# Developing three-dimensional co-culture systems using fibroblasts and breast tumor cells as a platform for screening of anti-cancer agents including Lycopene

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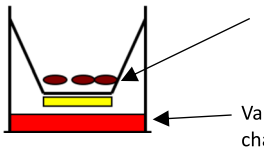
## Abstract

Three-dimensional (3D) models have been used to mimic the physiological tumor conditions *in vivo*, as cells growing in 3D spheroids tend to recapitulate those *in vivo* including cell-cell interaction, metabolism, and morphology (1). Breast tumor microenvironment encompasses many stromal cells including Cancer-associated fibroblasts, which have been found to support tumor initiation, invasion, and metastasis (2). Our study aims at developing a 3D culture system whereby tumor cells and fibroblasts are in direct physical contact. This system allowed us to investigate the reciprocal interactions between breast cancer cells (T47D,MCF-7) and human foreskin fibroblast (HFF), which still remains under investigation. By culturing cancer spheroids in fibroblasts conditioned medium (CM) and vice versa, we found that cancer CM induces behavioral changes in HFF spheroids. We also observed chemotaxis between both cell lines, when we added HFF cells/spheroid to already established tumor spheroids, and vice versa. This system also allows for testing against anti-cancer reagents like lycopene, the carotenoid found in red-colored fruits. Since lycopene mechanism of action remains unknown, we propose that lycopene exhibits anticancer effects on both breast tumor cells and fibroblasts, that negatively hinder their migration, survival, and proliferation. We support this hypothesis by recent evidence and a migration assay that exposed naive HFF cells to lycopene and resulted in a dose-dependent inhibition of migration of cells.


## Method:

**Formation of 3D cultures.** 3D cultures of T47D or MCF-7 and HFF cells were formed using 96-well plate Nunclon™ Sphera™ from Thermo Scientific. All spheroids were grown in 200 µl of DMEM containing 5% FBS and 1% penicillin-streptomycin mix. 3 conditions were presented with triplicate samples per condition. 50% of the medium was replenished every two days. Spheroids were maintained in a 5% CO2/95% air incubator at 37°C.

**Migration Assay**



100,000 Naive HFF cells +Variable concentrations of Lycopene seeded onto the top chamber of transwell filters (8-µm, Corning).

Variable %s of FBS medium in 24-well plate (lower chambers)

## Data:

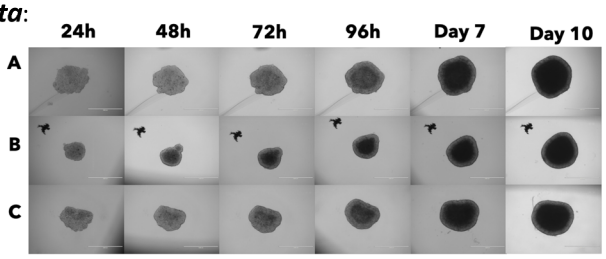



**Figure 1. Morphology of spheroids established from T47D Breast Cancer cells.** (A) T47D cells grown in DMEM , (B) T47D + HFF coculture in 1:1 ratio., (C) T47D cells grown in HFF conditioned medium. Imaging was done using EVOS FL Life Technologies Cell imager at 4x scale bar: 1000 µm.

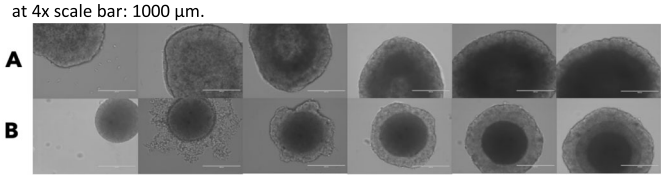



**Figure 2. Chemotaxis observed between T47D Breast Cancer cells and HFF fibroblast cells.** (A) T47D spheroid with 5000 HFF Cells added on day 4, (B) HFF spheroid with 5000 T47D Cells added on day 4. Imaging was done using EVOS FL Life Technologies Cell imager at 10x, scale bar: 400 µm.

## Analysis:

- Although spheroid compactness and layers formed by T47D cells have increased over time, there is no significant difference in compactness between all conditions at day 10 (Figure 1) .
- As shown after 6 hours, HFF cells have migrated faster than T47D cells to established spheroid. In condition B, at day 6 after addition, another new layer of cells has formed over the previously formed T47D cells (Figure 2).

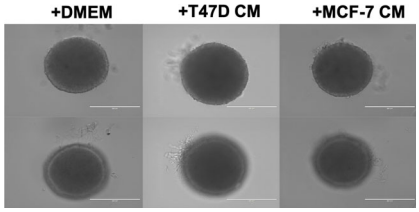



**Figure 3. Morphology of spheroids established from HFF fibroblasts cells incubated with cancer cell conditioned medium.** From left to right: HFF spheroid grown in normal DMEM medium for 10 days, HFF spheroid grown in T47D conditioned medium for 6 days, HFF grown in MCF-7 conditioned medium for 6 days. Imaging was done at 10x, scale bar: 400 µm.

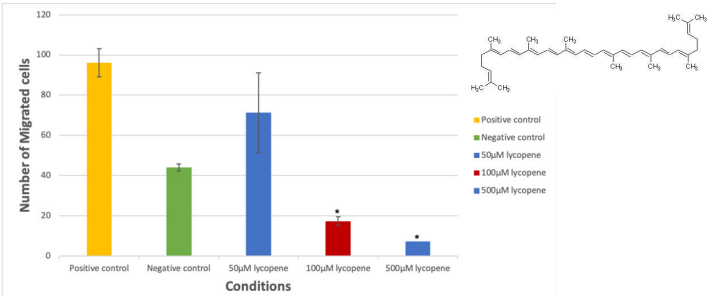



**Figure 4. Migration assay of 2D HFF cells at 24 h following exposure to different concentrations of lycopene.** 5 pictures were taken using EVOS FL Life Technologies Cell imager using normal light at 4x objective. Migrated cells were counted using ImageJ. Samples were run in duplicates and the average was calculated. (∗) indicates statistically significant differences by comparing the samples average values to the negative control ($p < 0.05$, t-test). On the right, lycopene chemical structure is depicted.

## Analysis:

- In figure 3, Cells of HFF spheroid grown in cancer conditioned medium are clearly seen migrating out of the spheroids.
- As shown in figure 4, a pattern appears where the higher the concentration of lycopene added, the lower the number of migrating cells. Both conditions (100, 500µM) are significant when compared to the negative control. The vehicle (DMSO) in the negative control, which is equal to that added in the highest lycopene concentration (500µM), has also affected the migration of cells, as it had a lower average of migrating cells than both wells with 50µM lycopene and the positive control.

## Conclusion:

- In conclusion, due to lycopene various ways of inducing apoptosis and impairing proliferation in breast cancer cells, we suggest that lycopene might represent a great potential therapeutic candidate that could be used in combination with other chemotherapeutic agents.
- As shown by morphological and EMT behavioral changes in HFF spheroids grown in cancer-derived conditioned medium , It is important to note the reciprocal interactions taking place between fibroblasts and cancer cells, which largely enhance tumor growth and inhibition
- We optimized a 3D co-culture system that includes fibroblasts and breast tumor cells, that can be used as a platform to screen anti-cancer agents including Lycopene.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