Presented by AGC Innovative Technology Research Center
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Test of anticancer drug sensitivity using EZSPHERE™

— comparison of 3D spheroid culture and 2D monolayer culture —

■**Cell species:**
- **DLD-1 (Colorectal cancer cell)**
- **MKN45 (Gastric cancer cell)**

Product of AGC Techno Glass Co., Ltd.
●EZSPHERE™ 96well microplate (#4860-900)
Diameter of micro-well: approx. 500μm
Depth of micro-well: approx. 100μm

■**Culture conditions:**

①Seed the cells into EZSPHERE™ 96well microplate (#4860-900) and tissue culture 96well microplate (#3860-096)
- DLD-1:
  3D spheroid culture --- a: 2×10^5 cells/well, 0.1mL/well / b: 1×10^5 cells/well, 0.1mL/well
  2D monolayer culture --- c: 1×10^4 cells/well, 0.1mL/well / d: 5×10^3 cells/well, 0.1mL/well
- MKN45: both 3D and 2D --- 1×10^5 cells/well, 0.1mL/well

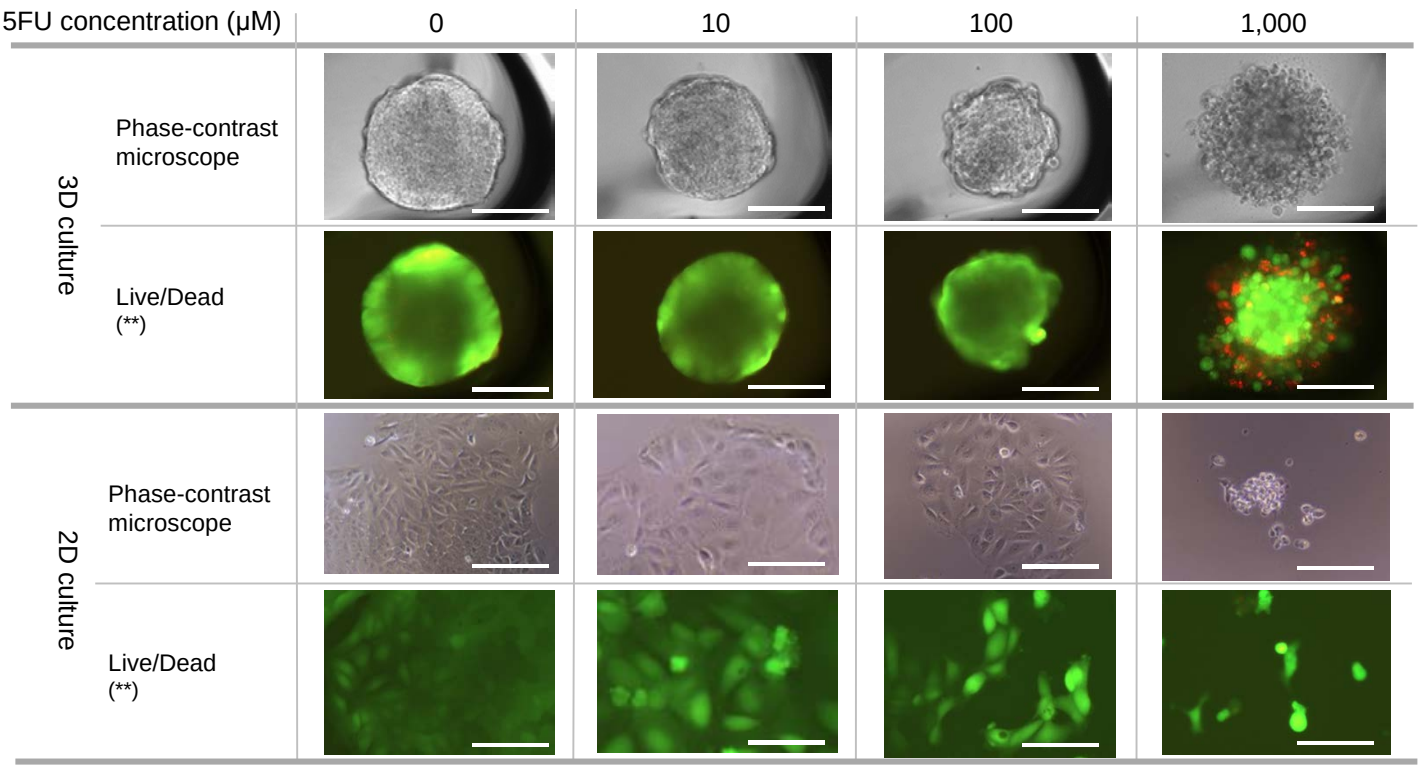
②The next day of seeding, aspirate 50μL medium from each well and add 50μL medium including anticancer drug 5-Fluorouracil (5FU)

③ATP measurement* and microscope observation were conducted to DLD-1 (5 days after seeding (4 days after addition of 5FU)) and to MKN45 (6 days after seeding (5 days after addition of 5FU))
(*) Reagent: Promega CellTiter®-Glo 3D Cell Viability Assay

＜Observation＞

●DLD-1 cell

・In 3D spheroid culture in EZSPHERE™, **solid spheroids** with strong cell adhesion were formed.
As concentration of 5FU got higher, spheroids became small. More than 300μM of 5FU, spheroids were lost the shape and the number of dead cells increased.
・In 2D monolayer culture, the number of cells decreased in more than 1μM of 5FU. Antiproliferative effect was shown in low concentration of 5FU compared to 3D culture.



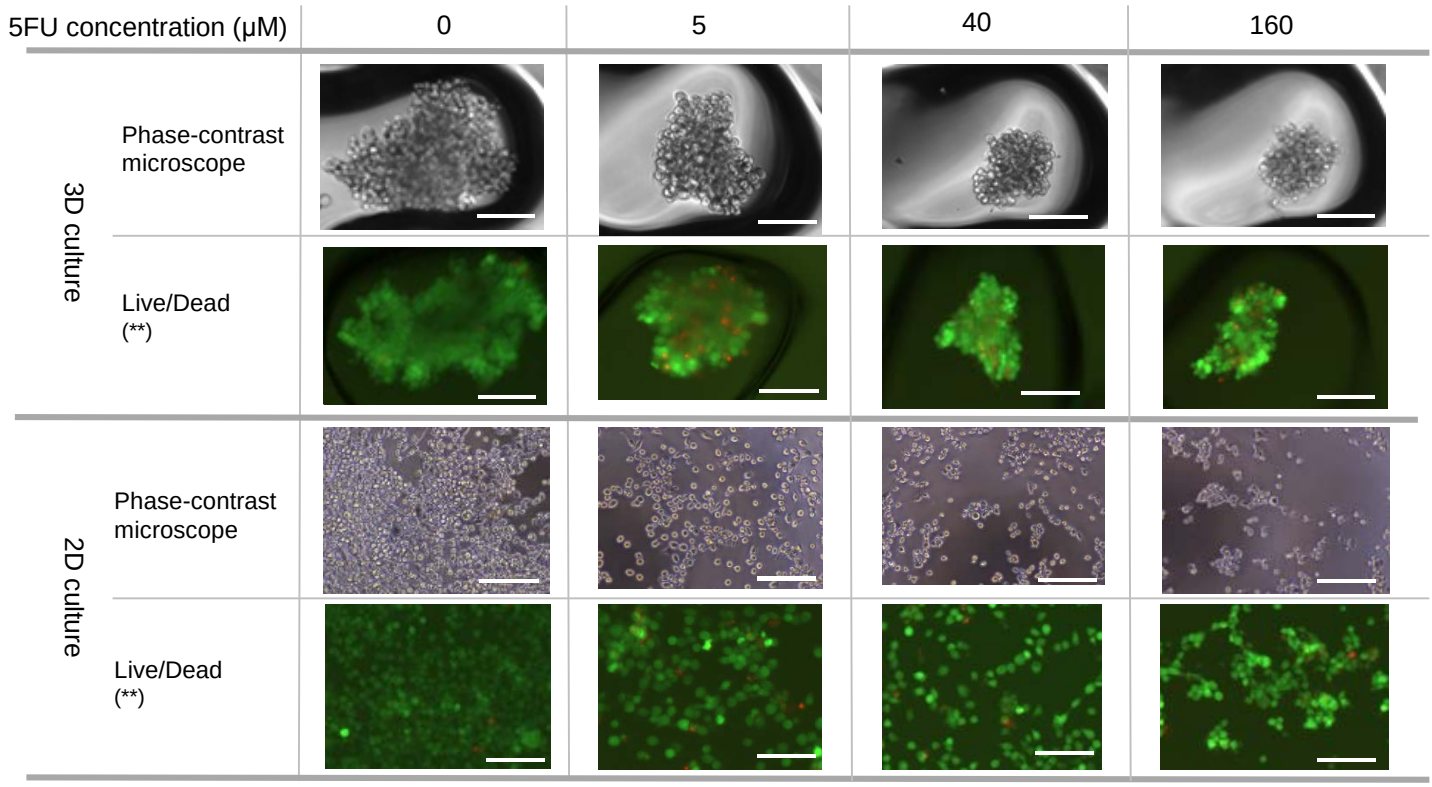
(3D spheroid culture: a. # of cells=2×10^4cells/well, 2D monolayer culture: c. # of cells=1×10^3cells/well)

scale bar = 100μm

(**) Reagent: PromoKine Live/Dead Cell Staining Kit Ⅱ Green=Live cell / Red=Dead cell

●MKN45 cell

・In 3D spheroid culture in EZSPHERE™, **soft spheroids** with soft cell adhesion were formed.
In low concentration of 5FU, spheroids were small and dead cells were found.
・Also in 2D monolayer culture, the number of cells decreased in low concentration of 5FU.

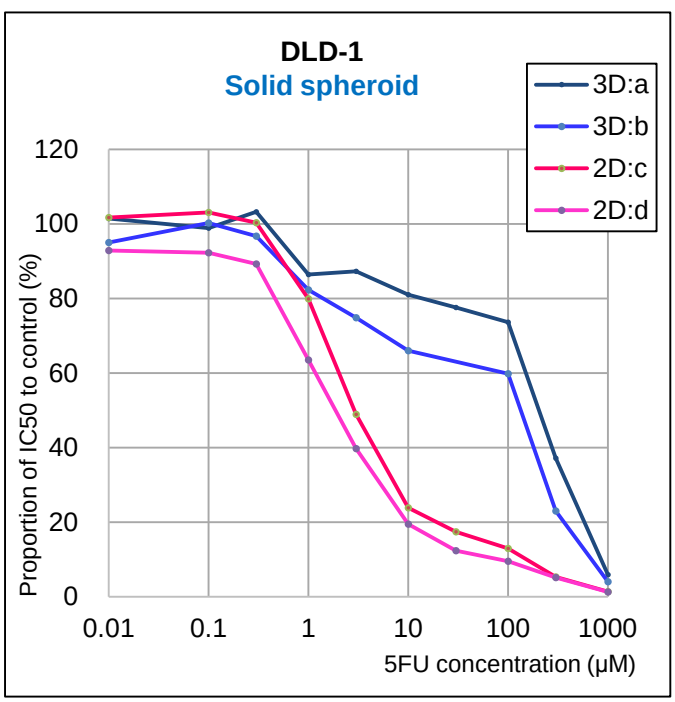


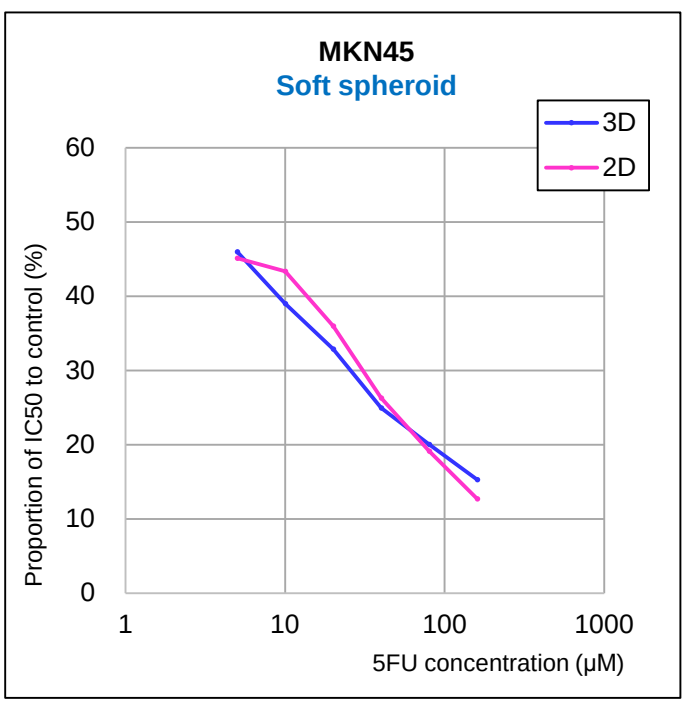
(**) Green=Live cell / Red=Dead cell

scale bar =100μm

＜Drug Sensitivity Test＞

ATP levels were measured as a barometer of the number of live cells. The ATP levels are shown in the graphs below as relative values against the level of 0μM of 5FU which is defined as 100%.
IC_{50} concentration of DLD-1 3D culture cells which form solid spheroid was 100 times as high as 2D culture cells, while IC_{50} concentration of MKN45 3D culture cells which form soft spheroid was as high as 2D culture cells.





EZSPHERE™ prevents good results of drug sensitivity test of cancer cell.
(The situations of spheroid formation and assay depend on cell species and culture conditions.)