A Co-Culture Model for Nanotoxicity and Tissue Engineering Studies

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ABSTRACT

Cell model systems *in vitro* play vital roles in recent advances in toxicological and tissue engineering research. Although the 3-D cell models better simulate the *in vivo* cellular architecture in tissues and organs, the advances made with such models were largely based on studies employing only a single cell type. Thus, there is a need for developing more co-culture cell systems because they provide different structural and functional perspectives that single-cell-type models do not offer. We have therefore developed a co-culture cell model for short- and longer-term nanotoxicity and tissue engineering studies. Our co-culture model consists of a monolayer of human astrocytoma U87 (astrocytes-like) cells onto which we seed human neuroblastoma SK-N-SH (neurons-like) cells. We have characterized and optimized the conditions whereby these two cell types could be co-cultured and have employed this co-culture model to further elucidate the cytotoxicity of metallic oxide nanoparticles.

Keywords: Co-culture model; tissue engineering; nanotoxicity; cell signaling

Topic Area: Cellular & Nanostructure Material Interactions (under BioNanoMaterials & Tissues)

1 INTRODUCTION

Evidence has been accumulating that *in vitro* cell model systems play vital roles in recent advances in toxicological and tissue engineering research. Cell models are particularly versatile in facilitating signaling and related mechanistic studies. Critical advances in recent designs of 3-D cell model systems are to better simulate the *in vivo* cellular architecture in tissues and organs [1-3].

The 3-D cell models have contributed significantly to a better understanding of the biological importance of designing the appropriate extracellular matrix (ECM) material such that the ECM provides a structurally and functionally adequate scaffold onto and around which cells proliferate and grow into the 3-D mode. Indeed, creative applications of nanomaterials (e.g., nanotubes and nanofibers) have led to the development of biodegradable ECM that could closely mimic the complex nanoscale architecture of the extracellular milieu along with providing some, if not all, of the topological molecular organization of biological cues that occur in native tissues and organs *in vivo*. Nevertheless, most of these advances were largely based on studies employing only a single cell type. [1].

We are aware of the need for developing more co-culture cell systems *in vitro* because they provide different structural and functional perspectives that single-cell-type models do not offer because the co-culture systems more closely simulate the native cellular heterogeneity and architecture in tissues and organs [1-3]. After all, in a native tissue or organ, more than one cell type co-exists in a layer and between layers, thereby forming heterogeneous cellular architecture within the tissue or organ. Furthermore, these models can also serve as artificial organs [4].

Our work on cerebrocortical neurons co-cultured on a monolayer of astrocytes reveals that neurons are more susceptible to manganese toxicity than astrocytes. More importantly, the astrocytes appear to provide some protective effect on the neurons in co-culture [5]. Based on these earlier findings of ours [5], we have therefore systematically developed a co-culture cell model for both short- and longer-term nanotoxicity and tissue engineering studies.

2 MATERIALS AND METHODS

2.1 Monotypic-culture of SK-N-SH or U-87 cells

Human neuroblastoma (neurons-like) SK-N-SH or astrocytoma (astrocytes-like) U-87 cells (ATCC, Manassas,VA, USA) were seeded into each well of a 96-well plate, allowed to attached to the bottom of each for 60-90 minutes, and cultured in an incubator at 37**°** C and 5 % CO_2 in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS). Then the cells were treated with specified concentrations of SiO_2 nanoparticles in MEM for 48 hours at 37**°** C. Subsequently, cell survival was determined using the MTT assay as described below.

2.2 Co-Culture Model of SK-N-SH Cells Growing on a Monolayer of U87 Cells

U87 cells were seeded into each well (at 4000 cells/well) of a 24-plate and allowed to attach and grow for 24 hours in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) in an incubator at 37^o C with 5 % CO_2. Then SK-N-SH (6000 cells per well) were seeded onto each well of a 24-well plate and allowed to grow at 37^o C with 5 % CO_2 for another 24 hours. Subsequently, the co-cultures were treated with SiO_2 nanoparticles at 1, 10, or 50 μg/mL for 48 hours at 37^{o}C. At the end of the treatment period, the survival of the cells in the co-culture was assessed the MTT assay as described below. Untreated cells served as the control.

2.3 MTT (Cell Survival) Assay

The MTT dye was added at the end of the treatment period (see above) to each well of the 96- or 24-well plates and the plates were incubated at 37^o C for 4 hours. The purple colored formazan product formed by viable cells was dissolved in dimethyl sulfoxide and the absorbance of the content of each well was measured at 567 nm using a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA) [6,7].

3 RESULTS AND DISCUSSION

Our co-culture model consists of a monolayer of human astrocytoma U87 (astrocytes-like) cells onto which we seed human neuroblastoma SK-N-SH (neurons-like) cells. We have characterized and optimized the conditions whereby these two cell types could be co-cultured.

We found that exposure of U87 (Figure 1) or SK-N-SH (data not shown) cells cultured as a single, monotypic culture to silicone dioxide nanoparticles induced cell damage and cell death in both cell types. For example, exposure of U87 cells to SiO_2 nanoparticles induced a dose-related decrease in the cell survival (Figure 1); exposure of U87 cells to the highest concentration of SiO_2 nanoparticles induced ~75% of cell death.

Similar to the effects on U87 cells (Figure 1), treatment of SK-N-SH cells cultured in a single, monotypic culture with SiO_2 nanoparticles also induced a concentration-related decreases in survival of SK-N-SH cells (data not shown).

Once we established the co-culture model, we employed the co-culture (i.e., SK-N-SH cells growing on a monolayer of U87 cells) to further elucidate the cytotoxicity of metallic oxide nanoparticles, employing SiO_2 nanoparticles as the test nanoparticles (Figure 2).

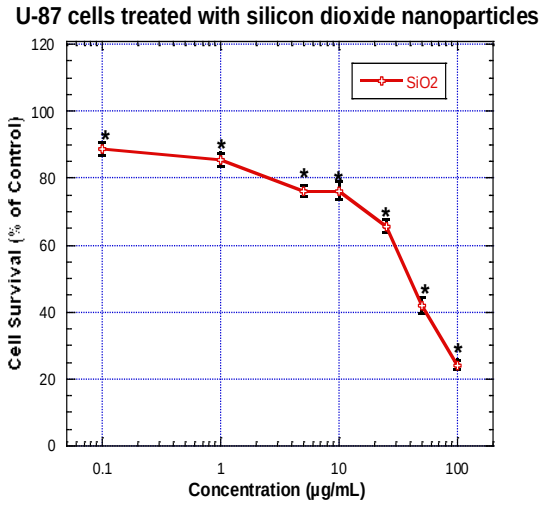

Figure 1. Treatment of SiO_2 nanoparticles induced dose-related decreases in U87 cells in monotypic cell culture. U87 cells were cultured alone and SiO_2 nanoparticles were added to the cultured medium of the U87 cells at the specified concentrations. Forty eight hours later, the survival of the U87 cells were determined using the MTT assay. Values are expressed as % of the control and are given as mean ± SEM of 6-10 determinations. *p< 0.05 versus control.

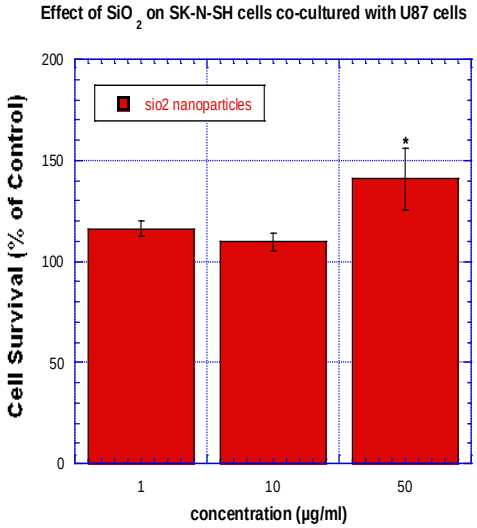

Figure 2. Effect of treatment with SiO_2 nanoparticles on SK-N-SH cells co-cultured with U87 cells. U87 cells were seeded into each well (at 4000 cells/well) of a 24-plate and allowed to attach and grow for 24 hours in MEM supplemented with 10% FBS in an incubator at 37° C with 5 % CO_2. Then SK-N-SH (6000 cells per well) were seeded onto each well of a 24-well plate and allowed to grow at 37° C with 5 % CO_2 for another 24 hours. Subsequently, the co-cultures were treated with SiO_2 nanoparticles at 1, 10, or 50 µg/mL for 48 hours at 37°C. At the end of the treatment period, the survival of the cells in the co-culture was assessed the MTT assay. Untreated cells served as the control. Values are expressed as % of the control and are given as mean ± SEM of four separate determinations. *p< 0.05 versus control.

Based on our prior experience with developing co-cultures of neurons growing on a monolayer of astrocytes [see 5 for discussion], we hypothesized that when two cell types are cultured in the contact-mode, they exert some protective effect on each other from the cytotoxicity of a toxicant to which they are co-exposed.

We therefore investigated our hypothesis by growing SK-N-SH (neurons-like) cells on a monolayer of U87 (astrocytes-like) cells and exposing both cell types simultaneously to different concentrations of SiO_2 nanoparticles. Based on the results we obtained with monotypic cultures of U87 and SK-N-SH cells (Figure 1), we anticipated to detect some effects of the SiO_2 nanoparticles on decreasing the survival of U87 and SK-N-SH cells.

Our results (Figure 2), on the other hand, strongly suggested that when the SK-N-SH cells were co-cultured

with U87 cells, both cell types appeared to exert some protective effect on each other against the cytotoxic effect of SiO_2 nanoparticles (Figure 2). Indeed, not only the SiO_2 nanoparticles did not apparently exert any cytotoxic effect on the co-cultured SK-N-SH and U87 cells, the cells appeared to survive better and proliferate even in the presence of the highest concentration of SiO_2 nanoparticles studied (50 μg/mL) (Figure 2).

We observed this "mutual protective" effect against the same range of SiO_2 nanoparticles even when we varied the seeding ratios of SK-N-SH versus U87 cells (data not shown).

4 CONCLUSIONS

When taken together, our results obtained from both the monotypic cell culture and the co-culture models revealed that both models are useful in elucidating the cytotoxicity mechanisms underlying the effects of metallic oxide nanoparticles and other nanomaterials. Moreover, as our results strongly suggested (Figures 1 and 2), the effects of these nanoparticles on SK-N-SH and U87 cells when cultured in a monotypic cell culture model differ markedly from those on SK-N-SH and U87 cells when cultured in the co-cultured mode (compare Figures 1 and 2). Consequently, our observation may have major implications in pathophysiological mechanisms underlying the cytotoxicity of metallic oxide nanoparticles on neural and other human cell types.

Additionally, this model system can also be exploited to investigate intercellular communications in studies, for example, to elucidate signaling mechanisms between two different cell types when cultured in the contact mode. This approach definitely contrasts with the capabilities of the co-culture cell model in the non-contact model we have designed and discussed elsewhere [see 8 for additional discussion].

By varying the cell types in our contact co-culture model, it provides a novel system whereby many different (including mechanistic) issues of tissue engineering research may also be addressed.

5 ACKNOWLEDGMENTS

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REFERENCES

[1] Fiegel HC, Kaufman PM, Bruns H, et al. Review: Hepatic Tissue Engineering. J Cell Mol Med Nov 16, 2007 [Epub ahead of print].

[2] Napolitano AP, Dean DM, Man AJ, et al. Scaffold-Free Three-Dimensional Cell Culture Utilizing Micromolded Nonadhesive Hydrogels. Biotechniques 43(4): 496-500, 2007.

[3] Du Y, Han R, Ng S, et al. Identification and Characterization of a Novel Prespheroid 3-Dimensional Hepatocyte Monolayer on Galactosylated Substratum. Tissue Eng 13(7):1455-1468, 2007.

[4] Nahmias Y, Berthiaume F, Yarmush ML. Integration of Technologies for Hepatic Tissue Engineering. Adv Biochem Eng Biotechnol 103:309-329, 2007.

[5] Lai JCK, Leung TKC & Lim L. Effects of Metal Ions on Neurotransmitter Function and Metabolism. In Metal Ions in Neurology and Psychiatry (Neurology and Neurobiology, Vol. 15) (Gabay S, Harris J & Ho BT, eds), Alan Liss, New York, pp. 177-197, 1985.

[6] Malthankar GV, White BK, Bhushan A, et al. Differential Lowering by Manganese Treatment of Activities of Glycolytic and Tricarboxylic Acid (TCA) Cycle Enzymes Investigated in Neuroblastoma and Astrocytoma Cells is Associated with Manganese-Induced Cell Death. Neurochem Res 29:709-717, 2004.

[7] Dukhande VV, Malthankar-Phatak GH, Hugus JJ, et al. Manganese Induced Neurotoxicity is Differentially Enhanced by Glutathione Depletion in Astrocytoma and Neuroblastoma Cells. Neurochem Res 31(11):1349-1357, 2006.

[8] Gao WJ, Dukhande VV, Jandhyam S, et al. A Non-Contact Culture Model for Investigating Cell Signaling and Nanotoxicity. In Proceedings of 2008 Nanotechnology Conference and Trade Show (in press), 2008.