Cancer Organoid Model

3D co-culture of MCF-7 cells and human primary dermal fibroblasts using the Cancer Cell Line Medium XF

Application Note

This application note describes a method for easy 3D organoid culture of Primary Human Dermal Fibroblasts (NHDF) and MCF-7 cells in our Cancer Cell Line Medium XF. As part of our cancer media toolbox the Cancer Cell Line Medium XF supports tumor-driving cancer stem cells and further differentiated cancer cells. However, the Cancer Cell Line Medium XF is also less stringent to other non-cancer cell types like immune cells, fibroblasts or vascular cell types. The Cancer Cell Line Medium XF provides a serum-free and xeno-free culture environment devoid of all stimuli originating from non-defined materials. It is particularly useful for researchers relying on well-defined cell culture conditions. The medium does not contain ill-defined components such as fetal calf-serum, extracts or hydrolysates and exhibits very low lot-to-lot variability.

The described method for 3D cancer organoid generation serves as a template for other cancer cells and non-cancer cells, which may be processed in the same way.

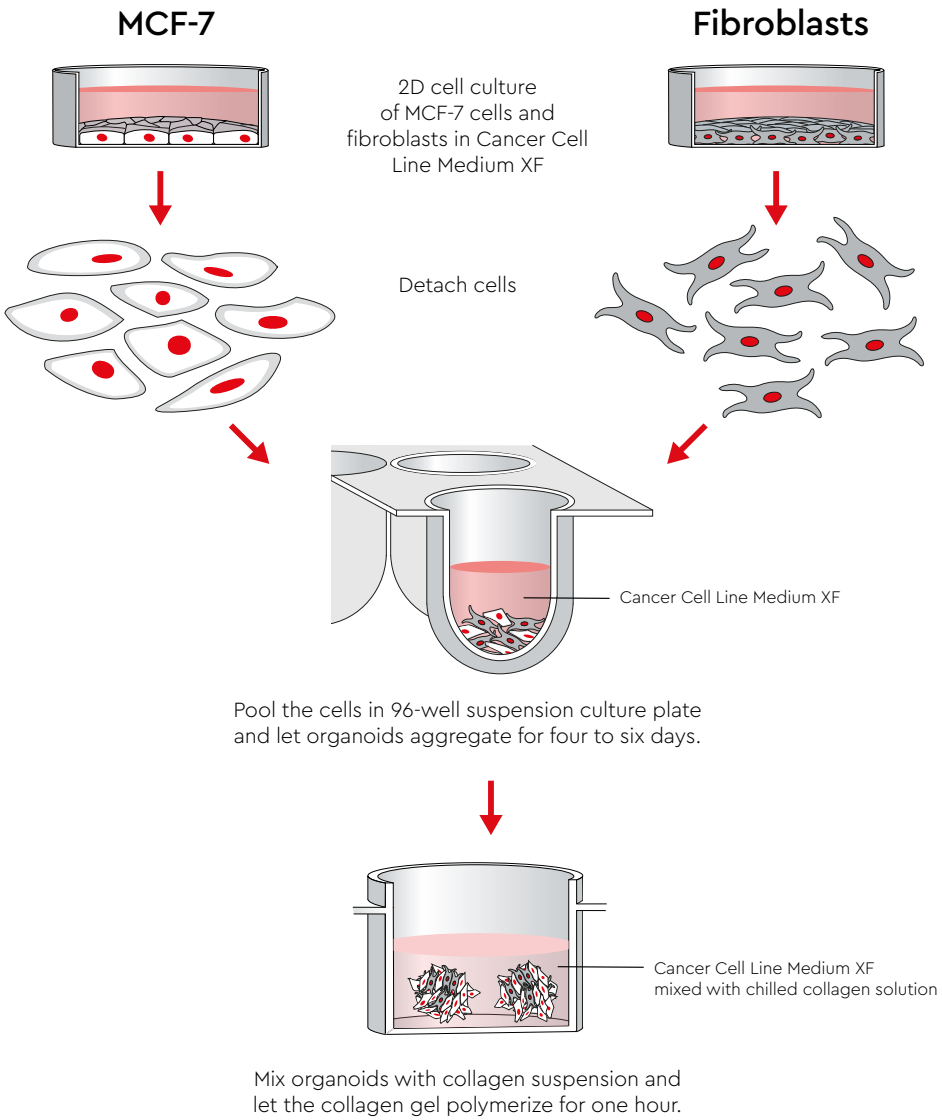

Figure 1: *Schematic overview of the essential steps for the generation of 3D cancer organoids using the Cancer Cell Line Medium XF.*

Background

The use of cell lines and primary cells in a classical 2D cell culture system has been successful for decades. In many areas of biomedical research, groundbreaking results have been obtained using cells growing in single 2D monolayers. The mammalian 2D cell culture provides a defined platform for investigating cell and tissue physiology outside of the organism. The advantages of 2D cultures are associated with **simple and low-cost maintenance of the cell culture** and with the performance of functional tests.

Although 2D cell culture is generally accepted and has increased our understanding of fundamental cell biological processes, there are limitations associated with it. In 2D cell culture, cells are grown as a monolayer on flat petri dishes or flasks optimized for cell attachment and growth. These artificial platforms result in unnatural growth kinetics and cell attachments. Therefore, cell architecture and behavior is not representative of that seen in natural microenvironments [1]. Recently, there have been improvements in the form of optimized *in vitro* models that resemble *in vivo* conditions. Three-dimensional cell cultures are an important example. They better mimic tissue physiology in multicellular organisms [2]. Although traditional monolayer cultures are still predominant in cellular assays, 3D cell culture techniques are making rapid progress.

3D cell culture was developed to improve the physiological relevance of *in vitro* experiments compared to *in vivo* conditions. 3D cell culture refers to the culture of living cells inside micro-assembled 3D devices mimicking tissue and organ specific microarchitecture [3]. Growth of cells in their 3D shape allows better cell-to-cell contact and intracellular signaling networks [4]. The 3D environment also facilitates developmental processes allowing cells to differentiate into more complex structures [5]. 3D cellular models offer an opportunity to better understand complex biology in a physiologically relevant context where 2D models have not been proven successful.

Over the past decade, several methods for 3D cell culture have emerged. Scaffold-based techniques as well as organoid cultures are used. Each method provides different advantage and limitations [6]. **It is important to select the most appropriate model for the specific cell-based assay.** Here, we describe a method for efficient organoid culture of our Human Primary Dermal Fibroblasts and MCF-7 cells in our Cancer Cell Line Medium XF as a useful *in vitro* tumor model.

Organoids are *in vitro* derived 3D cell aggregates derived from primary tissue or stem cells. They can self-organize in three-dimensional culture owing to their self-renewal and differentiation capacities. **Organoids overcome limitations of 2D models by providing a similar composition and architecture as primary tissue** and a stable system for extended cultivation. Organoids are physiological relevant, holding great potential in basic research as well as translational applications.

Organoid formation and culture require a defined 3D microenvironment [7]. The microenvironment can be made using an extracellular matrix hydrogel such as Matrigel or collagen. Organoids, which are typically a few millimeters in size, form either spontaneously by embedding the corresponding single cells in the hydrogel or are generated in advance and embedded as a whole. The organoid bodies can then be manipulated pharmacologically to study the influence of biochemical cues on tissue architecture.

Several organ architectures have been copied by using organoid techniques, including cerebral tissue, intestinal tissue, lung tissue or kidney tissue [8–11]. In this Application Note we describe the organoid culture of Normal Human Primary Fibroblasts (NHDF) and MCF-7 cells in the Cancer Cell Line Medium XF as a **model for tumor microarchitecture.** It has been shown that the growth and differentiation of normal and neoplastic epithelial cells may be regulated by the presence of stromal fibroblasts. Stromal fibroblasts have been reported to interfere with the proliferation, differentiation, and invasion of carcinoma cells [12]. In some cases, stromal fibroblasts enhance the malignant behavior of epithelial cells and have also been involved in the loss of epithelial integrity during neoplastic process [13, 14]. Fibroblasts can regulate the growth and differentiation pattern of MCF-7 mammary carcinoma cells through a process mediated by membrane-bound and soluble factors [15]. Therefore, studies on the interplay between fibroblasts and MCF-7 cells, especially in an *in-vivo*-like 3D environment, are of particular interest in tumor research.

Organoid Culture Protocol

The protocol describes the expansion of Primary Human Dermal Fibroblasts (NHDF) and MCF-7 cells in our Cancer Cell Line Medium XF and subsequent generation of a 3D organoid co-culture.

I. 2D Culture of MCF-7 Cells and NHDFs

Materials

- Fibronectin solution, human
- Normal Human Dermal Fibroblasts, juvenile foreskin (PromoCell, C-12300)
- MCF-7 cell line
- Culture vessels for adherent cell culture (e.g. Falcon, No. 353004)
- Phosphate buffered saline (PBS) without Ca^{++}/Mg^{++} (PromoCell, C-40232)
- Cancer Cell Line Medium XF (PromoCell, C-28077)

Use aseptic techniques and a laminar flow bench.

1

Coating of cell culture dishes

Dilute the fibronectin solution to 10 µg/ml in Dulbecco's PBS without calcium and magnesium. Overlay the culture surface of your tissue culture vessel with an amount of the diluted fibronectin solution sufficient to effectively coat the complete surface. Be sure that the entire surface is covered. Place flasks on a level surface at room temperature for at least 60 minutes.

2

Plate the cells

Thaw the cells from cryopreservation or harvest the cells from a pre-existing culture using your standard method. Resuspend the cells in the Cancer Cell Line Medium XF and plate them separately on pre-coated fibronectin surfaces. Use a plating density of 10.000 cells/cm² for MCF-7 cells and 5.000 cells/cm² for NHDFs.

3

Let the cells grow

Incubate the cells at 37°C and 5% CO_2 until they have reached 70–90% confluence. Change the medium every 2–3 days.

II. Initiation of Organoid Culture

Materials

- Cancer Cell Line Medium XF (PromoCell, C-28077)
- Phosphate buffered saline (PBS) without Ca^{++}/Mg^{++} (PromoCell, C-40232)
- Accutase (PromoCell, C-41310)
- 96-well suspension culture plate (e.g., Greiner Bio One, No. 650185)

Use aseptic techniques and a laminar flow bench.

1

Detachment of cells

Once the cells have reached 70–90% confluence, aspirate the medium and wash the culture with ambient tempered PBS without calcium and magnesium. Incubate the cells for 4–10 minutes with 100 µl/cm² Accutase at 37°C. Monitor the detachment process using a microscope. When the cells start to detach, facilitate their complete dislodgement by tapping the flask. Transfer the cell suspensions separately in centrifuge tubes using a 40 µm filter and dilute them 1:1 with the Cancer Cell Line Medium XF.

Note: NHDFs may need 4–5 minutes for complete detachment. MCF-7 should be incubated in Accutase for about 10 minutes for complete disaggregation of cell clumps. Be careful: Handle cell suspension with care. Do not over-triturate the cells during the detachment process. A high cell viability is critical for successful organoid aggregation.

2

Count the cells

Count the cells using your standard method.

Note: For optimal results, cell viability should be higher than 90%.

3

Seed the cells for organoid formation

Spin down each tube for 5 min at 300 x g. Adjust cell concentrations to 1 million cells/ml with Cancer Cell Line Medium XF. Plate an equal number of MCF-7 cells and NHDFs in a 96-well U-bottom suspension culture plate using Cancer Cell Line Medium XF.

Note: The more cells you use, the larger the organoids will be. Up to 3 × 10^5 cells per well can be plated.

4

Organoid formation

Organoids will form spontaneously within 24–48 hours of incubation. To generate tightly packed organoids, cells should be incubated for 4–5 days.

Note: Because of high metabolic activity, the color of the medium turns to yellow within one day. Ideally, the medium should be changed each day, or at least every two days. To change the medium, take off as much medium as possible using a 200 µl pipet without touching the organoid. Add new medium slowly and carefully. Do not stir or mix the organoids.

III. 3D Organoid Culture in Collagen Gels

Materials

- Cancer Cell Line Medium XF (PromoCell, C-28077)
- Nunclon Sphera 24-Well Multidish (Thermo Scientific, No. 174930)
- Collagen solution (e.g. 3D Collagen Cell Culture System, Merck, No. ECM675)

Use aseptic techniques and a laminar flow bench.

1

Collection of organoids

Transfer the organoid-containing medium from the 96-wells into 15 ml conical tubes using a 1.000 µl pipet. Allow the organoids to settle by gravity sedimentation for 10 minutes at room temperature. Aspirate the supernatant and resuspend the cells in a small volume (e.g., 30–50 µl) of Cancer Cell Line Medium XF per tube.

Note: Handle the organoids with care. Do not disturb the integrity of the organoid. Generation of single cells should be avoided.

2

Preparation of collagen gel solution

Prepare a collagen I solution of 2 mg/ml according to the manufacturer's instructions and keep it on ice.

Note: Preparation of the solution is a critical step. Collagen gel formation is highly dependent on temperature, pH, and the quality of the used collagen. We suggest using the 3D Collagen Cell Culture Kit from Merck because collagen gels form reproducibly when working according to the manufacturer's instructions. For organoids containing MCF-7 cells and fibroblasts use the 5x RPMI medium contained in the kit to guarantee physiological conditions.

3

Initiation of 3D culture in collagen gels

Mix the organoid suspension with the chilled collagen solution.

Note: Cell suspension should not be greater than 10% of the final volume. Formation of air bubbles should be avoided.
Add 500 µl of the organoid-containing collagen solution per well of a 24-well suspension culture plate. Be careful, not to introduce air bubbles in the suspension. Immediately transfer the plate to a 37°C incubator for 60 minutes to initiate polymerization of the collagen. After formation of the gel, cover the collagen gel with culture medium. Incubate cells overnight or several days at 37°C. Change the medium daily. Cells can be visualized using phase contrast microscopy and can be fixed and stained within the collagen. Exemplary results are shown in Figure 2.

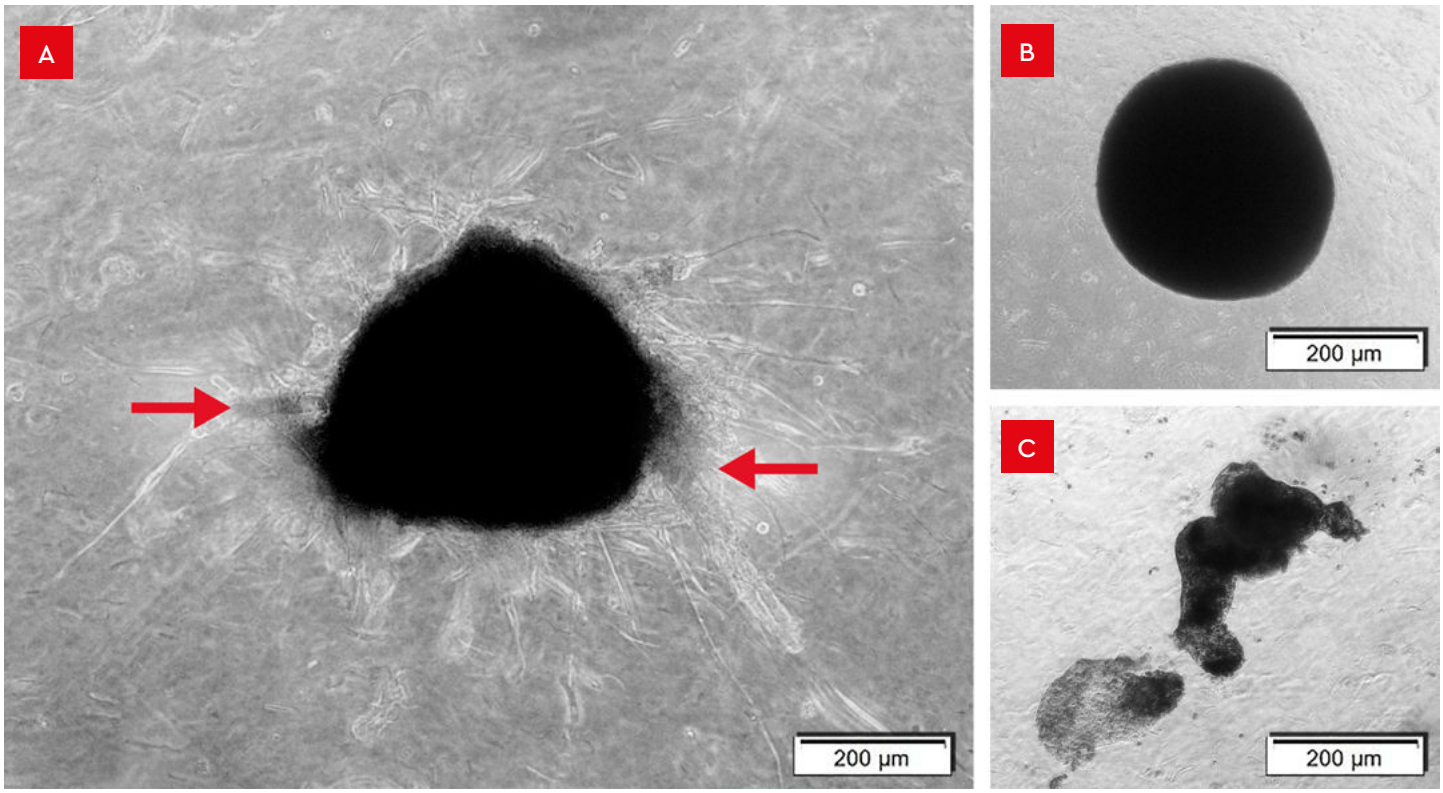

Fig. 2: Exemplary results after initiation of organoid culture in the collagen gel.
A: *Organoids containing MCF-7 cells and NHDFs. Organoids show a characteristic sprouting behavior (red arrows) after embedding in the collagen gel.*
B: *Organoids containing solely NHDFs. Organoids are tightly packed showing a round shape with clear edges. Sprouting is not as prominent as in Organoids containing both cell types.*
C: *Organoids containing solely MCF-7 cells. In contrast to NHDF-containing organoids, MCF-organoids seem to be loosely aggregated. No sprouting behavior was visible.*

Materials

Product	Size	Catalog Number
Cancer Cell Line Medium XF	250 ml	C-28077
Fibronectin solution, human (1 mg/ml)		
Phosphate Buffered Saline without Ca^{++}/ Mg^{++}	500 ml	C-40232
Normal Human Dermal Fibroblasts (NHDF) juvenile foreskin	500,000 cryopreserved cells	C-12300
MCF-7 Cell Line		
Culture Vessels for adherent cell culture (Falcon®)		353004
24-well plate Nunclon™ Sphera™ (Thermo Scientific)		174930
3D Collagen Cell Culture System (Merck)		ECMC75

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