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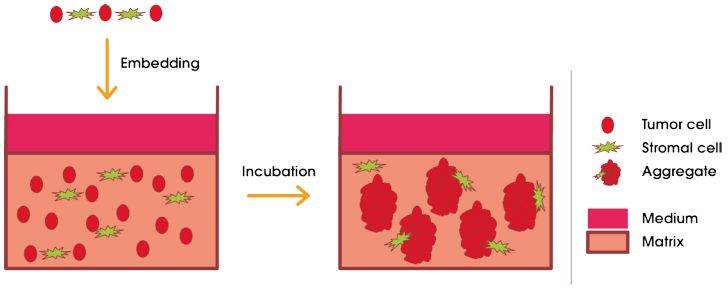
Application note

Syntrix-BM Tumor coculture

Key Features:

- Matrix embedded 3D co-culture of tumor cells and stromal cells in Syntrix-BM 3D Matrix
- 3D tumor model taking into account cell-cell and cell-ECM matrix interactions

Syntrix-BM for 3D Tumor Co-culture



Background

Cancer is complex and a heterogeneous pathological "organ" in dynamic interplay with their environment. Traditional *in vitro* 2D monoculture models fail to capture many important aspects of this complexity, including the 3D organization and the dynamic and reciprocal interactions of the tumor with stromal cells and with the extracellular matrix (ECM).[1]
3D spheroids have become an important platform in biopharmaceutical drug discovery, as they can model the 3D aspects of tumors.[2] In the case of co-culture with stromal cells, paracrine interactions in tumors are captured as well. Yet, they still miss an essential dynamic regulator of tumor biology, the ECM.[3]

Syntrix-BM is a fully synthetic, biomimetic matrix displaying cellular binding sites mimicking the important ECM components laminin, fibronectin, and collagen IV. This well-defined matrix can provide signaling cues via transmembrane receptors and the cytoskeleton to the nuclear matrix in a more controlled way than laminin-rich basal membrane extract gels of murine origin and could allow for cells to be analyzed for cell invasion and migration upon treatment with compounds.

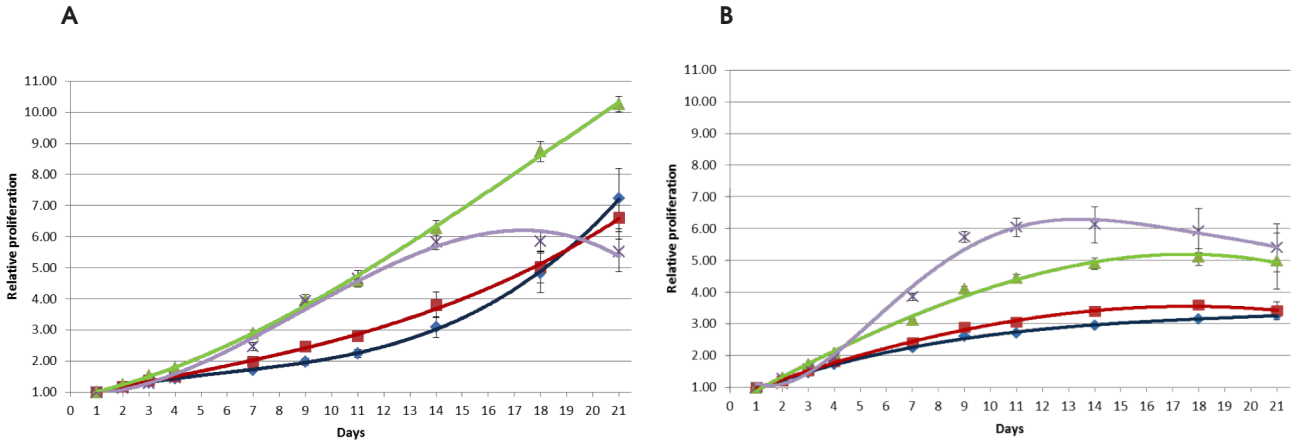


Figure 1: Relative growth curves of PC346c prostate cancer cells (20k starting cell count) in monoculture◆ or in 20:1■, 2:1▲, or 1:5× ratios with CAFs, as determined from the increase in fluorescence, in A) Syntrix-BM and B) BME matrix.

Application

Multiple 3D multicellular tumor growth assays have been developed to better mimic the *in vivo* situation of tumors by organizing a mixture of tumor cells and stromal cells in a laminin rich 3D extracellular protein gel. These models can be optimized and translated into screenable assays.

Here, we present a coculture model of the PC346c prostate cancer cell line labeled with RFP and Cancer Associated Fibroblasts (CAFs) labeled with GFP. Varying concentrations of CAFs were used to investigate the crosstalk between cancer and stromal cells. Both fluorescent labels are stably expressed.

Syntrix-BM is a fully synthetic, biomimetic matrix displaying cellular binding sites mimicking important ECM components present in laminin rich ECM gels, thereby offering a more defined alternative.

Cells were cultured for three weeks in 96 well plates using 100 µL of either Syntrix-BM 3D Matrix or Cultrex BME (Trevigen) as a control. RPMI supplemented with 2% serum was used as the culture medium. In the case of Syntrix-BM, the medium was further supplemented with multiple growth factors to optimize the gel for tumor growth.

The growth of PC346c was followed on an Opera High Content Imaging System using the increase in red fluorescence from the labeled cancer cells. Growth curves for 21 days are shown in Figure 1. Both matrix types allow for crosstalk between the two cell types, as PC346c proliferation increases with increased CAF concentration. In Syntrix-BM, the cells proliferate better, and cells can be kept in culture for a longer time, as evidenced by the absence of a decrease in fluorescence in the last week for all but the highest concentration of cells. The highest concentration of CAFs appears to high for long term cultivation under these conditions.

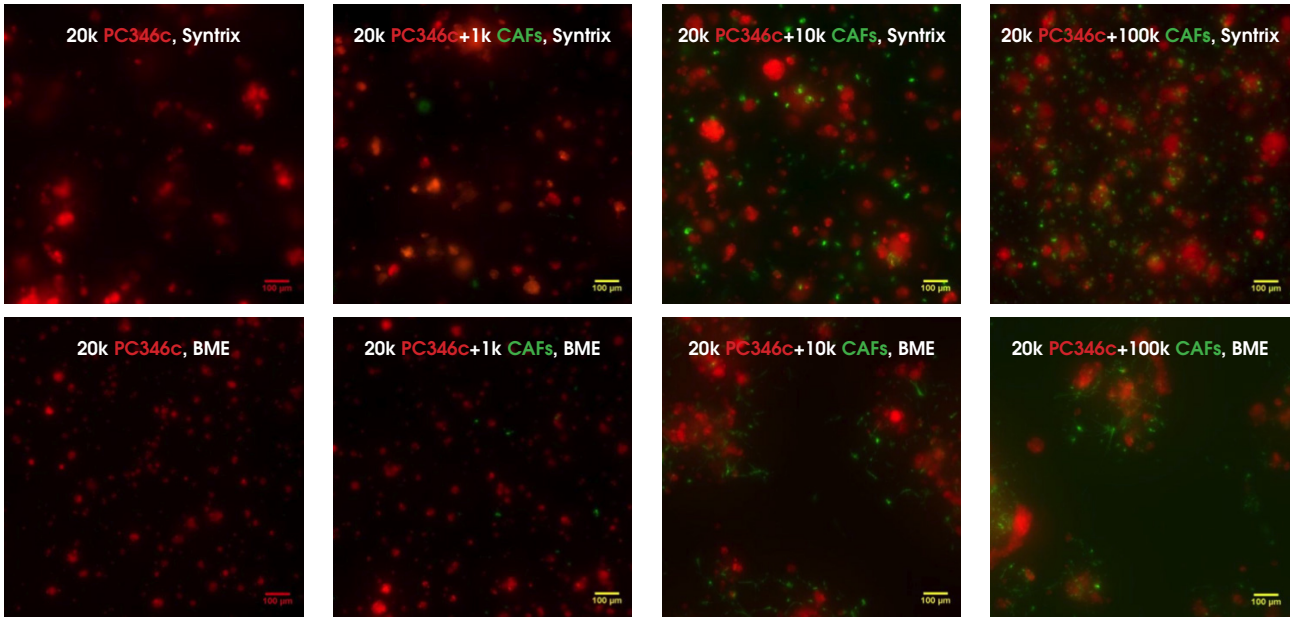


Figure 2: Live fluorescence images of PC346c and CAF cells cultured for 21 days in Syntrix-BM (upper panel) or BME (lower panel).

Confocal images taken after 21 days in culture (Figure 2) show that PC346c cells and CAFs are both still present. The PC346c cells form tumor spheroids, while the CAFs are interspersed with the PC346c clusters. At the higher concentrations, some clustering of CAFs with PC346c cells occurs in BME.

After 21 days in culture, cells were stained with Hoechst (nuclei), 5-ethynyl-2'- deoxyuridine (EdU) cell proliferation assay, and NucView 488 Caspase-3 apoptosis assay and images were taken in the Opera system. The images in Figure 3 show that after three weeks, proliferating cells are still present in the tumor spheroids in all samples. In addition, larger spheroids contain apoptotic regions, demonstrating that the tumor spheroids are densely packed.

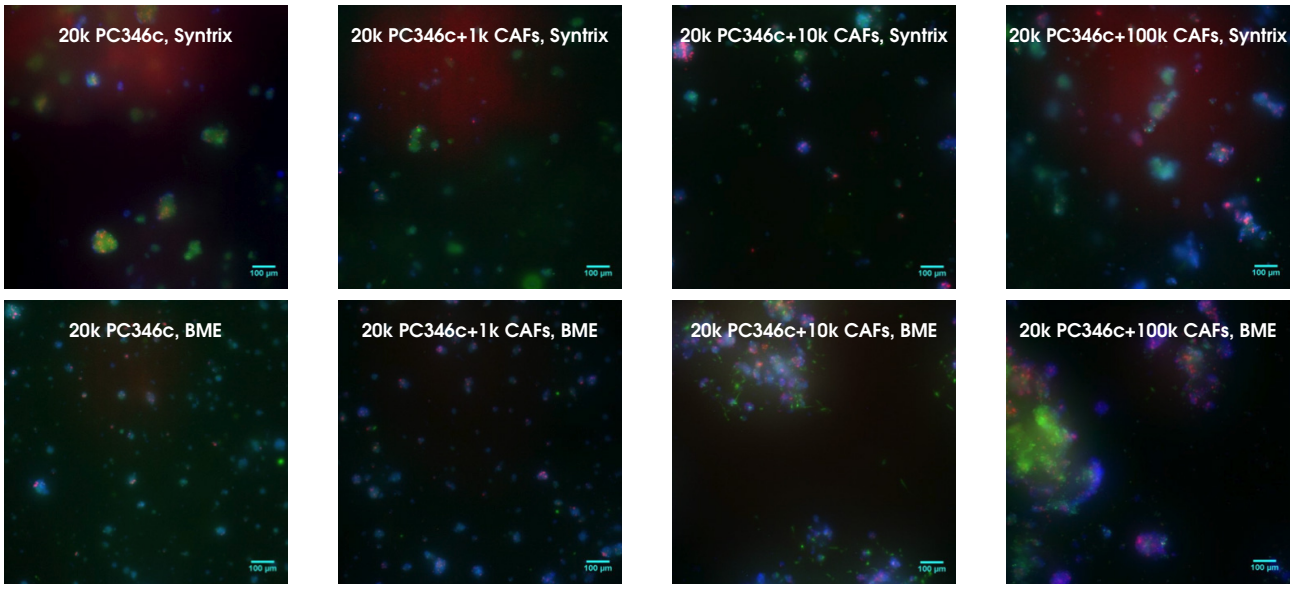


Figure 3: Fluorescence image of PC346c and CAF cells cultured for 21 days in Syntrix-BM (upper panel) or BME (lower panel) and stained with Hoechst (blue, nuclei), EdU (red, cell proliferation) and NucView 488 (green, apoptosis).

Conclusion

In this study, we compared the growth of 3D tumor microtissues in BME gels and Syntrix-BM. PC346C prostate tumor cells appear to proliferate better in Syntrix-BM and the high cell numbers can be better sustained.

Syntrix-BM allows for signaling between multiple cell types, as demonstrated by the increased proliferation of PC346C in the presence of CAFs.

Syntrix-BM allows for the optimization of the medium for specific purposes by fine-tuning of the growth factor composition.

The growth of fluorescently labeled cells grown in Syntrix-BM can be followed using live imaging techniques.

In summary, Syntrix-BM allows a new generation of matrix embedded cells as 3D cell models compatible with high content screening applications.

Cell culture data obtained by partner

1 Hickman, J.A.; Graeser, R.; de Hoogt,R.; Vidic, S.; Brito, C.; Gutekunst, M.; van der Kuip, H. *Biotechnol. J.* 2014, 9, 1115–1128.

2 Thoma, C. R.; Zimmermann, M.; Agarkova, I.; Kelm, J. M.; Krek, W.; *Adv. Drug Deliv. Rev.* 2014, 69–70, 29–41.

3 Weigelt, B.; Ghajar, C.M.; Bissell ,M.J. *Adv. Drug Deliv. Rev.* 2014, 69–70, 42–51.

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