Application Note

Spheroid Co-culture of Breast Cancer Cell Line (MCF-7) and Normal Breast Epithelial Cell Line (MCF-12A)

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Introduction

This application note describes the procedure for culturing the breast cancer cell line MCF-7 together with the MCF-12A normal breast epithelial cell line in the CelVivo ClinoStar System from a single cell suspension subculture. Co-culturing of different cell types adds to the complexity of the 3D tissue, and it is possible to study cellular communication. It is convenient to be able to track if the cell type of interest is present during live culture. The application note at hand describes a method using transient transfection of CellLight™ ER-GFP to track MCF-12A.

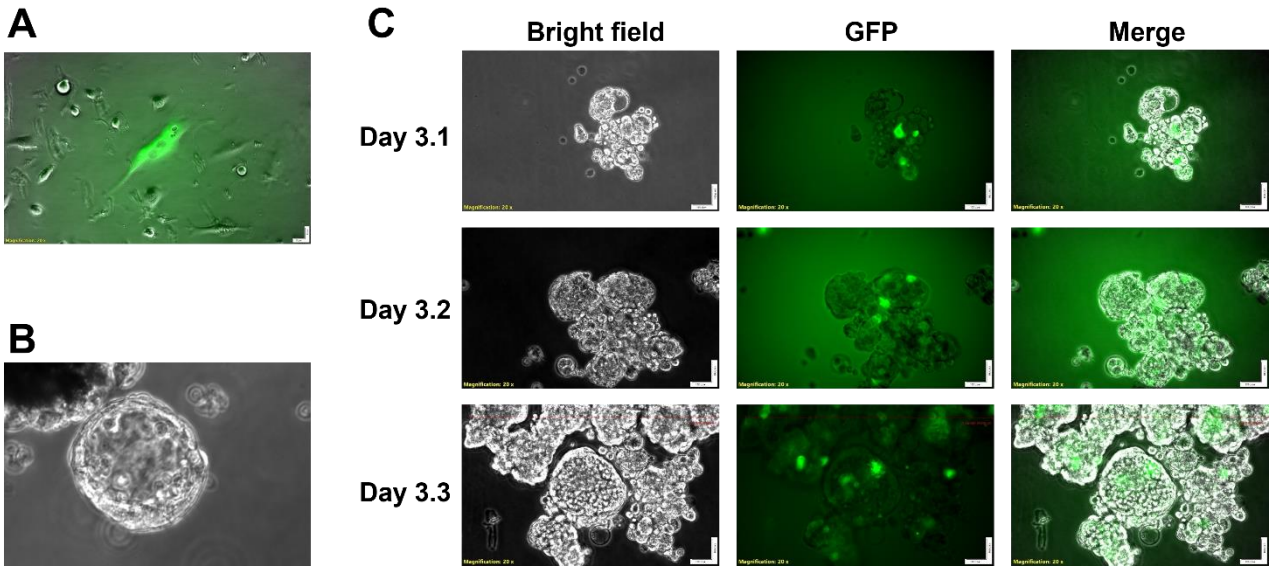

FIGURE 1 (A) Live imaging of 2D culture of MCF-12A 24 h after transient transfection with CellLight™ ER-GFP showing visible GFP expression. (B) Brightfield image of MCF-7 spheroid after 10 days in culture in ClinoStar system. (C) Three examples of MCF-12a expressing ER-GFP in co-culture with MCF-7 after 3 days in culture in ClinoStar System. GFP signals indicate that normal breast cancer cells are incorporated into the spheroids. Scale bar 100 µm.

Protocol

Preparation of initial 2D cell culture

1. MCF-7 or MCF-12A cells were thawed.
2. The cell suspension was seeded into T75 cm^2 cell cultureflasks.
3. The respective cell culture medium for monolayer cultures (see Reagents and Materials) was changed every two days.

4. When the cells reached 70-90% confluence, they were sub-cultured
    a. Medium was removed from the culture flask.
    b. The cell surface was washed twice with Gibco™ DPBS (14190144) without calcium and magnesium.
    c. 1 mL of Trypsin-EDTA (0.5%) solution was added and the flask was placed in the incubator for 10 min.
    d. 4 mL of the respective medium were added, the cells were washed off the surface of the flask and the liquid was transferred into a 50 mL-centrifuge tube.
    e. The cell suspension was centrifuged at 400 × *g* for 5 min. The supernatant was removed and 25 mL of the respective medium for every cell line were used to re-suspend the cells.
    f. MCF-7 or MCF-12A cells can be diluted up to 1:5, depending on the original confluence of the sub-cultured flask, the cells should be sub-cultured at least twice after thawing, before transferring into the ClinoReactor.

Preparing cells for transient transfection in 24 well plate

1. The cells were trypsinized for 10 min to be counted and then transferred to the 24 well plates.
2. One at least 80% confluent T75 cm^2 cell culture flask with MCF-12A cells was sub-cultured as described in the section above.
3. The cells were diluted at 1:2 in complete medium and Trypan Blue and counted using the Neubauer chamber (manually under the microscope).
4. $5×10^4$ cells were seeded per well in the 24 well plate.
5. 1 mL of their respective medium for MCF-12A (see Reagents and Materials) was added to the well.
6. The plate was kept in the incubator at 37°C, 5% CO_2 for 24 hours.
7. After 24 hours, the confluence was 70%, the cells were ready for the transfection reagent.

Transient transfection protocol for adherent cells

1. The cells should be between 70-80% confluent to obtain the best transfection performance.
2. The concentration used was 30 particles per cell, considering an initial 40,000 cells per well, we obtain we needed 12 µL of Cell Light Reagent per every well plate.
3. We transfected 6 wells of MCF-12A cells with CellLight™ ER-GFP.
4. The cells were left overnight in the incubator (>16 hours).
5. After 24 hours live images were obtained and shown in **Figure 1A**.

Culturing spheroids in the ClinoReactors

1. **ADDING THE TRANSFECTED CELLS.** The MCF-12A cells from the well plates were trypsinized (200µL trypsin) for 10 min to transfer them to the ClinoReactors®. The trypsinization was stopped with 300µL of medium. The contents of each of the 6 transfected wells were then transferred to the ClinoReactors.
2. **ADDING THE CANCER CELL LINE.** A minimum 80% confluent T75 cm^2 cell culture flask containing MCF-7 cells was sub-cultured as described in the above section.
3. The cells were diluted at 1:2 in complete medium and Trypan Blue and counted using the Neubauer chamber (manually under the microscope).
4. $5×10^5$ cells were seeded per previously equilibrated ClinoReactor.
5. Fresh pre-heated medium of every cell line was added carefully with a syringe (5 mL of each medium type) and needle 1.20 x 40 mm to maximum capacity to avoid bubbles in the cell chamber.
6. Any bubbles or clumps present were removed to avoid disturbing forces and to maintain only the spheroid culture.

7. The spheroids were monitored daily, and the rotational speed of the ClinoReactors was adjusted to counteract their increasing size.
8. The media was not changed during the experiment.
6. **Figure 1 C** displays three examples of MCF-12A and MCF7 co-culture after 3 days in culture arising from single cell suspension in 50:50 ratio.

Reagents and Materials

Culture media:

MCF-7: RPMI 1640 + 10 % FCS + 1% Pen/Strep
MCF-12A: DMEM/F12 (1:1) + 10% FCS + 1% Pen/Strep + 0.25% (=1.25 ml) MEGS

- Equilibrated ClinoReactor (3x)
- 1 x T75 cm^2 normal cell culture flask with MCF-7 breast cancer cells, 70-90% confluency
- 1 x T75 cm^2 normal cell culture flask with MCF-12A breast normal epithelial cells, 70-90% confluency
- Cell counter
- Sigma Fetal bovine serum (FBS) (F7524)
- Trypsin-EDTA (0.5%)
- 0.4% Trypan-Blue
- 70% ethanol solution
- Gibco™ DPBS, no calcium, no magnesium (14190144)
- 24 well plates Costar® 24-well Clear TC-treated Multiple Well Plates, Individually Wrapped, Sterile
- CellLight™ ER-GFP, BacMam 2.0 (cat no. C10590) Endoplasmic Reticulum, green
- 4% paraformaldehyde solution diluted in PBS

Conclusion

In conclusion, we were able to establish a method to co-culture breast cancer cell line (MCF-7) and normal breast epithelial cell line (MCF-12A) in the ClinoStar System. In addition, a method to distinguish MCF-12A from MCF-7 using CellLight™ ER-GFP transient transfection was demonstrated.

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