Production of spheroids from HT-29, Caco-2 and SW48 cell lines

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Background

Spheroids, which are known as microtumors, are well characterized models to mimic the natural environment for 3D culture. They can be used for assays that are drug screening, tumor growth and proliferation, immune interactions, invasion, matrix remodeling and angiogenesis. There are four general methods of spheroid formation; suspension culture, nonadherent surface methods, hanging drop methods, and microfluidic methods. The hanging drop technique is one of the simplest and cheapest methods inside of them [1,2].

Aims

In this study, it is aimed to use of different cell density for forming spheroids while focusing on cell growth conditions, cell proliferation and population and compare spheroids of three cell lines.

Materials and Methods

Sources of cell lines and material: Three human colon adenocarcinoma cells (Caco-2, HT-29 and SW48 cells) were obtained from the American Type Culture Collection (ATCC), USA. All reagents used for cell culture were purchased from Biological Industries, USA.. Perfecta3D™ 96-Well Hanging Drop Plate was purchased from 3D BiomatrixTM.

Cell culture: HT-29, SW48 and Caco-2 cells were grown respectively in RPMI 1640, DMEM, MEM with supplemented with 10% (v/v) Fetal Bovine Serum and 1% penicillin/ streptomycin.

Hanging Drop Plate Method: Briefly, when cells reached confluent monolayer in a T-75 flask, washed twice with PBS (pH 7.4) and treated with 0.25% trypsin-EDTA, then resuspended in fresh medium. After centrifuged, they were counted and more calculations were done to determine the volume needed to plate at density of 10^5, $2x10^5$ and $4x10^5$ cells per well. To dilute the total cell concentration in 40µL per well. The diluted solution was pipetted into each well [Figure 1]. The plate was placed in incubator.The fluorescence microscope examination and Image J software were used.

Figure 1. Method to manually pipette cell suspension into each well in the hanging drop plate.

Results

It was shown that the morphological appearance of spheroid was cell line dependent and the fluorescence microscope examination revealed that the general characteristics of spheroid formation were similar in HT-29 and SW48 cell lines, however;Caco-2 cells formed weaker than others. According to our findings, concentrations of cells, which are $2x10^5$ and $4x10^5$, were not found suitable for transferable spheroids. However, density of 10^5 cells were found transferable and remained proliferative by the end of the culture period.

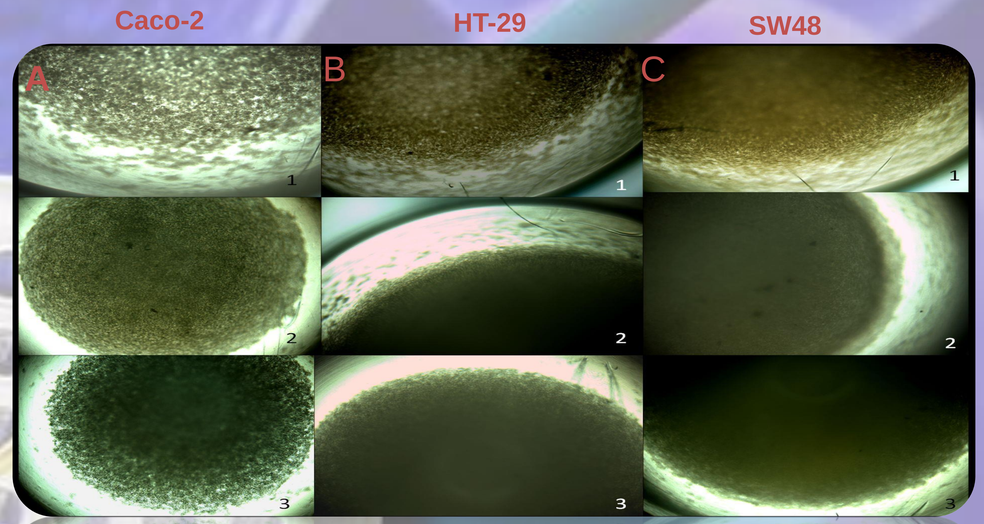

Figure 2. Images of spheroids (respectively: A,B,C) were belong to Caco-2, HT-29 and SW48 cell lines at density of 10^5, $2x10^5$ and $4x10^5$ (number of the images respectively: 1,2,3) and they were taken by fluorescence microscope (10x objective) The change over time of spheroids were determined using ImageJ program.

Discussion

There are some articles, which include the seeding density from as few as 50 cells to as many as 10^5 cells for many type of cells and [2]. In this study, we observed that density of 10^5 is useful for this method, but $2x10^5$ and $4x10^5$ are not suitable for long-term culture for these cell lines.

References

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