Generation of Spheroids

1. General Information

In recent years, three dimensional (3D) culture systems have gained increasing recognition as an effective tool for biological research. Cells cultured in 3D more closely mimic the physiological environment of living organisms compared to conventional monolayer culture systems. One widely used 3D culturing technique is the application of multicellular spheroids. Spheroids are microscale, spherical cell clusters which grow free of foreign material.

2. Principle

There are several methods available for the generation of spheroids which are used more or less frequently. All the methods available prevent cells from attaching to the culture ware substratum, thereby increasing interactions with neighboring cells and extracellular matrix. In this Application Note we use the liquid-overlay technique for spheroid generation. This method is one of the simplest approaches for spheroid formation and has several advantages. First, single spheroids can be generated and monitored allowing an easy evaluation of growth kinetics and spheroid characteristics. Second, a simple medium exchange allows longer cultivation times. Last, no special equipment is needed. In short, wells are coated with agarose before adding cell suspension. Besides providing a non-adhesive surface, the agarose-coated wells also increase cell-to-cell contact as cells collect on their concave bottoms.

3. Materials

For this protocol the following material is necessary:

- µ-Plate Angiogenesis 96 Well ibiTreat (ibidi, 89646)
- 1% agarose (dissolved in PBS) (e.g. Sigma-Aldrich Chemie GmbH, A9539)
- Trypsin or alternatives for cell detachment
- Cell culture medium

For this protocol the following equipment is necessary:

- Thermal incubator, microwave or autoclave to melt agarose
- Inverted microscope for determining growth kinetics
- Cell Culture incubator (typically 37°C, 5% CO_2 and 95% relative humidity)

4. Procedure

Preparation of the substrate:

1. Prepare 1% agarose in PBS.
2. Coat the wells with 30 µl of agarose. Agarose forms a gel and cools down to room temperature in less than 10 minutes. This volume is appropriate to entirely cover the surface of the wells and produce a concave surface.

Note: The agarose should be placed in a warm water bath to prevent the early gelation during pipetting. Also tips and plates should be placed at 50°C one hour before the preparation.

Spheroid formation:

1. Prepare cell suspension, as usual.
2. Enumerate the cell number and dilute the cell suspension to the desired cell concentration (consider Figure 1 as a reference).
3. Transfer 55 µl of cell suspension into each well.
4. Incubate plate in the cell culture incubator.
5. The culture medium should be gently changed every other day after an initial incubation time of two days. Minimize movement of the plate, particularly during spheroid initiation.
6. Spheroid formation and growth can be evaluated using phase-contrast microscopy and spheroid viability using fluorescein diacetate (FDA)/ propidium iodide (PI) staining as described in Application Note 33 “Live/dead staining with FDA and PI".

5. Notes:

- Size and thus characteristics of spheroids can be controlled by adapting seeding concentration or incubation time (Figure 1).
- Spheroid formation, growth kinetics and spheroid characteristics are cell type dependent (Figure 2, 3).
- Type and quality of serum critically affect the spheroid formation and growth.
- Irregular or insufficient agarose coating can result in formation of several irregular spheroids and/or cell adhesion per well. Do not allow agarose to cool down below 50°C to minimize the risk of gelation during dispensing.
- Formation of irregular and noncircular spheroids can be attributed to small particles in medium and/or serum. Monitor media and media supplements by microscopy and filter supplemented media through sterile filter if required.

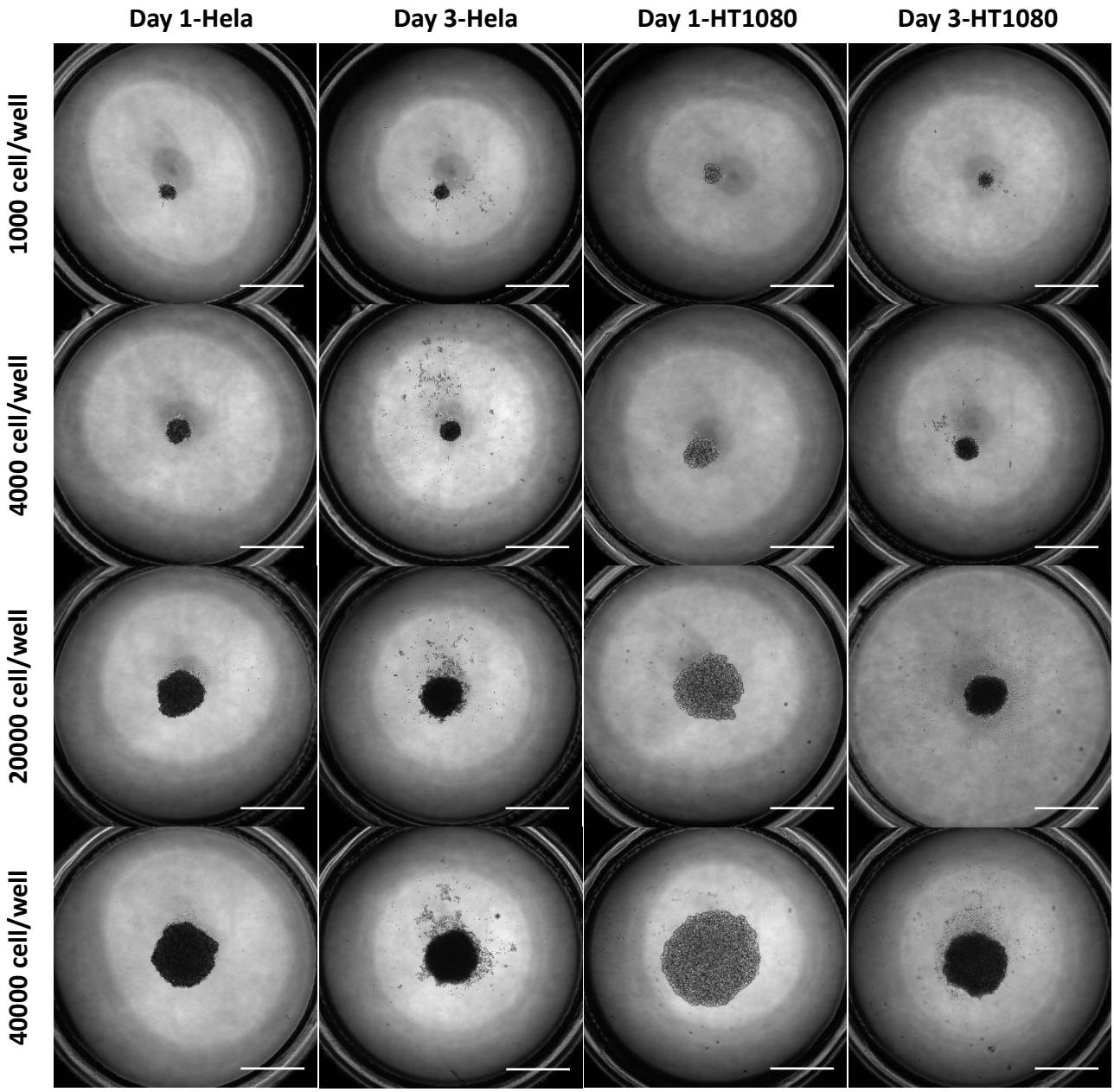

Figure 1. Spheroid formation and growth of HT-1080 and HeLa cell lines, seeded with different initial cell concentration in µ-Plate Angiogenesis 96 Well. (Scale bars: 800 µm)

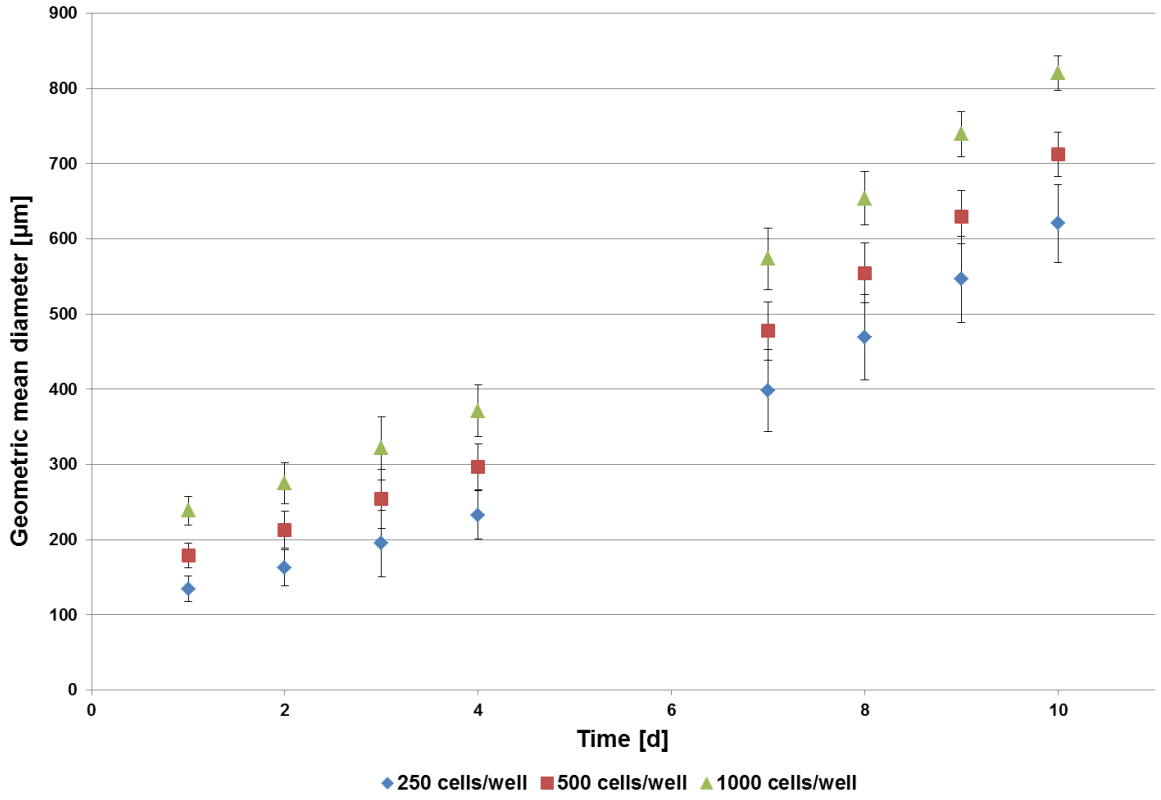

Figure 2 Spheroid growth of MCF-7 spheroids is dependent on the seeding concentration and the cultivation time.

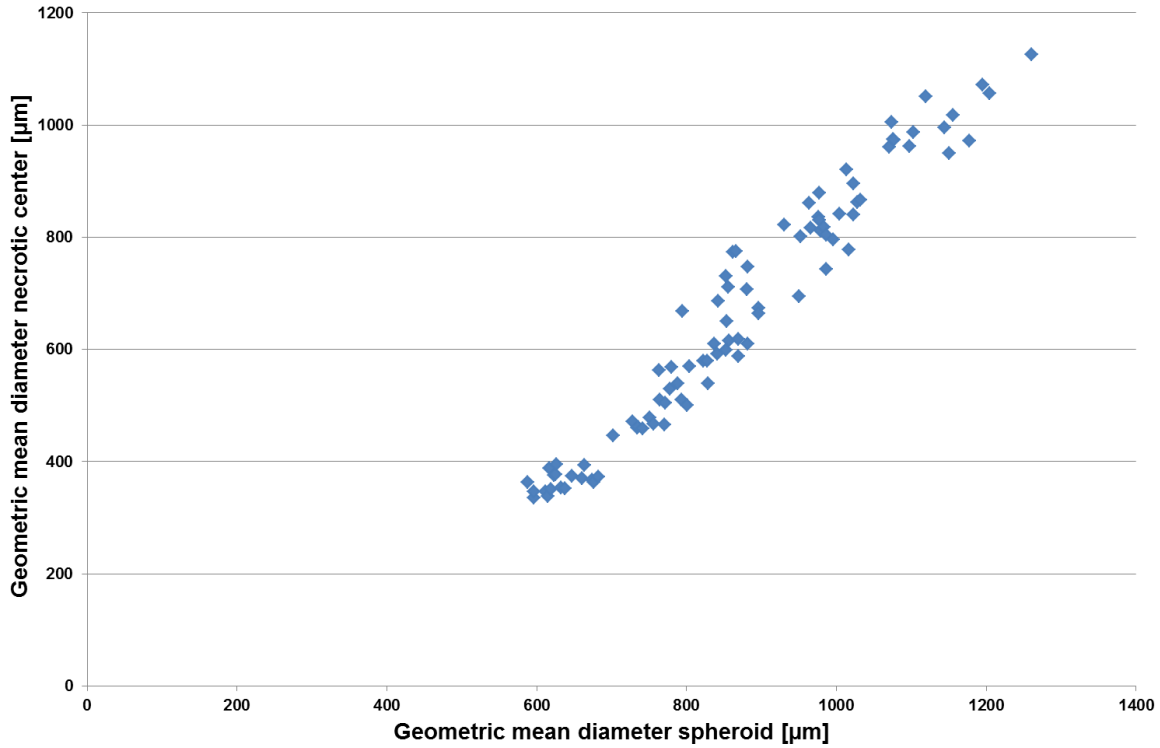

Figure 3 Correlation between MCF-7 spheroid size and the size of the necrotic center.