# SECM-Based Drug Sensitivity Test for Three Dimensional Cultured Cells

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The three dimensional cell culture technique has recently been received a great deal of attention in the cancer research field[1]. This culture technique realizes the original growth characteristic of the tumors and enables the evaluation of the drug sensitivity under *in vivo*-like conditions. Although many kinds of cell-based biosensors have been developed and applied for cellular activity monitoring, few studies directly evaluated the 3-D culture maintaining the primal cell nature. We previously reported a silicon-based biosensor developed using the 3-D cell culture, in which cells were embedded within the collagen gel, and applied it to test anticancer drug sensitivity[2]. The drug sensitivity was evaluated by monitoring the cellular proliferation rate using the scanning electrochemical microscopy (SECM) based on the cellular respiratory activity. In the present work, we investigate the drug sensitivity of a multicellular tumor spheroid[1] which is another 3-D cell culture technique without using an extracellular matrix. The differences of anticancer drug sensitivities in 2-D and 3-D cell culture conditions were discussed.

The respiratory activity of a multicellular tumor spheroid was non-invasively monitored by SECM. The hanging drop method was used to generate the spheroid consist of a human breast cancer cell line (MCF-7). The spheroids (ca. 200 μm in radius) were used for SECM measurements and analyzed using spherical diffusion theory[3]. The SECM measurement was carried out in a HEPES buffer solution[2] at room temperature. A probe microelectrode was scanned near the spheroid surface to monitor the oxygen reduction current (Figure 1). The oxygen consumption rate (F) of the spheroid was calculated to be $(1.40 \pm 0.27) \times 10^{-13}$ mol $s^{-1}$ and that of a single cell within the spheroid was estimated to be $(1.20 \pm 0.25) \times 10^{-17}$ mol $s^{-1}$. Thus, the SECM-based assay enables quantifying the oxygen consumption of a spheroid directly.

The respiratory activity of the spheroid was continuously monitored for 5 days with the objective to study anticancer drug sensitivity. The proliferation rate of cells grown within the spheroid was lower than that grown in a monolayer cell culture (2-D culture) and close to that grown within the collagen gel (data not shown). The effects of three anticancer drugs, cisplatin (CDDP, 0.2 μg $mL^{-1}$), 5-fluorouracil (5-FU, 1.0 μg $mL^{-1}$) and paclitaxel (TXL, 0.7 μg $mL^{-1}$) were evaluated by the SECM measurement, based on monitoring the F value. The spheroids were incubated in a culture medium with or without the presence of the anticancer drugs for 24 hours. These exposure conditions were determined so as to be pharmacokinetically equivalent to the anticancer drug plasma concentrations[2]. Figure 2 shows time course of the viability value for three anticancer drugs. Exposure to CDDP was found to be non-effective against the MCF-7 cells, since the viability value for CDDP was greater than 50% for 5 days. 5-FU and TXL was found to be effective, since the viability value for 5-FU and TXL was less than 50% on day 3 and 5; especially, TXL has a remarkable potency for inhibition of cellular growth. Moreover, these results were in good agreement with those obtained by a conventional colorimetric assay based on the activity of succinic dehydrogenase. Therefore, the SECM-based assay enables to continuously evaluate the drug sensitivity using the spheroid without any damages.

We also investigated the influence of the cell culture conditions upon the anticancer drug sensitivity. The present work suggests that cells grown within spheroid tend to be less sensitive against the anticancer drugs compared with that grown in 2-D cell culture and close to that grown within collagen gel. The drug sensitivity test in the 3-D cell culture seems to be important to obtain the meaningful results which can be extended to *in vivo* application.

**References**:

1) A. Abbott, *Nature*, **424**, 870 (2003); A. Reininger-Mack et al., *Trends Biotechnol.*, **20**, 56 (2002).
2) Y. Torisawa et al., *Biomaterials*, in press; Y. Torisawa et al., *Int. J. Cancer*, **109**, 302 (2004); Y. Torisawa et al., *Anal. Chem.*, **75**, 2154 (2003).
3) H. Shiku et al., *Anal. Chem.*, **73**, 3751 (2001); T. Yasukawa et al., *Biophys. J.*, **76**, 1129 (1999).

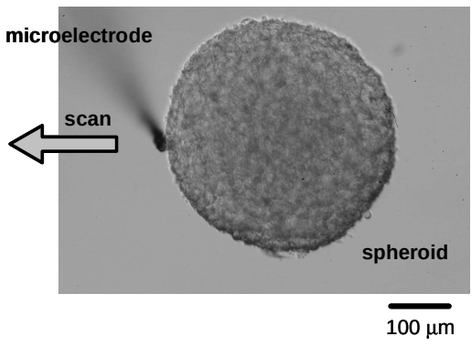


Figure 1. Optical micrograph showing the spheroid (ca. 200 μm radius) and the tip of probe electrode. The Pt microelectrode was set at the potential of -0.5 V vs. Ag/AgCl and scanned a 300 mm step at the scan rate of 19 μm $s^{-1}$. Bar is 100 μm

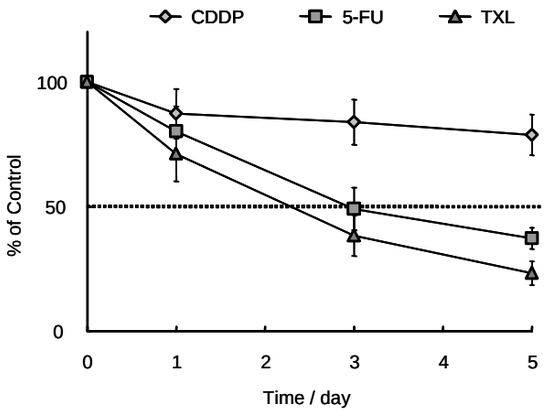


Figure 2. Time course of the viability value for three anticancer drugs measured by SECM. A multicellular spheroids were incubated in the presence of three anticancer drugs for 24 hours. Symbols; CDDP (◆), 5-FU (■), TXL (▲).