Supplementary information on

Highly stable polymer coated nano-clustered silver plates: A multimodal optical contrast agent for biomedical imaging

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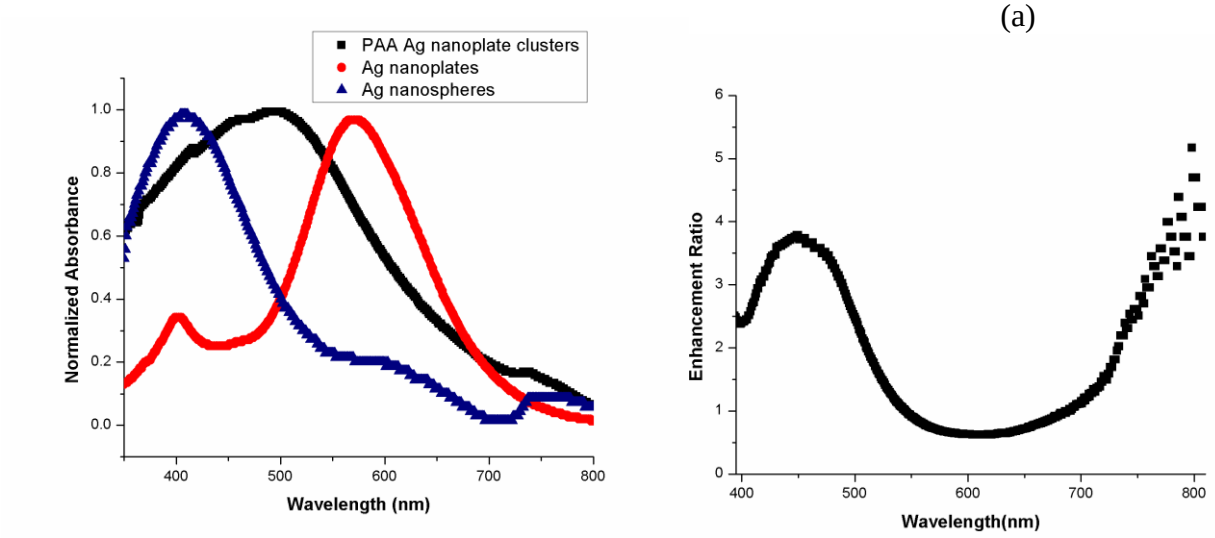
1. NP Characterization

We observe a significant broadening of the absorption peak for the NP's as compared to the single silver uncoated nanoplates. Additionally we also observe a blue shift in the absorption maxima. This can be attributed to the fact that the clusters containing the larger silver nanoplates could not be stabilized in the reverse micelles during the polymerization process. On average we observe the diameter of the plates inside the polymer matrix to be 25-30nm whereas the pure silver nanoplates on average are 30-40nm in size. The bigger particles and clusters precipitate and are separated during the polymerization process. However, the bigger particles can be incorporated into the NP's by developing more stable micelles by increasing the amount of the surfactant during the polymerization process.

(a)



(b)

Figure S1(a). Comparison between the absorption spectra of NP, the individual silver nanoplates and silver nanospheres. (b) Enhancement ratio of the absorption coefficient (NP and the individual silver nanoplates).

Hydrodynamic Particle Size Analysis

The mean hydrodynamic size of the PAA coated nanocluster was measured using light scattering (Malvern Particle size analyzer). The average size of the polymer coated nanocluster, as calculated by the software, is about 188nm. The size distribution is shown in Figure S1(c).

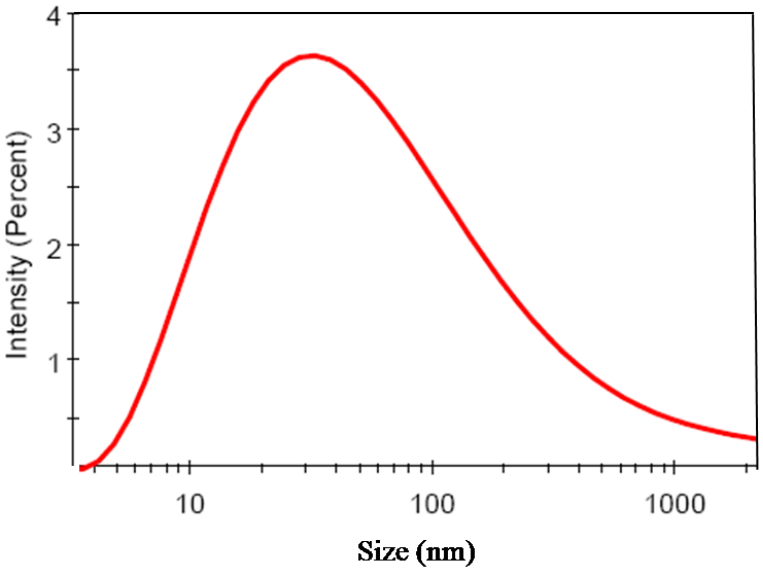


Figure S1(c). Hydrodynamic size distribution (by intensity)

2. Toxicity of Silver nanoplates

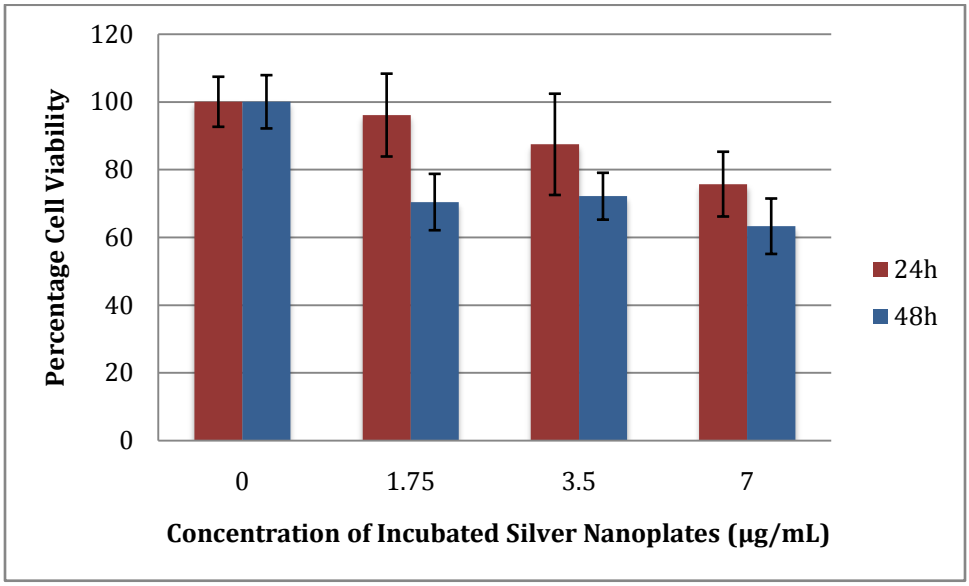
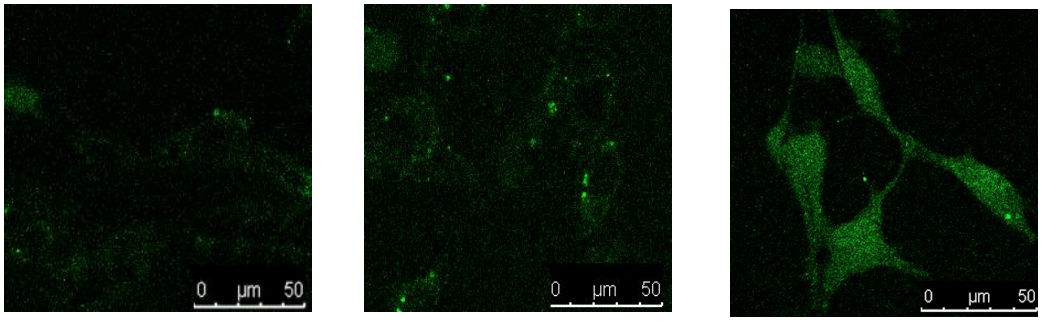


Figure S2: Concentration-Dependent Cell Viability for Silver Nanoplates

The amount of silver in the NP was determined by performing ICP OES. The comparisons between the toxicities of NP and the nanoplates were performed using equivalent amounts of silver. We start to observe cytotoxicity from the silver nanoplates

at a concentration of 1.75µg/mL, which is equivalent to 0.5mg/ml NP concentration. At increasing concentrations, we see a progressive increase in the cytotoxicity after 24 hours of nanoplate incubation. However after 48 hours of incubation, the cytoxicity levels are not significantly different for the various concentrations. The important point to note is that the uncoated nanoplates show slightly higher toxicity compared to the polyacrylamide coated silver nanoplates. This difference can be attributed to the direct interaction of the nanoplate surface with the cells, leading to increased electrocatalytic activity.

3. Monitoring the oxidative stress in 9L cells:



(a) (b) (c)

Figure S3: Fluorescence image of cells loaded with the dye DCF, for (a) 0mg/ml, (b) 2mg/ml and (c)4mg/ml NP concentration.

We observe very low levels of fluorescence signal from the DCF-DA inside the cells for the 0 mg/mL and 2mg/mL NP concentrations, indicating a lack of significant oxidative stress from the cells after 24 hours of nanoparticle incubation. This correlates with the results from our toxicity assay (MTT). While the fluorescence signal from cells incubated with 2mg/ml Ag-PAA is slightly higher than that of the control (0mg/mL) there is no significant difference to indicate the onset of oxidative stress. However the fluorescence signal from the cells at 4mg/mL NP concentration is significantly more intense than that of the control (0mg/mL) indicating that at a 4mg/mL concentration, the Ag-PAA particles induce oxidative stress in cells.

Specific Targeting of the Nanoparticles

The efficiency of the F3 peptide to selectively target the nucleolin over-expressing cells, is demonstrated by using the 9L and MCF7 cells. The 9L cells have a higher expression of nucleolin on the cell's outer membrane, compared to the MCF 7 cells. Both the targeted and non-targeted PAA NP's were incubated into the cells for 15 minutes before being washed away. The cells for each treatment were then spun down and collected separately, in the individual wells of a 96 well plate, and the amount of NP's inside the cells was measured using a plate reader. We observe a significant difference (3X) in the uptake of the F3 targeted *vs.* non-targeted NP's in the 9L cells. In contrast, for the MCF7 cell line there was no significant difference between the uptake of the F3 targeted and

non-targeted NPs. The overall uptake of nanoparticles by the MCF7 cell line was also ten and three times lower for the F3-targeted PAA NP and the non-targeted PAA NP, respectively, as compared to the 9L cells. This *in vitro* experiment demonstrates the ability of the F3 peptide to deliver the PAA NP's to the rat glioma cells.

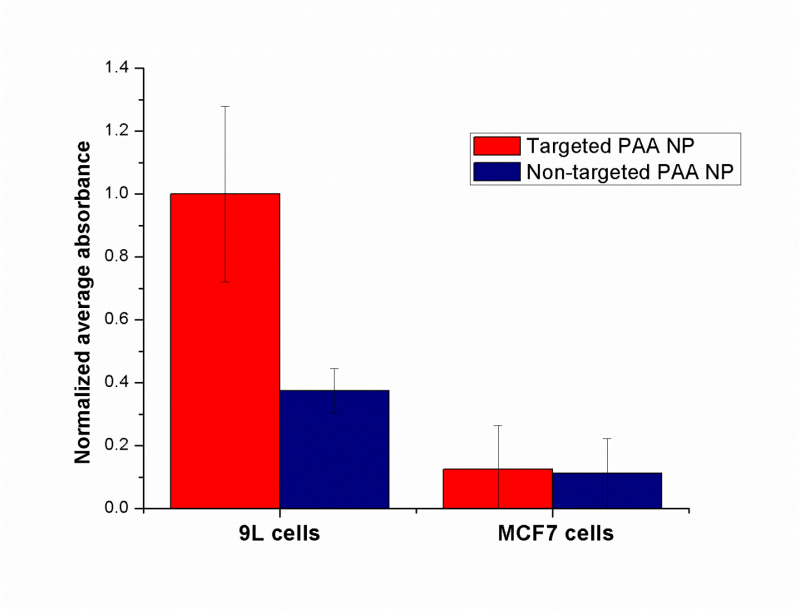


Figure S4: Targeting efficiency of the F3 peptide decorated particles, for different cells, with different nucleolin expression.