One and Two Dimensional Inorganic Nanoparticles' Interactions with Fibroblasts and Stem Cells

J. T. Rashkow*, Y. Talukdar* G. Lalwani* and B. Sitharaman*

*Department of Biomedical Engineering, Stony Brook University
Bioengineering Bldg, Room 115, Stony Brook University, Stony Brook, NY, USA,
balaji.sitharaman@stonybrook.edu

ABSTRACT

We report the effects of one and two dimensional transition metal dichalcogenide nanoparticles, tungsten disulfide nanotubes (WSNTs) and molybdenum disulfide nanoplatelets (MSNPs), on fibroblastic cell (mouse fibroblasts (NIH-3T3) and human adipose derived stem cell (MSC)) viability and MSC differentiation potential. Cytotoxicity of MSNPs and WSNTs dispersed in 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine conjugated polyethylene glycol (DSPE-PEG) was assessed by Presto Blue and lactate dehydrogenase (LDH) viability assays at concentrations ranging from 5 to 300 µg/ml for 6, 12 or 24 h. Viability assays showed no dose or time dependent increase in toxicity for NIH-3T3 cells treated with WSNTs or MSCs treated with MSNPs or WSNTs. Viability of NIH-3T3 cells treated with MSNPs at concentrations above 50 µg/ml was significantly lower compared to untreated cells. Analysis of MSCs differentiation to adipocytes and osteoblasts showed that MSC differentiation potential was not significantly affected after treatment with potentially safe low (10 µg/ml) and high (50 µg/ml) doses of MSNPs and WSNTs for 24 h. TEM analysis showed that both MSNPs and WSNTs are internalized into the cytoplasm by MSCs. The results lay the foundation to further explore the potential of these nanoparticles at potentially safe doses as multifunctional agents for biomedical applications.

Keywords: inorganic nanoparticles, stem cells, cytotoxicity, differentiaiton, tissue engineering

1 INTRODUCTION

Molybdenum disulfide nanoplatelets (MSNPs) and tungsten disulfide nanotubes (WSNTs) are layered inorganic compounds whose interesting physiochemical properties have been investigated for tribological and electronic applications [1]. Biomedical applications of MSNPs and WSNTs include investigation as lubricants for orthodontic wires and catheters [2]. Recently, we have found that these inorganic nanoparticles are able to reinforce polymeric nanocomposites for tissue engineering better than carbon nanotubes and graphene [3,4]. In this application, fibroblastic cell types, including mesenchymal stem cells (MSCs), would be exposed to these particles as the scaffolds degrade, necessitating investigation into the cytotoxicity of these particles. The few studies performed on the cytotoxicity of these nanoparticles have focused on cell types that would be affected during environmental exposure to the particles through inhalation or ingestion and not on stem cells. This study investigates the cytotoxicity of MSNPs and WSNTs to fibroblasts and human MSCs, the particles effect on differentiation potential of MSCs, and the uptake of these nanoparticles to determine potentially safe doses for biomedical applications.

2 MATERIALS AND METHODS

2.1 Synthesis and Characterization

MSNPs were synthesized using a high temperature reaction of MoO_3 and sulfur powder as described previously [5] while WSNTs were purchased from APNano (NY, USA). Particles were characterized by transmission electron microscopy (TEM) and Raman spectroscopy. The nanoparticles were water-solubilized with 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine conjugated polyethylene glycol (DSPE-PEG).

2.2 Cytotoxicity

For cytotoxicity assays, cells were treated with nanoparticle concentrations of 5, 10, 50, 100, or 300 µg/ml for 6, 12, or 24 hours. The two positive controls were untreated or treated with the DSPE-PEG, and the negative control was treated with 70% ethanol to kill the cells. Cytotoxicity was tested by Presto Blue and lactate dehydrogenase (LDH) assays.

2.3 Differentiation

For differentiation studies, cells were treated with 10 or 50 µg/ml of nanoparticles for 24 hours and then osteo- or adipogenic differentiation media was used to induce differentiation. Alizarin Red S staining and alkaline phosphatase and calcium assays were used to assess osteogenesis while Oil Red O staining and elution were used to assess adipogenesis against a control of DSPE-PEG treated cells.

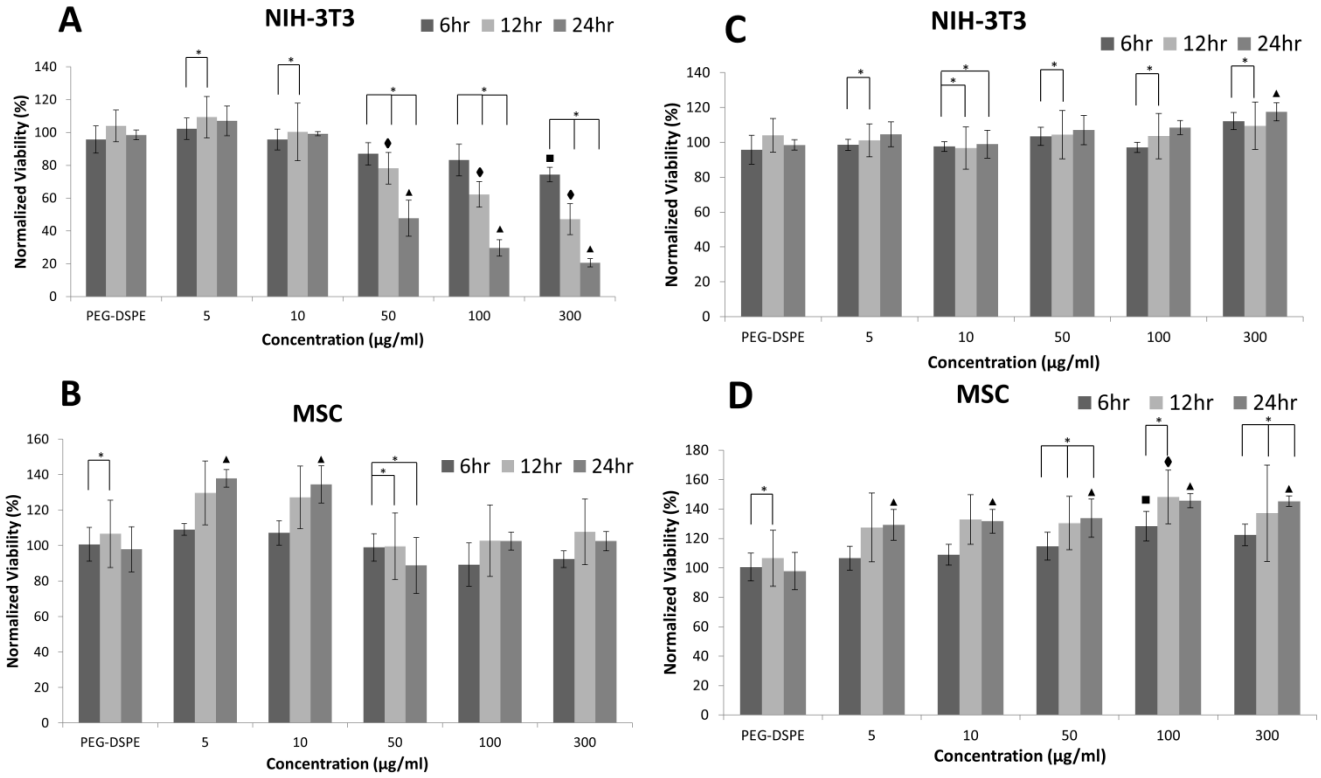

Figure 1. Presto Blue assay results at 6, 12, and 24 hours after treatment. For each nanoparticle, cells were treated with PEG-DSPE, 5 µg/ml, 10 µg/ml, 50 µg/ml, 100 µg/ml, and 300 µg/ml concentrations. MSNPs (A) and WSNTs (C) for NIH-3T3 fibroblasts; MSNPs (B) and WSNTs (D) for MSCs. Data are presented as mean ± standard deviation of percentage viability compared to untreated cells (n = 4). Statistical significance (p = 0.05) with respect to untreated groups at 6, 12 and 24 hours are denoted by (■), (◊), (▲) respectively. Statistical significance between time points within groups is denoted by (*).

2.4 Uptake

MSCs were seeded on ACLAR film and treated with 50 µg/ml of either MSNPs or WSNTs for 24 hours. After treatment, cells were fixed and processed for TEM.

2.5 Statistics

A sample size of four was used for all groups and Kruskal-Wallis with a Dunn post hoc was used to compare within the groups and with the controls.

3 RESULTS AND DISCUSSION

3.1 Characterization

MSNPs were round plates with diameters in the range of 60-90 nm and thickness of about 8 nm [3]. WSNTs were tube-like structures with diameters in the range of 50-100 nm and lengths in the range of 1-15 µm. Raman spectra of the nanoparticles showed the expected peaks for hexagonal molybenum disulfide and tungsten disulfide (data not shown).

3.2 Cytotoxicity

Only treatment of NIH-3T3 cells with MSNPs showed a decrease in viability with increasing concentration as compared to DSPE-PEG. Treatment of fibroblasts with MSNPs and treatment of MSCs with MSNPs or WSNTs showed little to no cytotoxicity at any of the concentrations (Figure 1). An increase in MSC proliferation was observed for all WSNT treatment concentrations compared to the DSPE-PEG control with the highest increase of 45% at 24 hours.

3.3 Differentiation

Figure 2 shows the results of the alkaline phosphatase and calcium assays. MSCs treated with low (10 µg/ml) and high (50 µg/ml) concentrations of MSNPs and WSNTs for 24 hours maintained their differentiation potential to adipocytes and osteoblasts.

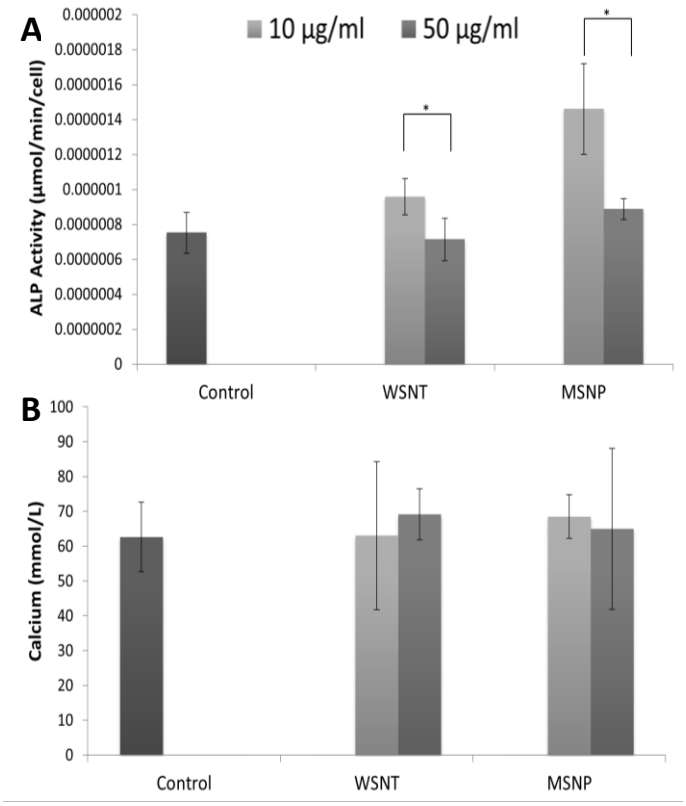

Figure 2**.** Osteogenesis results for MSCs after treatment for 24 h with either 10 or 50 µg/ml of MSNPs or WSNTs respectively, followed by 14 days incubation with osteogenic differentiation media. (A) ALP activity, (B) Calcium content. Data are presented as mean ± standard deviation (n = 3). Statistical significance ($p < 0.05$) was determined by the Kruskal-Wallis test with Dunn's post hoc. (*) indicates significance within groups.

3.4 Uptake

MSNPs are internalized in vesicles in the cells while WSNTs are internalized in vesicles as well as cytoplasmic matrix (data not shown).

4 CONCLUSIONS

The results indicate that concentrations less than 50 µl/ml of MSNPs or WSNTs do not significantly affect viability of NIH-3T3 cells or MSCs and do not affect the differentiation potential of MSCs. The results provide preliminary safety guidelines to further explore the potential of these nanoparticles at potentially safe doses as multifunctional agents for biomedical applications.

5 REFERENCES

[1] R. Tenne, "Inorganic nanotubes and fullerene-like nanoparticles," Nat. Nanotechnol. 1, 103-111, 2006.

[2] A. Katz, M. Redlich, H.D. Wagner, and R. Tenne, "Self-lubricating coatings containing fullerene-like WS_2; nanoparticles for orthodontic wires and other possible medical applications," Tribol. Lett. 21, 135-139, 2006.

[3] G. Lalwani, A.M. Henslee, B. Farshid, L. Lin, F. K. Kasper, Y-X. Qin, A.G. Mikos and B. Sitharaman, "Two-dimensional nanostructure-reinforced biodegradable polymeric nanocomposites for bone tissue engineering," Biomacromolecules, 14, 900-909, 2003.

[4] G. Lalwani, A.M. Henslee, B. Farshid, P. Parmar, L. Lin, Y-X. Qin, F.K. Kasper, A.G. Mikos, and B. Sitharaman. Acta Biomater. 9, 8365-8373, 2013.

[5] C.F. Castro-Guerrero, F.L. Deepak, A. Ponce, J. Cruz-Reyes, M.D. Valle-Granados, S. Fuentes-Moyado, D.H. Galván, M. José, "Structure and catalytic properties of hexagonal molybdenum disulfide nanoplates," Catal. Sci. Technol. 1, 1024-1031, 2011.