TEM) alone. Both energy dispersive x-ray spectroscopy (EDS) and electron energy loss spectroscopy (EELS) can be utilized to identify Gd and confirm the presence of Gd NPs locate them *in vitro*. Gadolinium has a characteristic x-ray peak at 6.056 keV for EDS analysis and a characteristic energy loss peak at ~148 eV for EELS analysis.

Gd NPs were imaged using an FEI Titan TEM with Cs image correction operated at 80 kV. The nanoparticles shown in figure 1(A) are very nondescript, appearing as dark blobs on the holey carbon film substrate. EELS, shown in figure 1(B), was utilized to confirm the presence of Gd indicating that these nanoparticles are indeed Gd NPs. Compared with inorganic Au-Si core shell nanoparticles shown in figure 1(C) these nanoparticles are very non uniform without distinct features that would make them identifiable *in vitro*.

Gd NPs treated with lipofectamine, to induce uptake, were incubated with rat adipose derived stem cells (rADSC). The cells were then fixed, stained, dehydrated and embedded following standard protocols. 150 nm thick sections were cut and placed on 100 mesh formvar coated Cu grids and imaged using the FEI Tecnai F20 TEM operated at 200 kV. Gd NPs were expected to be located in the vesicles of the cells. Figure 2(A) shows a vesicle with a number of nanoparticles inside. The darker nanoparticles originally thought to be Gd NPs, figure 2(B), are actually comprised largely of iron oxide. The lighter nanoparticles, shown in figure 2(C), are Gd NPs as confirmed by EDS in figure 3.

Locating and characterizing Gd NPs is a difficult task in the TEM requiring the use of advanced analytical techniques including EDS and EELS. With the addition of these tools, it is possible to locate and identify Gd NPs inside stem cells.

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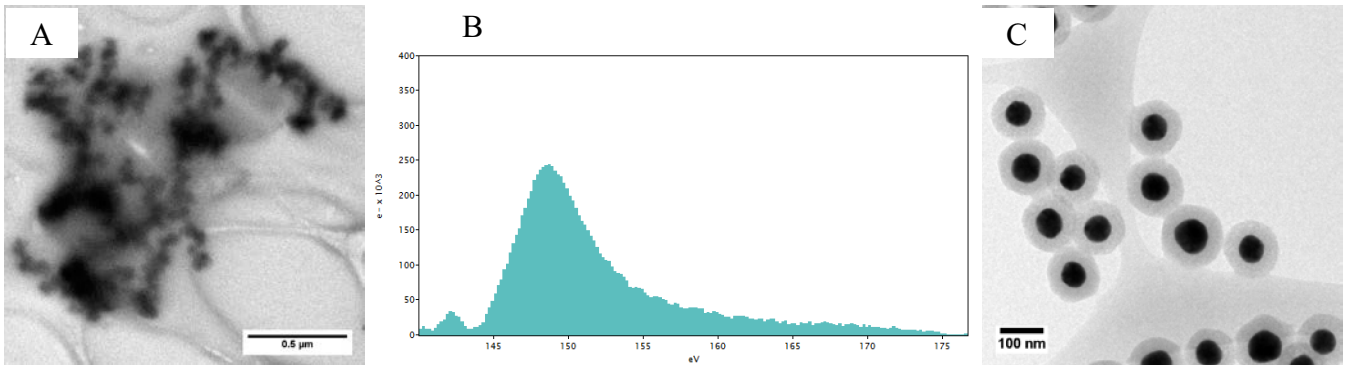

Figure 1: (A) TEM image of Gd NPs showing that the nanoparticles are non-uniform and non-descript. (B) EELS spectra confirming the presence of Gd in the nanoparticles. (C) Inorganic Au-Si core-shell nanoparticles highlighting the difference between amorphous organic nanoparticles and inorganic nanoparticles.

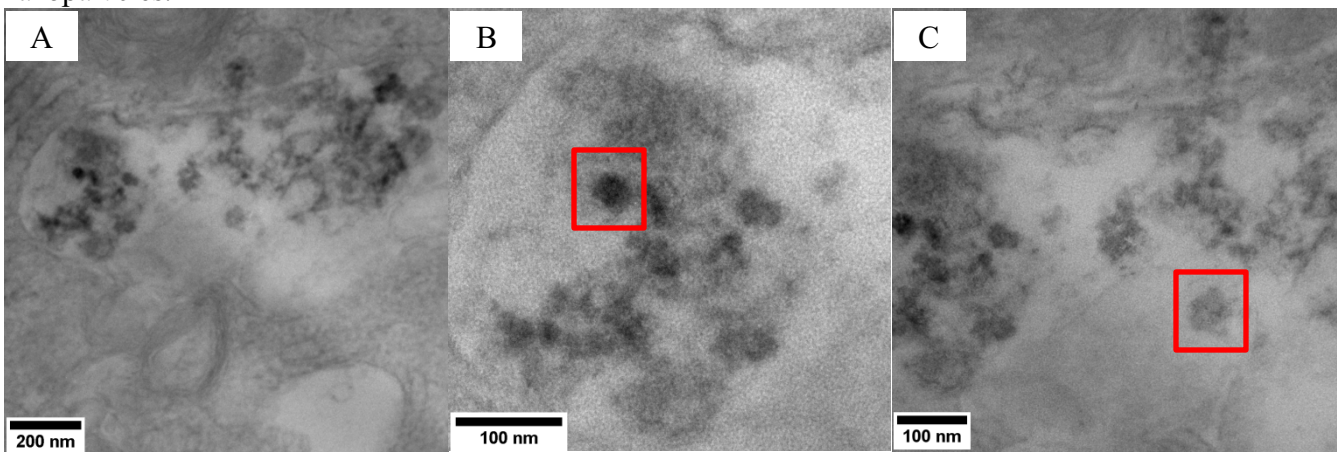

Figure 2: TEM images of (A) vesicle inside a stem cell showing a large amount of nanoparticulate. The darker nanoparticles (B) are comprised largely of iron oxide while some of the lighter nanoparticles (C) are Gd NPs.

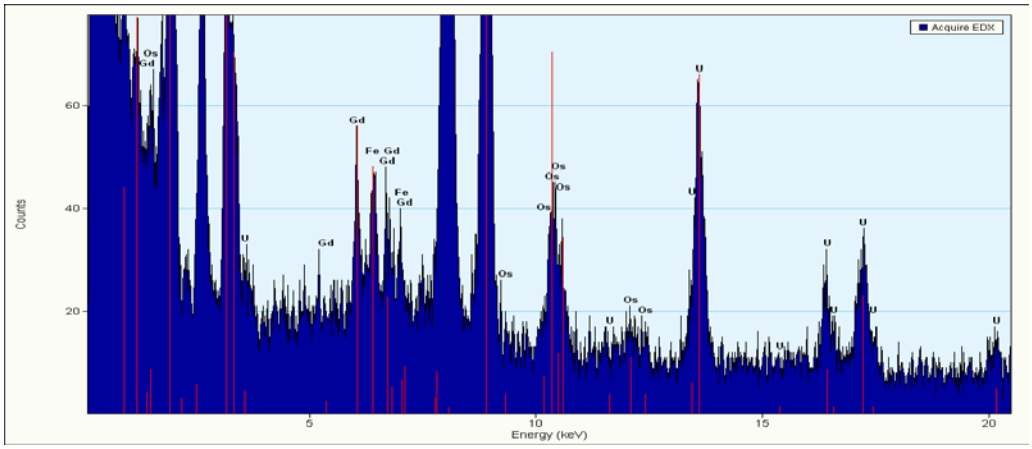

Figure 3: EDS spectrum taken from the nanoparticle shown in figure 2(C) showing the presence of Gd in the nanoparticle.

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