A drug screening system: Preparation of cell islets on the hydrophilic surface

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Abstract

Individual surface(hydrophilic/hydrophobic) were prepared and mammalian cells were cultured on the hydrophilic region. For drug test, cancer and normal cells were treated with Taxol, as an example. Our system was compared with MTT assay. CHO cells were resistant to Taxol up to 100 nM in both Methods. However, A549 cells was sensitive at 100 nM Taxol in the 2 day-treatment. Cervical carcinoma cell, HeLa, was very sensitive to Taxol. In our system, the cells were not shown from above 20 nM Taxol treatment. Our system was competitive to MTT assay in animal cells for drug test.

Introduction

For drug development, animal test is an effective mean because it shows directly effectivity and toxicity. However, considering increasing trend animal protection and ethics, many researchers have been studied for the substitution of animal test with animal cell culture. Among alternations, *In vitro* cell culture had been used by researchers for drug development. The cells or tissues in bio-screening system are affected by chemical or biochemical agents. Hydrophilic regions supply adhesive surface for animal cells. APTS (3-aminopropyl triethoxisilane) is a silane derivative containing amino group as a hydrophilic region. It was reported that APTS can form chemical bond with OH- group on glass. Purpose of this work is to develop a rapid, simple and costly effective system.

Materials and Methods

Glass cleaning

Slide glasses (75 mm×25 mm, 22 mm×25 mm)were obtained from Marienfeld. Glasses were dipped into MeOH : HCl (1:1) solution for over 30 min, then washed with MeOH and dH_2O 3 times. After drying, the glasses was immersed into conc. H_2SO_4 solution, followed by rinsing with MeOH and dH_2O, again.

APTS (3-aminopropyl triethoxysilane) coating

In order to immobilize amino group on the glass surface, 2% APTS (Sigma-Aldrich) in

dH_2O was used. By spotting 10 $\mu\ell$ of 2 % APTS on a glass, hydrophilic region was produced. After 30 min, the glasses were dried in a 60℃ dry-oven.

Sigma cote coating

In order to prepare hydrophobic background around hydrophilic region, the glasses were coated with Sigma cote® solution (Sigma-Aldrich). Sigma cote had shown hydrophobic property. After drying for 2 days in a dry-oven (60 ℃), the glasses were washed out with EtOH and dH_2O 3 times. Water drop, were attached only on hydrophilic region. At last, the glasses were sterilized by ultra violet lamp for 30 min a in laminar fluid cabinet.

Cell culture

CHO K1 (Chinese hamster ovary; normal cell), A549 (lung cancer cell), HeLa (cervix cancer cell) cell lines were obtained from KCLB (Korea cell line bank). All cell lines were cultured with IMDM (Iscove's Modified Dulbecco's Medium, Nunc) with 10 % heat-inactivated bovine serum. Mammalian cells were cultured totally on the modified glasses in humidified 5 % CO_2 at 37℃ for 30 min. By rinsing with PBS (Phosphate buffer saline) solution, draft cells on hydrophobic region were washed out. After incubation for one day, the cells on hydrophilic region were treated by each concentration Taxol in well plates.

MTT assay

The assay was based on the protocol as Doyle et al. described[1]. Each cell (1×10^4 cells/well) was cultured in 96-well plates in 100$\mu\ell$ media for one day. Then 10 $\mu\ell$ MTT(Sigma-Aldrich) stock solution (5 mg/ml) was added into each well. The plates were incubated in humidified 5% CO_2 at 37 ℃ for three hours until formazan crystal was appeared. The insoluble blue formazan crystals was dissolved by pipetting after addition of 100$\mu\ell$ 0.04 M HCl in propanol-2-ol. Cell viability was measured using ELISA reader (Bio-rad) at dual wavelength (570/630nm).

Staining

Cells on surface-modified glass were stained with methylene blue basic fuchsin (Sigma)[2]. The photograph of glass surface was taken by a digital-camera, Sony DSC-F505V,

Result

Biomaterial researchers have used chemical binding groups that are available to cell-adhesive surface. Amino group was effective for adhesion and proliferation of mammalian cells in serum-free and serum-rich culture condition. Filippini et al.[3] reported that irrespectively its surface group, amino or carboxyl group, growth profile of mammalian cells was similar to the proliferation on clean glass. In this work, amino group on the surface of glass was prepared by treatment of APTS. Amino group was

reacted with $KMnO_3$ (strong oxidant), and detected by its yellow color. We prepared individual two regions, hydrophilic islets in hydrophobic region. To access the potency of our system for drug screening, Taxol, an anti-cancer drug, was used. Taxol was treated to CHO, A549 and HeLa cells.

Fig. 1 shows CHO cell viability in MTT assay and in vitro assay of cell islets. Both assays showed that CHO cells were resistant against with Taxol up to 100 nM. In MTT assay, CHO cells were resistant to 100 nM Taxol. Cell viability was maintained at 85% of control. Also, *In vitro* assay of cell islet, CHO cells were resistant to Taxol. So we tried to treat Taxol to other cells. A549 cells, lung cancer cells, were assayed with MTT assay and in vitro assay of cell islets(Fig. 2). In MTT assay, though A549 was sensitive depending on the concentration of Taxol, their viability was above 85% except the treatment of 100 nM for 2 days. *In in vitro* assay of cell islets, with treatment of 100 nM Taxol for 2 day, cell viability decreased much 20% more compared with control.

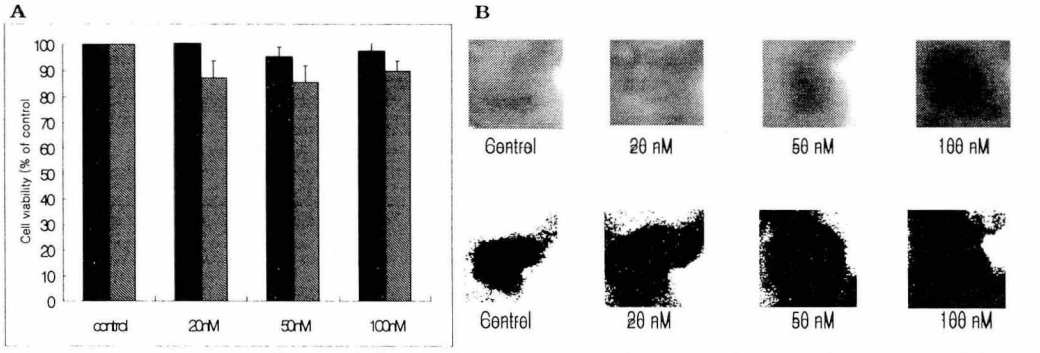

Figure 1. Comparison of MTT assay with *in vitro* assay of cell islets of CHO. A. MTT assay of CHO cell treated with Taxol. Black bar(■) showed the viability of CHO cells after one day cultivation with each concentration Taxol, slant bar(▨) after two day. B. *In vitro* assay of cell islet on a glass after Taxol treatment (up; after one day, down; after two day), CHO cells were stained with methylene blue basic fuchsin, Diameter of cell islet was about 5 mm.

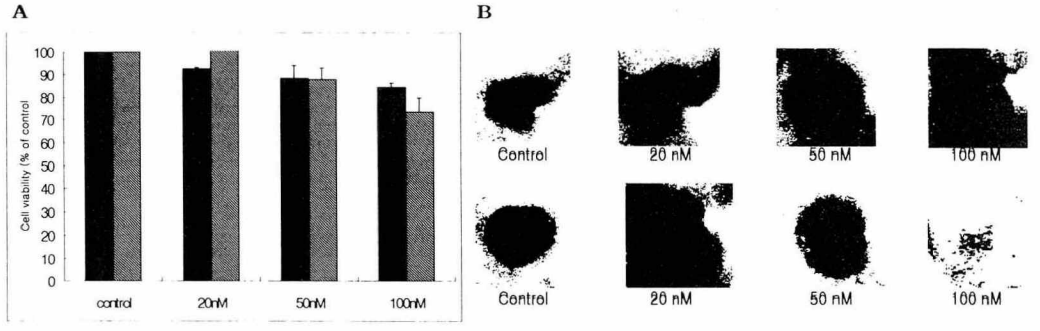

Figure 2. Comparison of MTT assay and in vitro assay of cell islets of A549. A. MTT assay of A549cell treated with Taxol. Black bar(■) showed the viability of A549 cells after one day cultivation with each concentration Taxol, slant bar(▨) after two day. B. *In vitro* assay of cell islet on a glass after Taxol treatment (up; after one day, down; after two day), A549 cells were stained with methylene blue basic fuchsin, Diameter of cell islet was about 5 mm.

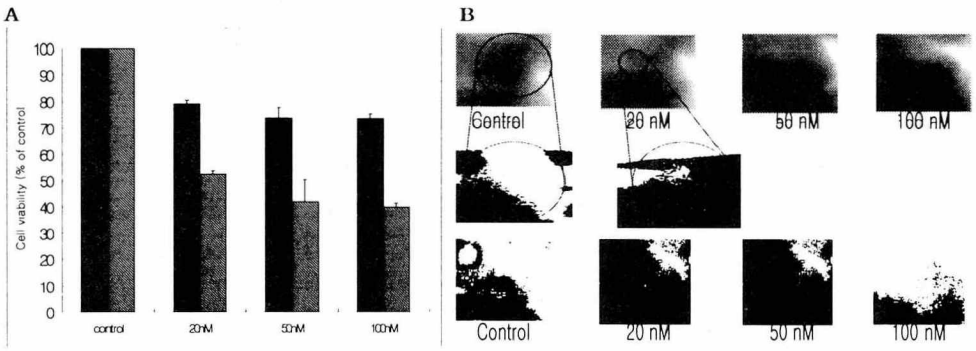

Figure 3. Comparison of MTT assay with *in vitro* assay of cell islets of HeLa. A. MTT assay of HeLa cell treated with Taxol. Black bar(■) is viability of HeLa cells after one day cultivation with each concentration Taxol, slant bar(▨) after two day. B. *In vitro* assay of cell islet on a glass after Taxol treatment (up; after one day, down; after two day), HeLa cells were stained with methylene blue basic fuchsin. Diameter of cell islet was about 5 mm.

MTT assay showed about 80% viability. When treated with Taxol, HeLa cells were very sensitive in both assays (Fig. 3). After one day treatment with 20 nM Taxol, *in vitro* assay of cell islets showed small blue colonies compared with control. In the other hands, MTT assay showed 80% cell viability. In the treatment of above 50 nM Taxol, viable cells were not detected in *in vitro* assay. Our result s indicate that cell islet assay system can be used as a substition of MTT in drug screening assay.

Reference

1. A. Doyle, J. B. Griffiths, D. G. Newell. "Cell & Tissue cluture; Laboratory procedures", John Wiley & Sons. 4B:6.1-2(1993)

2. G. Clark, W. J. Doughty, F. H. Kasten. "staining procedure 4th ed. In; Clark G, editor General methods". Baltimore ; Wiliams & Wilkins. p34-35(1981)

3. P. Filippini, G. Rainaldi, A. Ferrante, B. Mecheri, G. Gabrielli, M. Bombace, P. L. Indovina, M. T. Santini. "Modulation of osteosarcoma cell growth and differentiation by silane-modifed surfaces". Biomed Mater Res 55: 338-349(2001)