A novel 3D microwell array for the analysis of adhesion independent micro-tumours

Andreas R. Thomsen[a,b], Christine Aldrian[a,b], Yi Thomann[c], Anca-Ligia Grosu[a,b], Peter Bronsert[b,d], Marco Leu[e], Per G. Lund[a,f]

[a]Department of Radiation Oncology, Medical Center – University of Freiburg, Germany. [b]German Cancer Consortium (DKTK), Partner Site Freiburg and German Cancer Research Center (DKFZ), Heidelberg, Germany
[c]Freiburg Material Research Center and Institute for Macromolecular Chemistry, Department of Chemistry, University of Freiburg, Germany [d]Institute for Surgical Pathology, Medical Center – University of Freiburg, Germany
[e]abc biopply ag, Solothurn, Switzerland [f]Department of Radiation Oncology, Ortenau-Klinikum, Offenburg, Germany

Background

Multicellular organoids, namely micro-tumours, represent a well-established 3D model to study tumour response to radiation and anti-neoplastic treatment *in vitro*. The analysis of certain cancer cell lines was impeded by the inability of those cells to form stable spheroidal structures. Furthermore, co-culture experiments represent an important tool to understand key questions in tissue development and disease biology. Yet, the use of feeder cells together with test cells in the generation of 3D organoids led to heterogeneous cell aggregates whose treatment response could be interpreted ambiguously.

Here we present a novel and innovative 3D microwell array that circumvents those issues by offering a number of unique features. It´s conical geometry allows the formation of aggregates of cell lines and cell types that did not form spheroids using other approaches. Furthermore, feeder and test cells can be cultivated in contact co-cultures as well as in a distance co-culture setup whereby the two cell types are physically separated, hence homogenous 3D aggregates are maintained.

Materials and Methods

The microwell arrays are generated from agarose by using a high-precision replica moulding technique. The resulting arrays comprise several hundreds of cone-shaped microwells each and fit into 6 well plates.

To generate 3D micro-tumours, a cell suspension is seeded into the 3D CoSeedis™ micro-well array. Cells then sediment into microwells, where aggregate formation is supported by the confined space within the non-adhesive hydrogel. For co-culture, the microwell array is placed on top of a layer of adherent stromal cells, so that both cell types remain in separate compartments, but may communicate via soluble factors that diffuse through the permeable hydrogel. As a readout, light and fluorescence microscopy, optical scanning or even paraffin histology is used.

Results

The 3D microwell array allows the formation of spheroidal and non-spheroidal cell aggregates and consequently enables the examination of tumour cells in 3D structures that have so far been elusive.

Seeding of feeder cells underneath the 3D-microwell array enables to assess the aggregate growth in presence of secreted feeder cell-derived factors omitting a direct physical contact (distance co-culture). However, different cell types may also be assembled as mixed cell aggregates (contact co-culture). Validated read-out methods, namely volumetric and histological protocols, enable a rapid and reliable analysis and warrant reproducible results over different experiments and treatments.

Conclusions

The 3D CoSeedis™ microwell array is well-suited to grow cells as 3D aggregates that would normally not do so, e.g. MIA PaCa-2 cells, thus extending the range of cells to be used in analyses of the micro-tumour treatment response.

Special emphasis has been put on the interaction between stromal and cancer cells. Both, contact and distance co-culture effects can be easily addressed with the presented 3D microwell array.

3D CoSeedis™ allows to monitor dose-dependent effects of complex treatments using both cell aggregate volume and marker expression as a read-out.

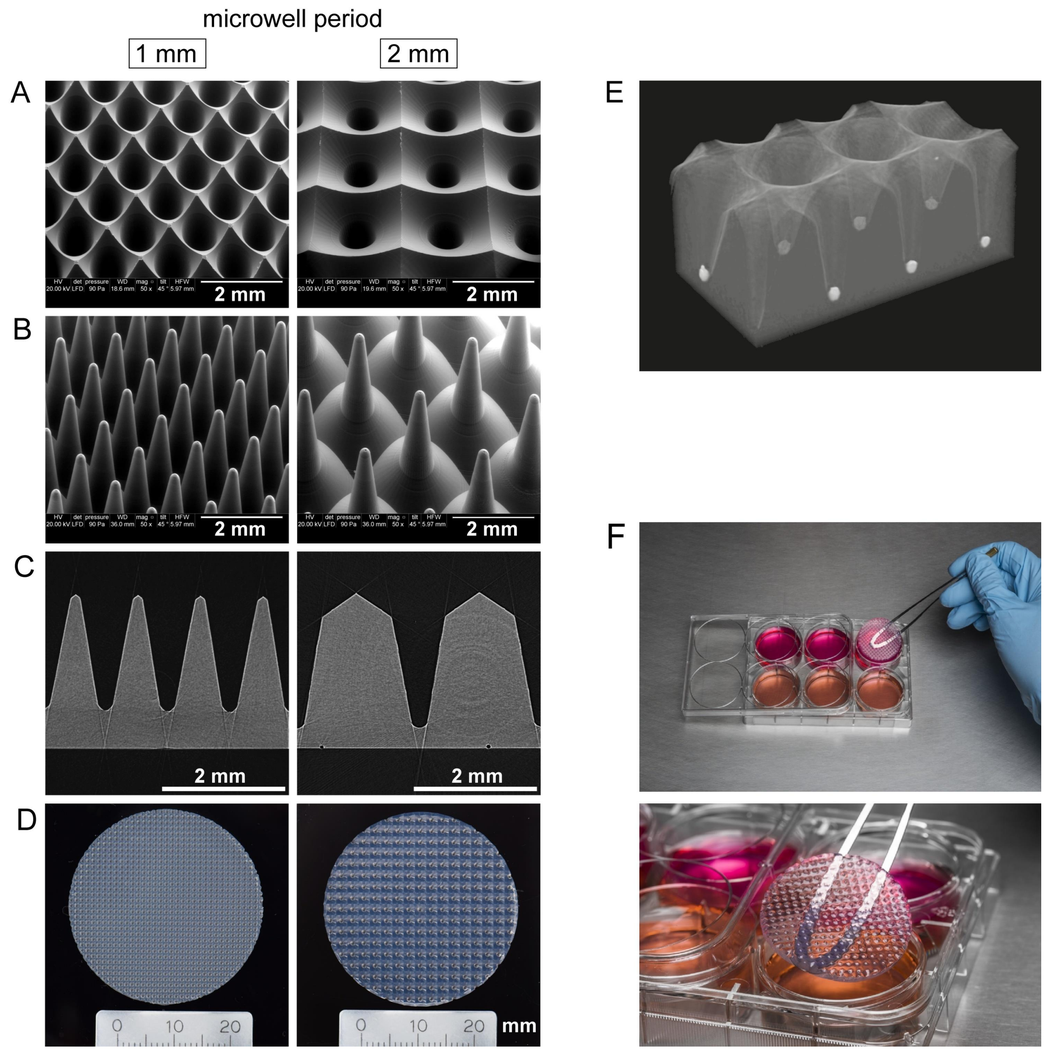

Fig. 1 Properties of the conical agarose microwell arrray
(A) Scanning electron microscopy of master structure and (B) of the PDMS moulds. (C) Profiles of two epoxy resin replicates made out of micro computer tomography (µCT) datasets. (D) Photography of the agarose array. (E) µCT reconstruction of an array with T47D spheroids in the microwells. (F) Handling of array.

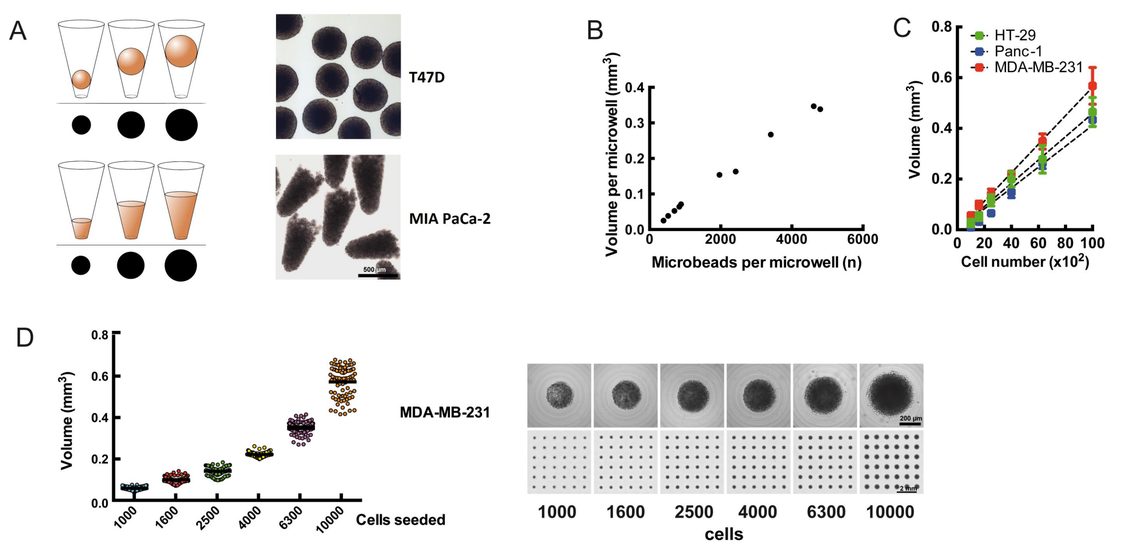

Fig. 2 Volume determination and calibration of cellular aggregates in the array
(A) Determination of spheroid (upper row) and cell aggregate (lower row) volume. Volumes correspond to the projected area seen from the bottom. Spheroids of T47D breast and conical cell aggregates of MIA PaCa-2 pancreatic cancer cells after harvesting. (B) Calibration of volume to microbead numbers. Microbeads were loaded onto the array, centrifuged, scanned and photographed. From each microwell the microbeads were extracted, counted and correlated to the calculated aggregate volume. (C) Calibration of cell aggregate volume against cell number. Three cell lines were seeded at defined cell numbers. (D) Volumes of MDA-MB-231 cell aggregates seeded at defined numbers (left). Corresponding single cell aggregates and scans of array regions (right).

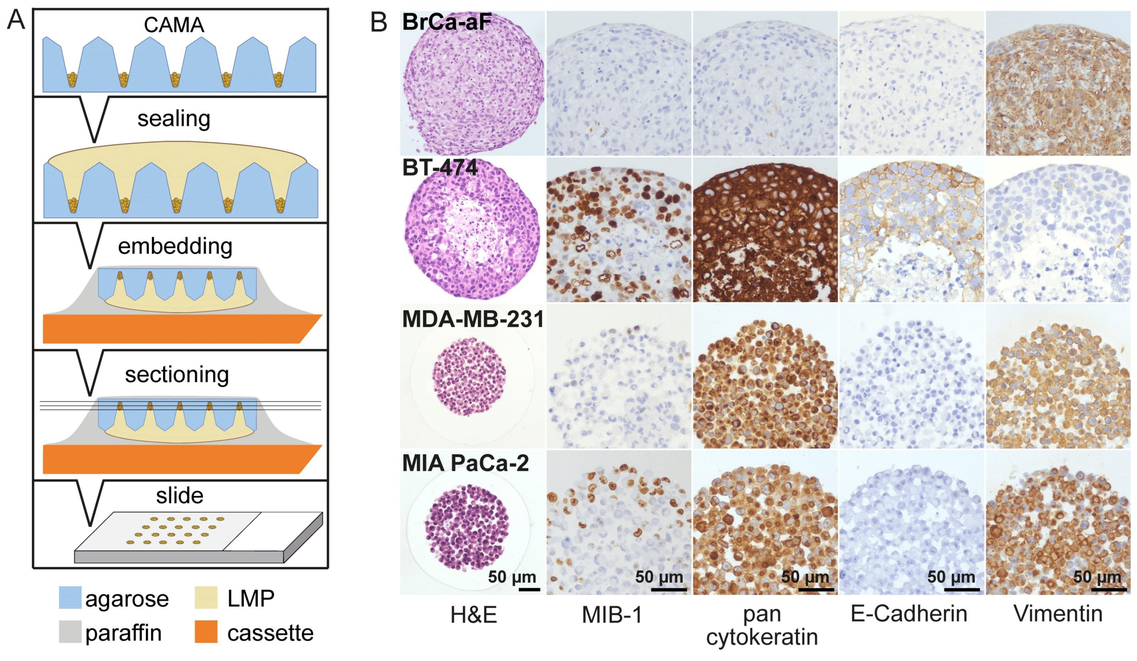

Fig. 5 Histological processing of 3D cell cultures in 3D CoSeedis™ micro-well array
(A) Preparation of histological slides. Formalin-fixed microwell arrays are sealed with low melting point (LMP) agarose. Following automatised dehydration and paraffin infiltration, the array is turned upside down, embedded by addition of paraffin and mounted onto a cassette. Sections of 2 µm are prepared for subsequent standard staining procedures. (B) Histology of breast cancer associated fibroblasts (BrCa-aF), BT-474 and MDA-MB-231 breast cancer and MIA PaCa-2 pancreatic cancer cells .

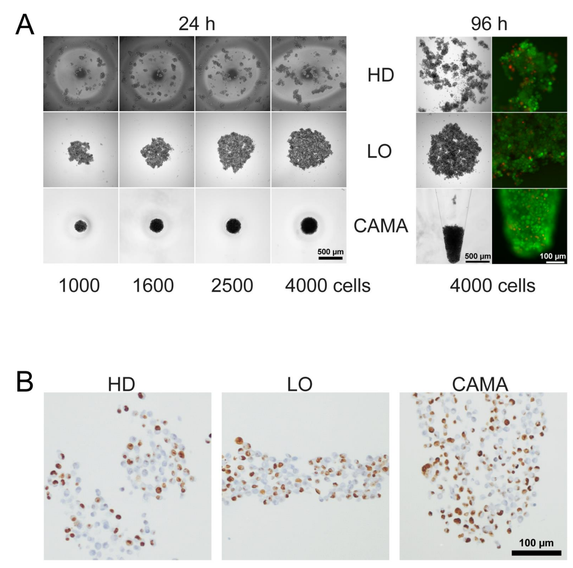

Fig. 3 Cell aggregate growth in Hanging Drop (HD), Liquid Overlay (LO) and the conical agarose microwell array (CAMA).
(A) Images of single cell aggregates. Fluorescence microscopy images of FDA- (green/viable cells) and PI- (red/dead cells) stained aggregates.
(B) MIB-1 staining for proliferating cells. Proportion of MIB-1 positive cells is mainly unchanged in the three sytems.

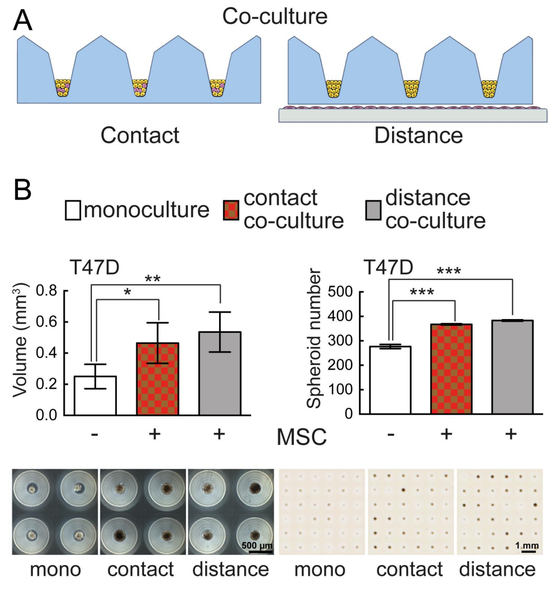

Fig. 4 3D co-culture in the 3D CoSeedis™ micro-well array (A) For contact co-culture the distinct cell types are seeded together into the array For distance co-culture one cell type is seeded into the array, then the array is placed on the monolayer of another cell type. (B) Effect of human MSC on growth and spheroid formation of T47D breast cancer cells in contact and distance co-culture. Cell seeding: T47D (10 cells per microwell (cpm)); MSC for contact co-culture (10 cpm); MSC for distance co-culture (10^3 cells/cm²). Volume was measured on d20 (n=3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.