Large size and small size co-culture tumor-like 3D spheroids formation and new model of cancer cell migration

introduction

Cancer cells form a three-dimensional tumor microenvironment in vivo together with many types of stromal cells such as fibroblasts, vascular endothelial cells, and inflammatory cells and extracellular matrix. These complex interactions between cancer cells and their microenvironment have been reported to play important roles in tumor progression, including cancer cell proliferation, infiltration, and metastasis (1, 2). Therefore, in recent years, by mixing cancer cells and stromal cells to produce three-dimensional chimeric (co-cultured) spheroids, the tumor microenvironment can be imitated in the culture vessel, and the function of cancer cells can be more accurately performed. It is expected that the effectiveness of anticancer drug candidate compounds can be analyzed.

AGC has two types of 3D culture vessels, EZ-BindShut and EZ SPHERE, and the optimum vessel shape can be selected according to the purpose of the research. Since the surface of EZ-BindShut has a low adhesive coating, cells do not adhere to the container, but cells aggregate to form spheroids. In EZSPHERE, in addition to the low adhesive coat, fine holes (fine wells) are formed on the culture surface without gaps. Therefore, when cells are sown in EZSPHERE, a uniform number of cells are depressed in each microwell to form spheroids of uniform size. Thus, EZSPHERE is capable to form large numbers of uniformly sized spheroids at one time.

In this application note, we will introduce a method of co-culturing cancer cells and fibroblasts using our EZ-BindShut and EZSPHERE. We will also introduce a cancer cell migration assay method based on a new cancer cell migration / infiltration model recently reported by Dr. Miyazaki (3, 4).

Key word

co-culture spheroid、3D culture 、cancer cell、fibroblast、Cancer cell migration

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Cells

Article	Origin	Provider
GFP-labeled A549	human adeno carcinoma cell line derived from lung cancer (Delivered from JCRB, GFP labeled at our lab)	JCRB
OUS-11	normal human fibroblast line from lung cancer patient	JCRB

Media

Article	Provider	Cat. No
DMEM/F12		
5×DMEM/F12		
fetal bovine serum (FBS)		
0.3% bovine dermis native collagen	Koken Atelo Cell	IAC-30
0.05M NaOH/0.26 M $NaHCO_3$		

AGC's Culture Supporting Vessel

Article	Culture surface	Cat. No
EZSPHERE 96well plate type900	MPC-coating and micro-fabricated Φ500μm dimple	4860-900
EZ-BindShut SP round bottom 96-well plate	SP-coating	4870-800SP
EZ-View LB glass bottomed 96-well plate	TC-treated	5866-096
Φ12 ㎜ glass based 35-mm dish	TC-treated	3961-035

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Optimization of initial cell number and mixing ratio for co-culture spheroid of lung cancer cell and fibroblast in EZ-Bindshut and EZSPHERE microplate

1. Mix single cell dissociated GFP-A549 and OUS-11 at 1:1, 1:2 or 1:5 ratio in DMEM/F12 +10% FBS.
2. Seed the mixed cells into EZ-Bindshut round bottom microplate at 3,000cells/well or 30,000cells/well and allowed culture for 1 day.

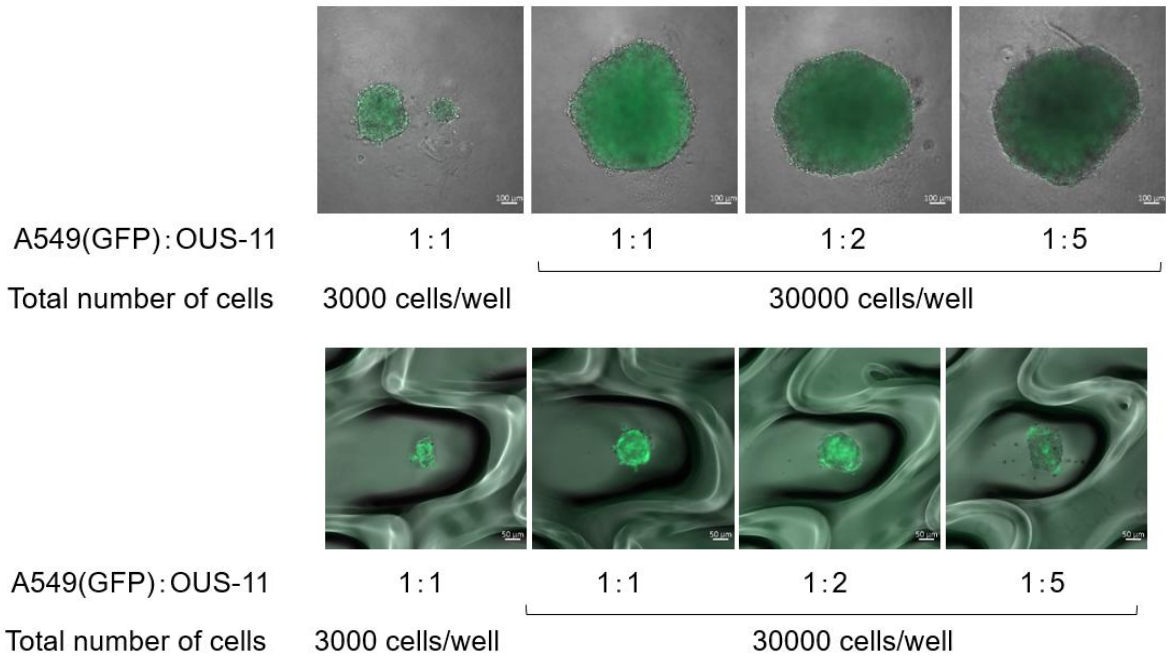

Figure 1. Co-culture spheroids of GFP-A549 and OUS-11 in EZ-BindShut round bottom microplate

Merged microscopic images of phase contrast and GFP channel fluorescent (green).

We recommend that select EZSPHERE or EZ-BindShut according to the size of the co-culture spheroid.
EZSPHERE micro-fabricated vessel for small size
EZ-BindShut round bottom microplate for large size

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lung cancer cell and fibroblast were rapidly aggregated and form co-culture spheroids.

1. OUS-11 was stained with a red fluorescent dye (Cell Explorer Live Cell Tracking Kit Red Catalog No.22623).
2. Mix single cell dissociated GFP-A549 and OUS-11 at 1:1 ratio in DMEM/F12 +10% FBS
3. 30,000cells/well of mixed cells was seeded into EZ-Bindshut round bottom microplate and EZSPHERE
4. Put EZ-BindShut or EZSPHERE microplate on the on-stage incubatore of microscope and obtain time-laps image every hour.

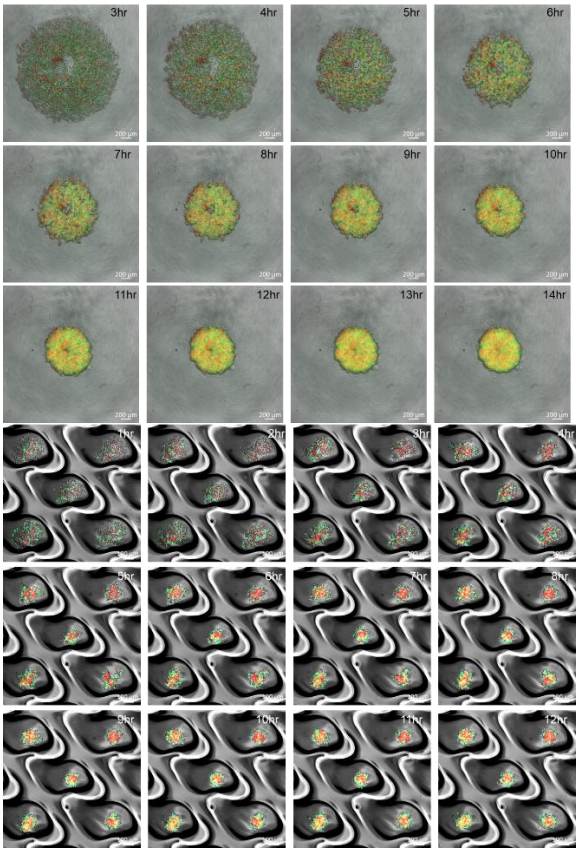

Figure 2.　Time-laps image of co-culture spheroid formation using EZ-Bindshut® and EZSPHERE

In a mixed state of A549 (Green) and OUS-11 (Red), cells gathered at the bottom of the round bottom EZ-BindShut and micro-well of EZSPHERE microplate and allowed to form spheroids 10 hours after seeding.

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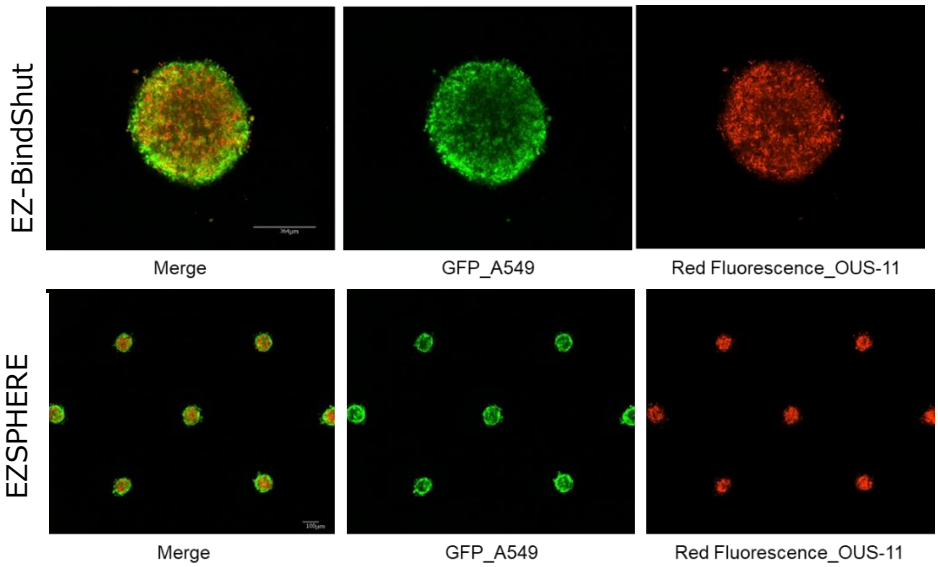
Confocal microscopy of co-cultured spheroids formed on EZ-BindShut and EZSPHERE microplate

A) Slice Image(x10)



B)MIP Image(x20)

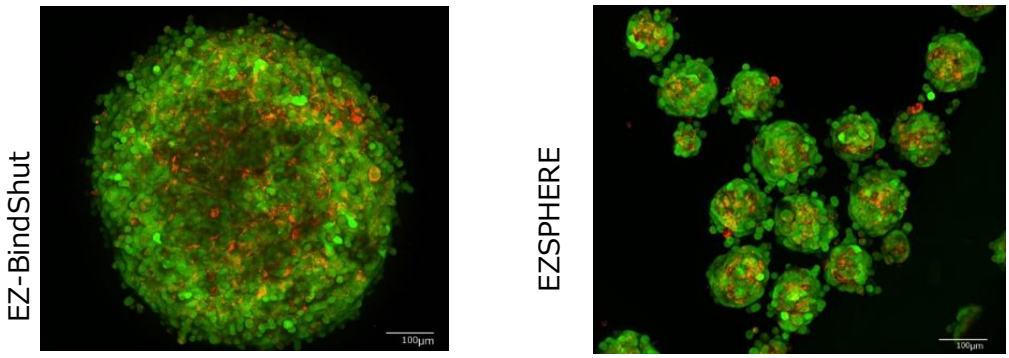


Figure 3　A549 (Green) and OUS-11 (Red) co-cultured spheroids

A) Slice image of co-culture spheroids formed in EZ-Bindshut and EZSPHERE

B) MIP image of spheroids formed in EZ-Bindshut® and EZSPHERE. To obtaind MIP images, spheroids were moved to a glass bottom plate (EZ-View LB MP96 Code No. 5866-096).

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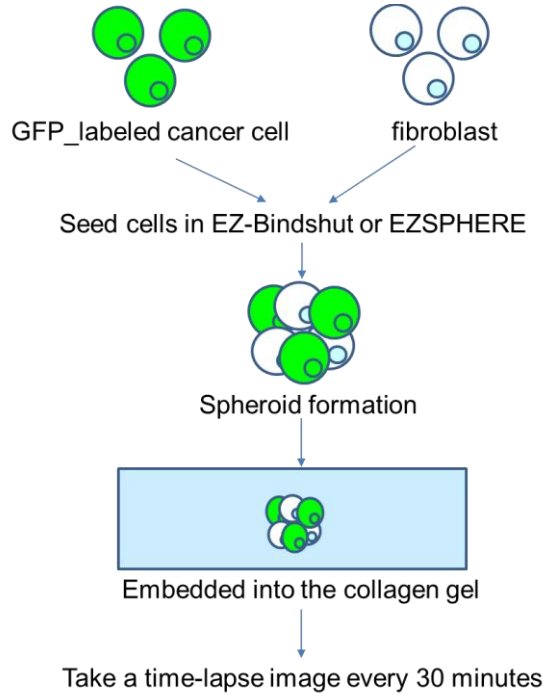
New model of cancer cell migration using co-culture tumor-like spheroids

Dr. Miyazaki *et.al* reported a new model of cancer cell migration / invasion [1, 2]. Embedding a co-cultured spheroid of cancer cells and fibroblasts in 3D collagen gel, it was observed that the cancer cells move in the gel along the fibroblasts. Interaction between integrin α5β1of cancer cell and fibronectin expressed on the surface of fibroblast probably contribute to this migration. This application note shows detail method of cancer cell migration assay using A549 cancer cell and OUS-11 fibroblast co-cultured spheroids formed in EZ-BindShut and EZSPHERE.

Over view of cancer cell migration assay



Form co-culture spheroid with chimera of GFP-laberld cancer cells and fibroblasts on EZSPHERE or EZ-BindShut. Then spheroids are embedded in 3D collagen gel and analyse cancer cell migration by fluorescence microscope with on-stage incubator.

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Cancer cell migration assay in 3D collagen gel

1. To form co-culture spheroids, mix GFP-labeled A549 : OUS-11 = 1:1 in DMEM/F12+10% FBS and seed 30,000 cell/well into EZ-BindShut or EZSPHERE and allowed culture for approxmetly 23 hours.
2. Prepare collagen gel coated plate or dish before.
   96-well glass bottomed plate: Put and spread 40 µl of collagen/medium solution (0.3% bovine dermis native collagen: 5×DMEM/F12: 0.05M NaOH/0.26 M $NaHCO_3$: FBS=7: 2: 1: 1) into well center of EZ-View LB glass bottomed 96-well plate followed by incubation (37℃, 5% CO2) for 30 min.
   Φ12㎜ glass based dish: Put and spread 70 µl of collagen/medium solution into well center of Φ12㎜ glass based dish followed by incubation (37℃, 5% CO2) for 30 min.
3. Collect co-culture spheroids from EZ-BindShut or EZSPHERE by gentle pippeting to 1.5ml tube.
4. Precipitate spheroids on the bottom of tube for 2 min or centrifugation (800rpm, 2min) and remove supernant carefully.
5. Add 100 µl of collagen/medium solution to tube on ice and mix gently.
6. Transfer 50 µl of collagen/medium solution containing spheroids to collagen gel coated plate and incubate at 37℃ for 10 min to allow the gel harden followed by adding 100 µl (96-well plate) or 2ml (glass based dish) of cultuer medium.
7. Put the collagen gel plate/dish on on-stage incubator of the fluorescence microscope Axio Observer 7 (Carl Zeiss) and obtain phase contrast and GFP-channel fluorescent images every 30 min.

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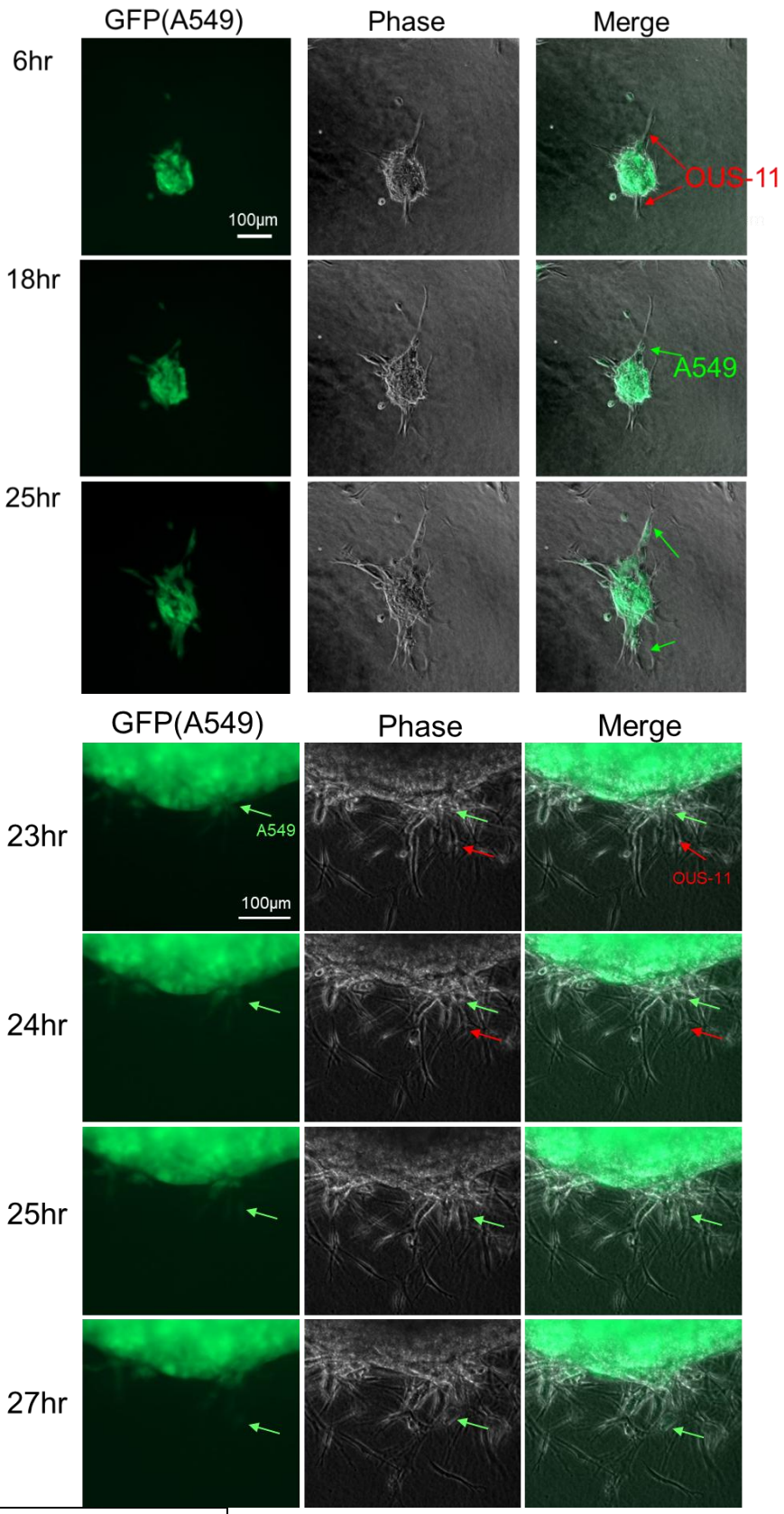

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Figure 4.　single cell invasion in 3-D collagen gel of A549 cells

Time course of A549 cell migration from co-culture spheroid with OUS-11 fibroblast formed in EZSPHERE (A) or EZ-BindShut (B). GFP channel fluorescent images (left), phase contrast images (center) and of GFP-A549 and OUS-11 co-culture spheroid. OUS-11 cells migrate into 3D collagen gel first (red arrow) and then A549 cells migrate (green arrow) along OUS-11 cells.

Both EZSPHERE and EZ-BindShut are useful tool to form cancer and fibroblast co-culture spheroid. For cancer cell migration assay in 3D collagen gel, EZSPHERE can be the best tool, because spheroids with 100 – 200 μm diameter optimal for focusing. Furthermore, Dr Miyazaki et.al. reported that this cancer cell migration model can work with cancer associated fibroblast (CAF) instead of fibroblast cell line.

Reference

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