Presented by AGC Innovative Technology Research Center
Advised by K. Miyazaki, Professor Emeritus, Yokohama City Univ.

Growth of cancer cell spheroid in EZSPHERE™

■Cell species: DLD-1
Colorectal cancer cell (adenocarcinoma)

■Culture conditions:
①Seed the cells into EZSPHERE™
Medium: DMEM/F12+10% FBS
The number of cells: 2×10^5cells/mL , 0.1mL/well

②Collect the spheroids 1 day, 3 days and 4 days after seeding for observation and measurement of size and growth of spheroids

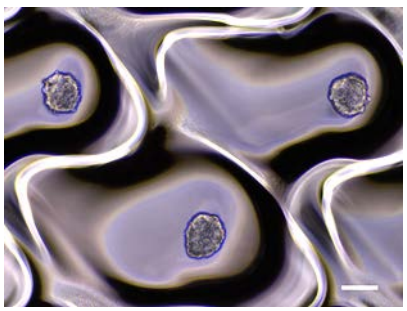
Product of AGC Techno Glass Co., Ltd.
●EZSPHERE™ 96well microplate (#4860-900)

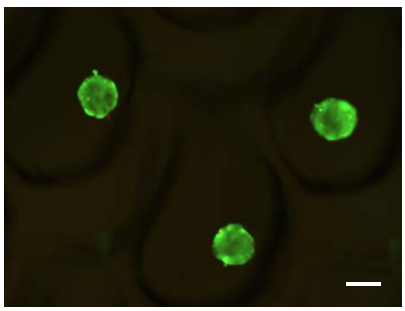
Diameter of micro-well: approx. 500μm
Depth of micro-well: approx. 100μm

＜Observation＞ objective lens ×10, scale bar: 100μm

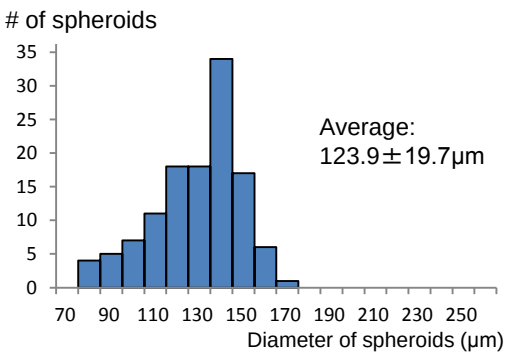
＜Measurement of size＞

1 day after seeding

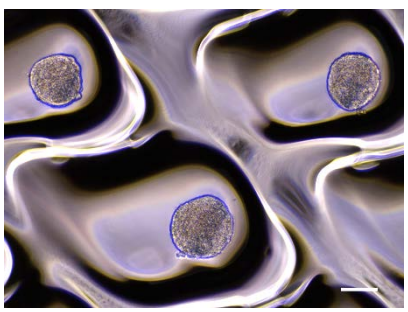


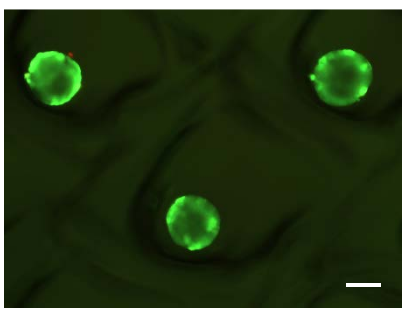


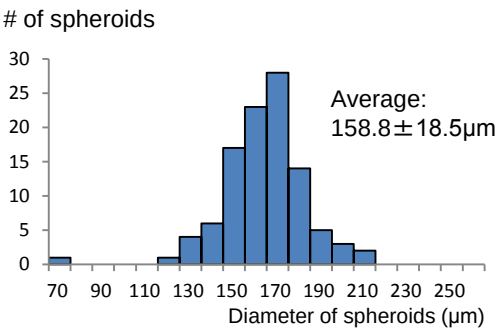
Green=Live cell / Red=Dead cell (*)



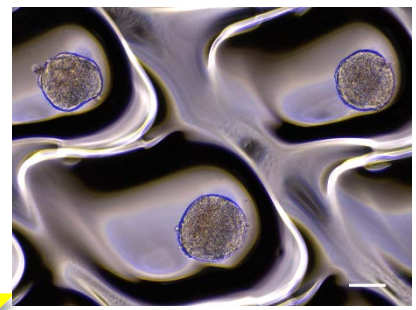
3 days after seeding

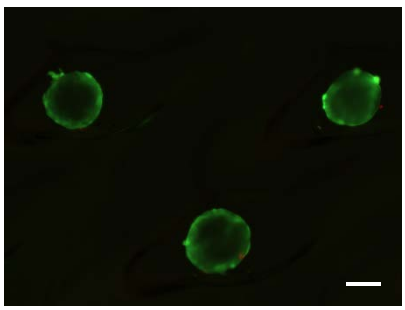


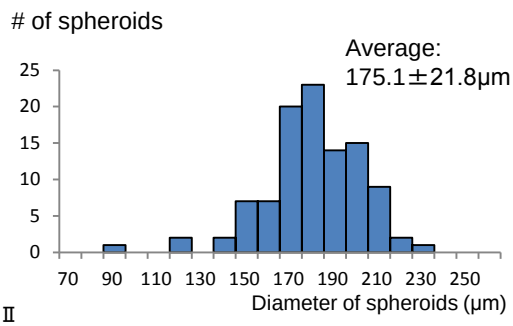




4 days after seeding





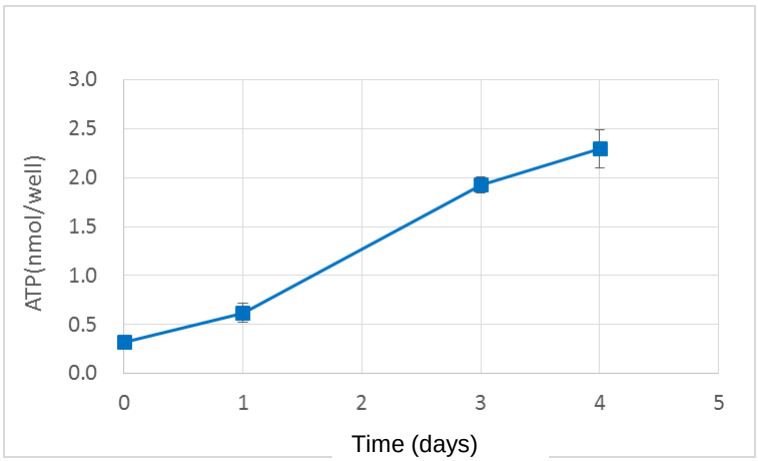


(*) Reagent: PromoKine Live/Dead Cell Staining Kit Ⅱ

＜Measurement of growth＞

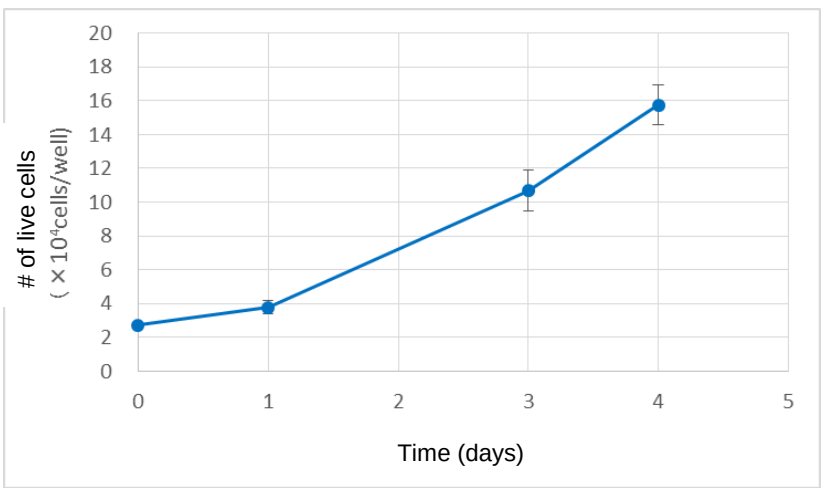
Measurement of ATP level

ATP measurement method – Promega CellTiter®-Glo 3D Cell Viability Assay

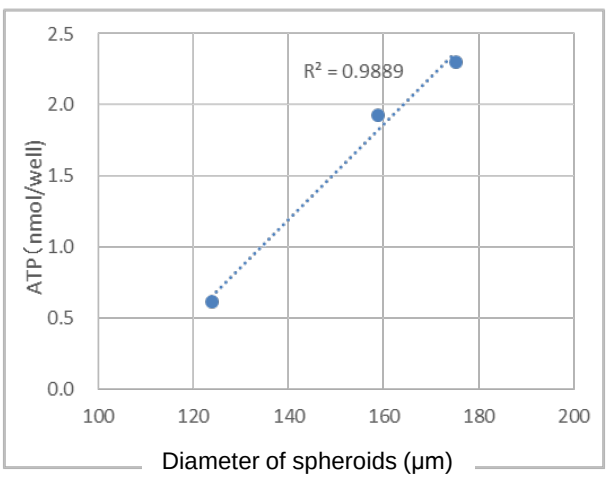


Count of live cells

Spheroids were separated by trypsin and live cells were counted with trypan blue stain



Correlation between ATP level and diameter of spheroid



In 3D spheroid culture, DLD-1 cells formed spheroids of the same size in EZSPHERE™.
Maintaining their high survival rate, the spheroids were grown and the size of spheroids were getting large.
And a significant correlation between ATP level and spheroid size was found.

EZSPHERE™ prevents good formation of cancer cell spheroid.
(The situation of spheroid formation depends on cell species and culture conditions.)