A Non-Contact Culture Model for Investigating Cell Signaling and Nanotoxicity

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ABSTRACT

There is general agreement that *in vitro* cell model systems offer many advantages over *in vivo* model systems in elucidation of cell signaling and other mechanisms underlying a toxicant's effect. While the 3-D cell models simulate the *in vivo* cellular architecture in tissues/organs, many 3-D cell models only focus on one cell type. Consequently, there is need for additional and alternative approaches to design cellular models with and without physical contact. We design a "non-contact culture model" whereby two cell types are separated by a nanopore membrane. We use this approach to investigate cell-cell communication and interaction without physical contact by growing a monolayer of SK-N-SH neuroblastoma cells on the bottom of 12- or 24-well plates with hanging inserts with polyethylene terpthalate nanopore membrane onto which is seeded a monolayer of U87 astrocytoma cells. We use this model to characterize intercellular communications and the cytotoxicity of toxicants, including metallic oxide nanoparticles.

Keywords: Non-contact culture model; cell signaling; nanotoxicity; tissue engineering; artificial organs

Topic Area: Artificial Organs (under BioNanoMaterials & Tissues)

1 INTRODUCTION

There is general agreement that *in vitro* cell model systems offer many advantages over *in vivo* model systems in elucidation of cell signaling and other mechanisms underlying a toxicant's effect [1-3]. While the 3-D cell models simulate the *in vivo* cellular architecture in tissues/organs, the provision of an adequate extracellular scaffold similar to native extracellular matrix to support cellular growth in 3-D still poses some challenges [1]. Moreover, many 3-D cell models only focus on one cell type [1-3]. Consequently, there is need for additional and alternative approaches to design cellular models that simulate cell-cell interaction with and without physical contact. Furthermore, these models can also serve as artificial organs [4].

We therefore design a "non-contact culture model" whereby two cell types are separated by a nanopore membrane. We use this approach to investigate cell-cell communication and differential cytotoxicity of toxicants.

2 MATERIALS AND METHODS

2.1 Co-culture of SK-N-SH and U-87 cells

Human neuroblastoma (neurons-like) SK-N-SH and astrocytoma (astrocytes-like) U-87 cells (ATCC, Manassas,VA, USA) were cultured in an incubator at 37° C and 5 % CO_2 in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS). SK-N-SH cells were plated on 24 well plates. U-87 cells were introduced by using Millipore polyethylene terpthalate (PET) hanging culture inserts (pore size 0.4 μm) to achieve co-culture condition.

2.2 MTT (Cell Survival) Assay

SK-N-SH cells were treated with rotenone (100 nM, 200 nM, 500 nM and, 2000 nM) and MTT dye was added at the end of 24 hrs incubation. Purple colored formazan product formed by viable cells was dissolved in dimethyl sulfoxide and absorbance was measured at 567 nm using a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA) [5,6].

2.3 Reactive Oxygen Species (ROS) Generation

Carboxy H_2DCFDA (2',7'-dihydrodichlorofluorescein diacetate) is an indicator of ROS. SK-N-SH cells were treated with rotenone (1000 nm), BSO (1 mM, 2.5 mM) and combination of these two for 24 hours and then were incubated with 10 μM of carboxy H_2DCFDA dye (dissolved in DMSO) for 45 minutes and then the medium was replaced with sterile PBS. The fluorescence formed from the oxidized dye was measured at excitation of 492 nm and emission of 521 nm employing a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA).

2.4 Protein Determinations

Bicinchoninic acid protein assay kit from Pierce (Rockford, IL, USA) was used to determine protein levels in cell homogenates and or extracts as described previously [5].

3 RESULTS AND DISCUSSION

To facilitate the elucidation of intercellular interaction(s) without physical contact, we designed a "non-contact culture model" by growing a monolayer of SK-N-SH neuroblastoma cells on the bottom of 12- or 24-well plates with hanging inserts with polyethylene terpthalate nanopore membrane onto which was seeded a monolayer of U87 astrocytoma cells.

In this model, the U87 cells appeared to exert some protective effect on SK-N-SH cells against oxidative stress. For example, when SK-N-SH cells by themselves were exposed to increasing concentrations of rotenone (which is a metabolic poison that inhibits mitochondrial complex I activity), their survival was decreased with increases in rotenone concentration (Figure 1). At concentration of 500 or 2000 nM, rotenone treatment resulted in 40% SK-N-SH cell death, indicating that rotenone is a potent metabolic poison for these cells (Figure 1).

On the other hand, in the presence of U87 cells in the non-contact mode, the rate of increase in the death of SK-N-SH cells when exposed to increasing concentrations of rotenone was slowed, with only 13% and 27% cell death, respectively, when SK-N-SH cells were exposed to 500 and 2000 nM of rotenone (Figure 1). Thus, these results (Figure 1) are consistent with the hypothesis that when SK-N-SH cells are co-cultured with U87 cells in the non-contact mode, the U87 cells protect the SK-N-SH cells against oxidative stress induced by treatment with rotenone.

To further test the hypothesis stated above, we investigated the protection paradigm of this non-contact co-culture model by examining the effects of agents that are known to induce oxidative stress either singly or in come combination on the production of reactive oxygen species (ROS) by the model (Figure 2). For these studies, we chose two agents that are known to exert oxidative stress on cells, especially neural cells — rotenone and L-buthionine-(S, R)-sulfoximine (BSO).

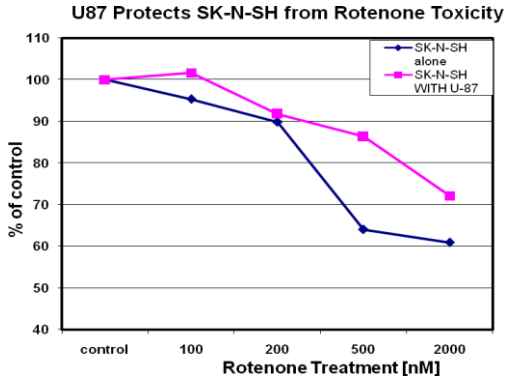

Figure 1. Co-culture with U87 cells protects SK-N-SH from rotenone-induced decreases in cell survival. SK-N-SH cells were cultured alone (blue diamond) or with U87 cells in the non-contact mode (purple squares) and the rotenone was added to cultured medium of the SK-N-SH cells at the specified concentrations. Twenty four hours later, the survival of the SK-N-SH cells only were determined using the MTT assay. Values are the mean of two separate determinations.

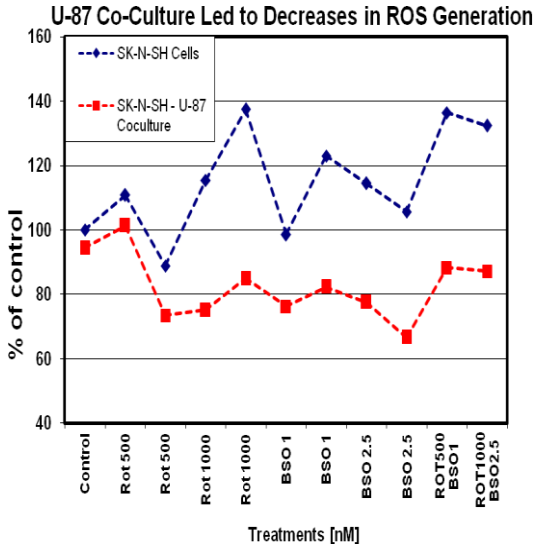

Figure 2. Effects of treatment with rotenone (Rot), L-buthionine-(S, R)-sulfoximine (BSO), or some combination of Rot and BSO on reactive oxygen species (ROS) production by SK-N-SH cells cultured singly (blue diamond) or as the co-culture in the non-contact mode with U87 cells (red square). SK-N-SH cells were treated with Rot, BSO (in mM), or Rot plus BSO (in mM) for 24 hours and then ROS production by the treated SK-N-SH cells were determined as described in MATERIALS AND METHODS. Values are the mean of two separate determinations.

Rotenone is a known metabolic poison that selectively inhibits mitochondrial Complex I activity and has been employed to generate animal and cell models of Parkinson's disease. BSO is a selective inhibitor of glutathione synthesis and glutathione is one of the most important intracellular naturally-occurring antioxidant. Indeed, BSO has been often employed to induce oxidative stress [see 6 for discussion].

As shown in Figure 2, rotenone treatment at the higher concentration of 1000 nM and to a lesser extent BSO treatment at both concentrations (in mM) induced SK-N-SH cells to produced more ROS compared to control (i.e., untreated) SK-N-SH cells. Similarly, SK-N-SH cells treated with a combination of rotenone and BSO also produced more ROS compared with untreated SK-N-SH cells although the effect of rotenone and BSO was not additive (Figure 2).

On the other hand, when the SK-N-SH cells were co-cultured with U87 cells in the non-contact mode, their production of ROS were lower than those of control SK-N-SH cells regardless of the treatment or treatment combination (Figure 2). The results of these ongoing studies suggested that co-culturing with U87 cells in the non-contact mode rendered SK-N-SH less able to generate ROS. Alternatively, another explanation for our results (Figure 2) may be that the U87 cells even in the non-contact mode, might have produced antioxidants (e.g., reduced glutathione) [6] to eliminate the ROS produced by SK-N-SH cells. Irrespective which is the more likely mechanism, the conclusion that co-culturing with U87 cells affords SK-N-SH cells protection against oxidative stress may still be tenable. Clearly, this is an interesting mechanistic avenue for further investigation.

4 CONCLUSIONS

Our ongoing studies demonstrate that the non-contact cell model consisting of two cell types can be gainfully employed to elucidate cytotoxicity mechanisms in one cell type and the protective paradigm when the first cell type is co-cultured with a second cell type. 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 non-contact mode. This approach definitely contrasts with the capabilities of the co-

culture cell model in the contact model we have designed and discussed elsewhere [see 7 for additional discussion].

By juxtaposing the two cell layers separated by a nanopore membrane of known pore size, we can investigate intercellular communications between these cell types when they are singly or simultaneously exposed to an agent such as a metabolic poison (such as rotenone that has been employed in our studies) or metallic oxide nanoparticles.

Our model can thus simulate an artificial organ having two different cell types separated by capillaries (i.e., simulated by the nanopore membrane). By varying the cell types in our model, it provides a novel system whereby many different (including mechanistic) issues of tissue engineering research may be addressed.

5 ACKNOWLEDGMENTS

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