Use of Transwell® Cell Culture Inserts to Study Trans-endothelial Transport of Polymeric Doxorubicin Nanoparticles Derived from an Injectable Nanoparticle Generator

Application Note

CORNING

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Introduction

Breast cancer is the most common malignant disease in women. During breast cancer development, breast cancer cells can reach the basement membrane and spread to distant organs via blood vessels and lymphatic systems and form metastases, which is the major cause of death. In the clinic, the lung is the most preferential organ of metastases, and it is difficult to be cured[1]. Metastatic breast cancer usually requires systemic treatment of chemotherapy. To conquer lung metastatic breast cancer, an injectable nanoparticle generator (iNPG) loaded with polymeric doxorubicin (pDox) was developed[2]. To effectively kill cancer cells after systemic administration of chemotherapeutics, one of the key points is whether drugs can overcome a series of biological barriers and achieve sufficient drug accumulation in tumor lesions. Among the barriers, the association and extravasation of particles across tumor-associated vascular endothelial cells are of great importance. Upon injection of particles into the blood stream, drugs are separated from tumor nodules by blood vessel endothelium; consequently, the outcomes of the therapy are determined in part by how the iNPG-pDox is processed by endothelial cells and the efficiency of drug release and transport across the endothelium into tumor cells.

Here, we used a Transwell two-chamber system to test the transcytosis of iNPG-pDox by endothelial cells. This system provides a method to evaluate whether endothelial cells can uptake and process iNPG-pDox particles and transfer drugs effectively to tumor cells.

Transwell Cell Culture Inserts

The Transwell inserts (Corning Cat. No. 3413) with polycarbonate 6.5 mm membrane diameter and 0.4 µm pore size were used to test the transport of drugs from human umbilical vein endothelial cells (HUVECs) to breast cancer cells MDA-MB-231. Transwell inserts provide a cell co-culture environment that mimics *in vivo* conditions. The 0.4 µm pores in the insert membrane prohibit the movement of microparticles but permit the transport of released nanoparticles from the upper chamber to the lower compartment. Cells were seeded on the upper and lower surface of the membrane, which allowed the direct drug uptake by the cells in the lower surface and prevented the dilution of released drugs in the culture media.

Result

HUVECs were maintained in Medium 200 supplied with Low Serum Growth Supplement (complete M200, Invitrogen). MDA-MB-231 cells were cultured with Dulbecco's Modified Eagle's media containing 10% FBS (complete DMEM). The schematic in Figure 1A shows the design of the co-culture system using Transwell cell culture inserts. Transwell inserts were placed

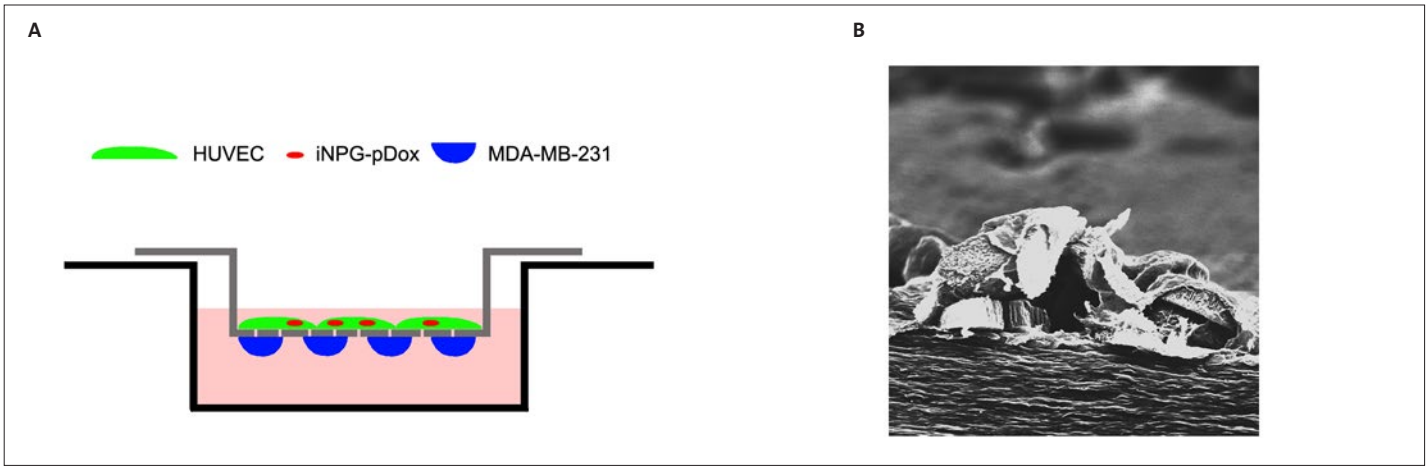

Figure 1. (A) Schematic diagram of the Transwell co-culture system; (B) SEM image of iNPG-pDox particles in HUVECs.

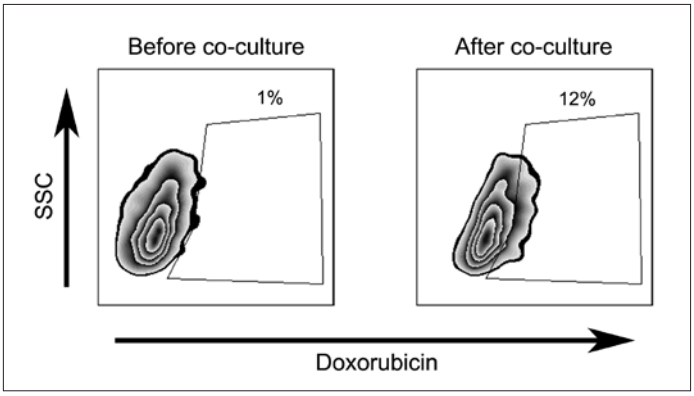

Figure 2. Uptake of doxorubicine by MDA-MB-231 cells after co-cultured with HUVECs with iNPG-pDox.

upside down on a Petri dish and 5 x 10^4 MDA-MB-231 cells in 100 µL complete DMEM were added on the membrane and cultured in a cell incubator to allow cells to attach. Warm media should be supplemented before the membrane turns dry. After 2 hours, the Transwell® inserts were transferred to a 24-well plate in normal hanging position with equally mixed (v/v) complete M200 and complete DMEM overnight. HUVECs were fed with iNPG-pDox microparticles (cell-to-particle = 1:50) for 2 hours followed by PBS wash and cell sorting by a flow cytometer to isolate HUVECs with iNPG-pDox and discard/eliminate free particles. 5 x 10^4 HUVECs with iNPG-pDox were placed in the upper compartment of the Transwell inserts with MDA-MB-231 cells (pre-seeded at the basal side of the membrane) and co-cultured for 48 hours. After co-incubation, the membrane in the insert was withdrawn with a scalpel, torn, and coated for scanning electron microscope (SEM) imaging. In a separate experiment, MDA-MB-231 cells on the lower surface were trypsinized and collected for measurement of doxorubicin content with a flow cytometer.

As shown in Figure 1B, iNPG-pDox microparticles can be visualized from the breakage of the cells. All the particles were taken up by HUVECs and covered by cell membrane. Due to the large size, iNPG-pDox particles cannot pass directly through the pores in the polycarbonate membrane. In Figure 2, doxorubicin content were detected with flow cytometry in MDA-MB-231 cells on the lower surface, which demonstrated that doxorubicin can penetrate out of endothelial cells and reach tumor cells. This result confirmed that cargos loaded in our delivery system can transfer across endothelium barriers and take effect in tumor cells.

Prospective Research

Transwell cell culture inserts provide a very helpful platform for co-culture of different cells and explore short-distant interplay among various cells types. The tumor microenvironment is very complicated. It involves a large amount of cells that interact with each other. Drugs may indirectly interact with a unique subpopulation of cells via many distinct ways. With this system, we can further investigate the multiple effects of particles and drugs in the tumor microenvironment; the interplay among drug, intermediate cells and target cells; and the kinetic process.

References

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