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Effects of Gold Nanoparticle Size and Functional Group on Adipogenesis of Mesenchymal Stem Cells

Peter Su
Chemical and Biomolecular Engineering, University of California, Berkeley

NNIN iREU Site: National Institute for Materials Science (NIMS), Tsukuba, Ibaraki, Japan
NNIN iREU Principal Investigator: Dr. Guoping Chen, Tissue Regeneration Materials Unit, International Center for Materials Nanoarchitectonics, National Institute for Materials Science, Tsukuba, Ibaraki, Japan
NNIN iREU Mentor: Dr. Jasmine Li, Tissue Regeneration Materials Unit, International Center for Materials Nanoarchitectonics, National Institute for Materials Science, Tsukuba, Ibaraki, Japan
Contact: su.peter92@gmail.com, guoping.chen@nims.go.jp, li.jasmine@nims.go.jp

Introduction:

Mesenchymal stem cells (MSCs) are extremely useful in generating a multitude of cell lineages for tissue regeneration applications. The microenvironment of these MSCs is critical in regulating their differentiation, including soluble factors that bind to various cell receptors. Meanwhile, gold nanoparticles (AuNPs) have shown great potential in biological research due to their ability to interact with biomolecules. Previous work has shown AuNPs of different sizes to affect the regulation of adipogenic (fat cell) differentiation of MSCs [1]. Thus, the objective of this research was to expand on previous research by examining the effects of AuNPs of two different sizes (20 and 90 nm) and functional groups (citrate and β-mercaptopropionic acid (COOH)) on MSC growth, morphology, and degree of adipogenic differentiation.

Experimental Procedure:

Gold nanoparticles were synthesized via the citrate reduction method. Trisodium citrate was added to a 100 mL solution of 0.29 mM tetrachloroauric acid in a reflux setup, heated at 110°C, and stirred at 700 rpm for thirty minutes. AuNPs of diameters 20 nm and 90 nm were produced by varying citrate concentrations [2]. After synthesis, the AuNPs were purified via centrifugation. Then, they were characterized for size via dynamic light scattering (DLS) and ultraviolet-visible light (UV-vis) spectroscopy, charge and stability via zeta potential measurements, and morphology using scanning electron microscopy (SEM). Finally, to functionalize the AuNPs with β-mercaptopropionic acid, a ligand-exchange reaction was performed at pH 11 in dark conditions for twenty-four hours. These AuNPs were then characterized as well.

Citrate conc. (mM)	Ligand	DLS - Particle diameter after resuspension (nm)	UV-vis peak (nm)	Zeta potential (mV)
1.70	Citrate	23.4 ± 5.0	520	-40.6 ± 1.4
1.70	β-mercaptopropionic acid	19.7 ± 5.6	521	-33.6 ± 2.0
0.34	Citrate	86.8 ± 46.3	568	-47.2 ± 3.0
0.34	β-mercaptopropionic acid	95.8 ± 39.9	586	-46.1 ± 0.8

Table 1: AuNP synthesis and characterization results.

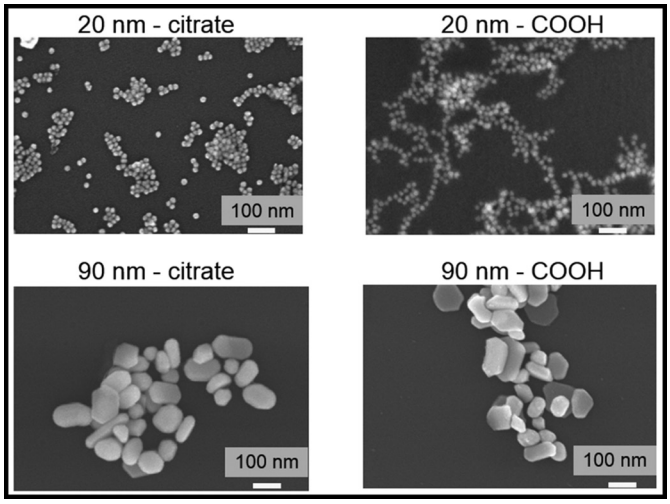

Figure 1: SEM images of the AuNPs (platinum-coated) showing morphology and size distribution.

MSCs were seeded in four 24-well plates at a density of 5 × 10^3 cells/cm^2. Three biological replicates of each condition were used. After one day of culture, 1 mM of AuNPs were added in, along with adipogenesis induction media for two plates (the other two plates were negative controls). Media was changed once every three days. After seven days, growth and morphology were examined via optical microscopy, and adipogenic differentiation was quantified via an alkaline phosphatase (ALP) activity assay and an Oil Red O staining assay. ALP activity was measured using the Anaspec Sensolyte® kit. Oil Red O staining was carried out by fixing then staining the cells with Oil Red O. Cells were then imaged before the oil was eluted and measured for absorbance at 500 nm. Cells were counted using a hemocytometer under an optical microscope.

Results and Discussion:

Table 1 summarizes nanoparticle synthesis and characterization. Zeta potentials were below -20 mV, showing the AuNPs to be stable, while size and morphology of the AuNP (Figure 1) using both high and low concentrations of citrate was consistent with literature [3].

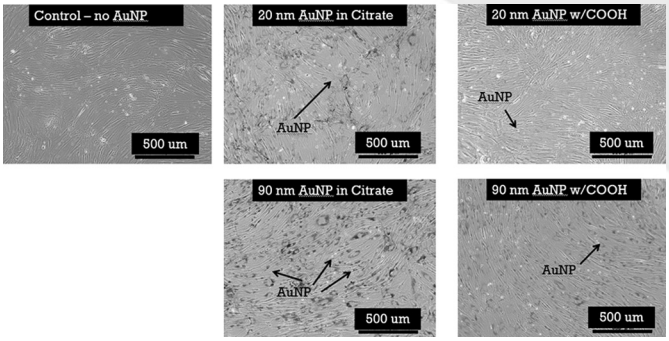

Figure 2: Undifferentiated MSCs grown in growth media after seven days. The arrows indicate AuNPs internalized by the MSCs.

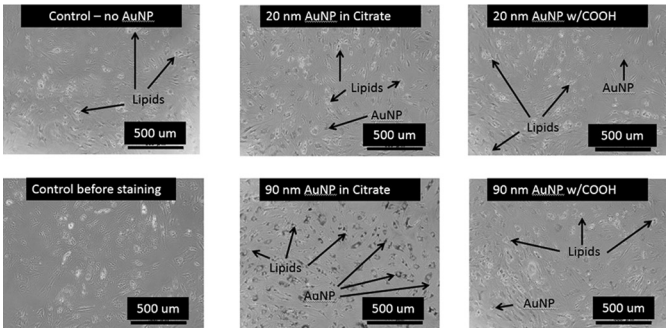

Figure 3: Oil Red O staining of MSCs cultured with adipogenic media after seven days. Arrows indicate AuNPs and lipids inside the MSCs.

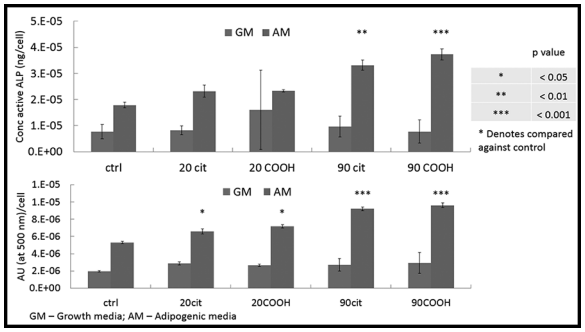

Figure 4: ALP activity assay results (top) and Oil Red O absorbance measurements (bottom) for each condition. Statistics were determined using a one way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test.

		Total Number of Cells/well	
Sample Conditions	Initial	Growth Media	Adipogenic Media
Control	10000	24480	13080
20 citrate	10000	20400	10880
20 COOH	10000	21040	10240
90 citrate	10000	23280	9200
90 COOH	10000	19200	7760

Table 2: Cell number data after seven days of culture.

Figure 2 shows undifferentiated MSCs, while Figure 3 shows Oil Red O images of MSCs with adipogenic media. Adipogenesis was evident by rounding of the MSCs as well as formation of lipid droplets, but in all cases no morphological changes were visible compared to the controls. Additionally, the 90 nm AuNP-citrate condition seemed to show the most cellular uptake. Cell number data (Table 2) clearly shows that growth was inhibited by the 90 nm AuNPs functionalized with β-mercaptopropionic acid.

ALP activity results (Figure 4) demonstrated a significant increase in ALP activity for the two 90 nm AuNPs, more so for the 90 nm COOH AuNP. Additionally, Oil Red O absorbance readings showed an increase in lipid droplet formation per cell for all AuNPs, but significantly more so for 90 nm AuNPs.

These findings indicate that larger AuNPs-COOH seem to not only inhibit growth, but also favor adipogenesis simultaneously. Additionally, there was also less uptake of the 90 nm AuNPs-COOH, indicating that the functional group on this larger AuNP has strong biological implications. These AuNPs may be interacting with a variety of receptors both on the cell surface and inside the cytoplasm. One possible cause of such behavior is that the AuNPs may disrupt F-actin cytoskeleton filaments inside the MSCs, an early step in adipogenic differentiation.

Conclusions and Future Work:

We have demonstrated the successful synthesis of two different sizes and functional groups of AuNPs, and shown that larger AuNP tend to help MSCs favor adipogenesis while inhibiting growth. Although the mechanism of interaction still remains to be elucidated, these findings show interesting implications of β-mercaptopropionic acid functionalized gold nanoparticles with a diameter of around 90 nm.

For future studies, examining expression levels of genes related to adipogenesis would be useful. Additionally, a more exact concentration of AuNPs before treatment and after culture can be taken in order to quantify cellular uptake. Finally, exploration of other biologically active functional groups on the AuNP surface is already being conducted in our laboratory, which can give further information about their effects on MSC adipogenesis.

Acknowledgements:

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References:

[1] Kohl, et al. "Effect of AuNP on adipogenic differentiation of human mesenchymal stem cells." J Nanopart Res (2011) 13:6789-6803.

[2] Long, et al. "Synthesis and optical properties of colloidal AuNP." Journal of Physics: Conference Series 187 (2009) 012026.

[3] Kimling, et al. "Turkevich Method for Gold Nanoparticle Synthesis Revisited." J. Phys. Chem. B 2006, 110, 15700-15707.