An in vitro tumor-stroma co-culture model using a biomimetic hydrogel and Raman spectroscopy analysis

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1. Introduction

The microenvironment of tumors plays a significant role in tumor progression. 3D *in vitro* models are now widely developed to investigate mechanisms of tumor progression and the relationships between tumor and stroma. In addition, 3D *in vitro* models are needed to screen for drugs targeting these mechanisms and interactions.

Collagen gels, often used for modeling the stroma, are not always ideal when changes in the extracellular matrix (ECM) are to be investigated. A defined matrix, free of ECM proteins and growth factors would be preferred as a „clean" background for the detection of newly synthesized extracellular components or changes in the miroenvironment.

Raman spectroscopy (RS) is a highly sensitive molecular spectroscopic technique, that can be used to profile the entirety of biomolecules in a cell or in a cellular substructure. RS gives insight into the metabolome of cells in a completely non-destructive way and can be used as "photonic marker" to characterize cells and cell states.

In this study we show how Raman spectroscopy can detect changes in fibroblasts and their microenvironment when co-cultured with tumor cells in chemically defined hydrogels.

2. Materials and Methods

3000 MCF-7 breast epithelial tumor cells and 10,000 primary human dermal fibroblasts were grown alone or in co-culture for 14 days in 30 µl dextran gels (*3-D Life* Hydrogel; Cellendes GmbH). The gels had been modified by covalently attaching 0.5 mmol/l RGD Peptide and crosslinked with the MMP cleavable crosslinker CD-Link at a final concentration of 3 mmol/l reactive groups. Raman Spectra were taken with the BioRam® confocal Raman laser trapping system (Cell Tool GmbH). Statistical data evaluation was performed using Prinicipal Component Analysis (PCA).

3. MCF-7 cell culture: survival and morphology in defined hydrogels

MCF-7 cells form spheroids in dextran gels in a round (Fig. A), mass (B) or grape-like (C, arrow) phenotype. Most of the cells are alive (green) in all phenotypes and only a few dead cells (red nuclei) are randomly distributed throughout the cell clusters.

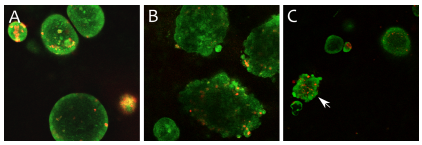

Fig 1: Epifluorescence microscopy of MCF-7 spheroid phenotypes and cell survival. Live (green) and dead (red nuclei) were stained with calcein and ethidium homodimer-1, respectively. Size bar, 100 µm. Arrow in C indicates the grape-like phenotype.

4. Co-culture of MCF-7 cells and fibroblasts

In co-culture with fibroblasts MCF-7 cells also form mainly round spheroids (Fig. 2 C, C'). In mono-culture fibroblasts display a well spread, phenotype with randomly oriented protrusions and few cell-cell contacts (Fig. 2 B, B'). In contrast, when co-cultured with MCF-7 cells fibroblasts have a more slender appearance with fewer protrusions and aggregate in a parallel alignment to form dense cell clusters (Fig. 2 C, C'). This change in fibroblast morphology and intercellular arrangement suggests that a tumor cell - fibroblast interaction occurs in this hydrogel matrix and fibroblasts may have turned into cancer-associated fibroblasts.

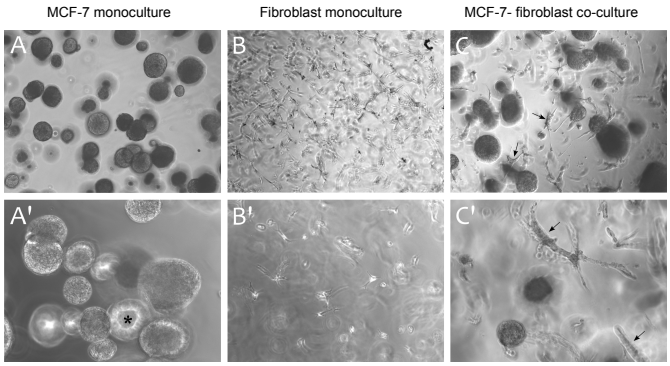

Fig 2: Phase contrast microscopy of cell phenotypes of MCF-7 cells and primary dermal fibroblasts in mono- and co-culture. The asterisk in A' indicates a lumen inside the spheroid similar to an acinus. When MCF-7 cells and fibroblasts are grown together, fibroblasts form long shaped aggregates with tightly packed cells (arrows in C and C'). Size bar, 100 µm.

5. MCF-7 spheroids increase in size when co-cultured with fibroblasts

The size of MCF-7 spheroids increases when they are co-cultured with fibroblasts (Fig. 3). This may indicate a tumor promoting effect of fibroblasts on MCF-7 cells.

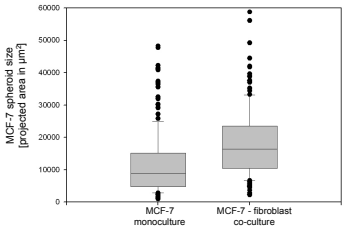

Fig. 3: Comparisopn of spheroid size of MCF-7 cells grown in mono- and in co-culture with fibroblasts.
After a 14 day culture gels were dissolved by the addition of dextranse. Sedimented spheroids were photographed and the projected area of each spheroid was measured with Image J. The projected area was taken as a measure of spheroid size. 180 spheroids of each culture condition were processed. A Mann-Whitney test shows a significant increas of spheroid size (p=0,00079) in co-cultures.

6. Raman analysis of mono- and co-cultured fibroblasts

Raman spectra of fibroblasts in mono-culture differ from those of co-cultured cells in nucleic acid, protein and collagen content (Fig. 4 A). The PCA analysis also reveals a group of co-cultured cells behaving more like the mono-cultured fibroblasts Fig.4 B, encircled data in light blue). In a detailed analysis this differences can be assigned to the collagen content (Fig.4 C).

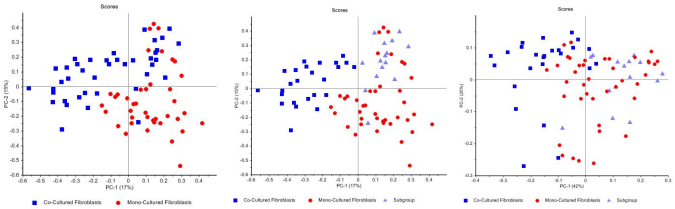

Fig 4: PCA analysis of mono-cultured Fibroblasts (red) and co-cultured Fibroblasts (blue).
A: PCA analysis with respect to the overall spectrum.
B: PCA analysis with respect to the overall spectrum, showing subgroup of co-cultured cells (light blue) overlapping with mono-cultured cells.
C: PCA analysis with respect to the collagen relevant spectral data only.

7. Summary

- MCF-7 cells and fibroblasts can be successfully co-cultured in dextran-based biomimetic hydrogels.
- Fibroblasts change their morphology in co-culture with MCF-7 tumor cells resembling the phenotype of cancer-associated fibroblasts.
- MCF-7 spheroids increase in size when co-cultured with fibroblasts
- Raman spectroscopy identifies a different metabolome in co- and mono-cultured fibroblasts (different content of nucleic acid, protein and collagen).
- Raman spectroscopy identifies a subgroup of co-cultured fibroblasts behaving more like mono-cultured cells with respect to collagen.

8. Conclusions

- Dextran-based biomimetic hydrogels are a suitable 3-D matrix for the development of tumor-stroma models *in vitro.*
- The combination of chemically defined hydrogels and Raman spectroscopy provide a platform for the non-invasive and label-free investigation of tumor-stroma interactions.

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