The application of the Cell MicroArray technology to study 3D cell culture systems

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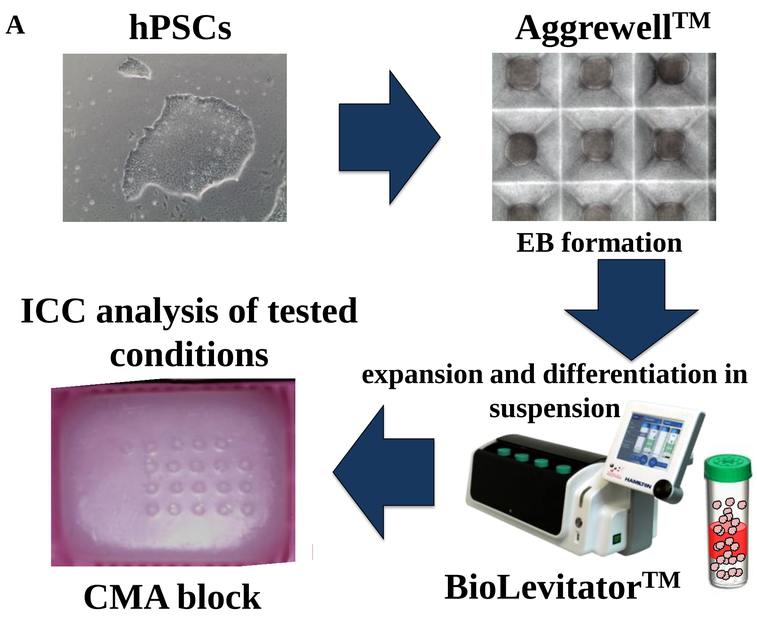
BACKGROUND

Recently it was demonstrated the enormous self-organizing capacity of pluripotent stem cells to form whole tissues (Lancaster MA et al., 2013) and that extracellular matrix (ECM) proteins promote organoid development by acting as a 3-D matrix for structural support and instructive signaling. The generation of e.g. cerebral organoids was described as a multi-step culture system including aggregation of ESCs in embryoid bodies (EBs): suspension in neural induction medium to form symmetric neuroectoderm followed by the manual deposition of each EB in a droplet of Matrigel and final expansion and tissue maturation in a spinning bioreactor (Rodrigues GM et al., 2014). In order to test the influence of different matrix compositions during dual-SMAD mediated neural induction we cultured the EBs under variant ECM protein conditions.
Here we describe the application of CMA technology to investigate human stem cell pluripotency and differentiation capacity using 3-D iPS suspension cultures.

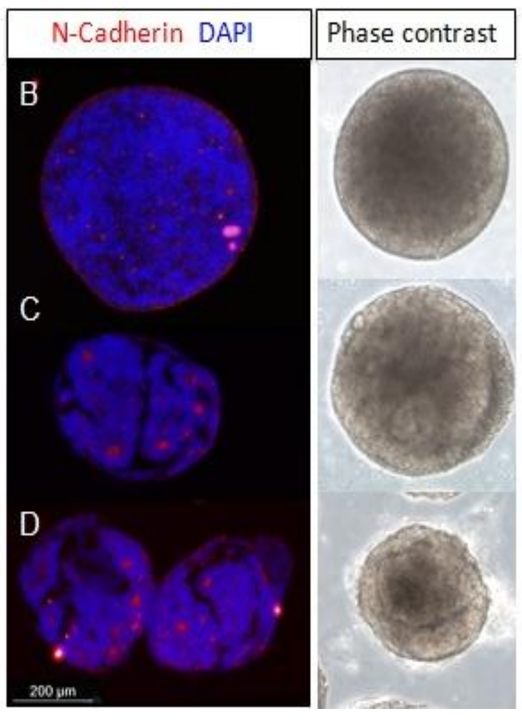
Tissue or Cell MicroArray (TMA/CMA) platform is a high-throughput experimental method that can be exploited to study 3-D cell culture systems. Cells cultured as 3-D models exhibit features that have proven to recapitulate the *in vivo* conditions (Vinci M et al., 2012). This characteristic is mainly due to the behaviour of cells when grown in matrices and scaffolds. In the area of stem cell research, 3-D cultures have been widely studied in tissue engineering, basic research (good source for the continuous supply of specifically differentiated cells), therapeutic applications and/or drug testing (studying the synergistic effects of biologically important substances on cells).

Generation of cortical organoids



Schematic representation of the experimental procedure (A). In presence or absence of ECM proteins, we generated uniform EBs from hiPSC cultures using Aggrewells (STEMCELL Technologies), which were transferred to a parallel bioreactor system (BioLevitatorTM) after 5 days of static cultivation (Elanzew A et al., 2015). At day 12 of neural differentiation aggregates were collected, fixed in 4% PFA and resuspended in 1.5% low-melting agarose. For Cell MicroArray (CMA) construction aggregates were embedded in paraffin with an automatic tissue processor. Cell cores were then transferred and assembled in a recipient block using the Galileo TMA CK4500 platform (ISENET).

CMA cores *vs* live aggregates



Shown are stained cell cores (B-D) of a cell array slice (5 µm) obtained from the CMA with corresponding representative phase contrast images of live aggregates from three tested culture conditions. At early stages of neural differentiation neural rosettes and cavities are formed, which show a characteristic apical localization of the neural specific N-cadherin. CMA analysis showed that the size of cavities/lumen of neural rosettes and the morphological complexity of the neural aggregates increased in the presence of ECM proteins. Figure (B) Cultivation without ECM proteins, (C) with ECM proteins from day 5 of differentiation onwards, (D) with ECM protein addition from day 0 (starting during EB formation).

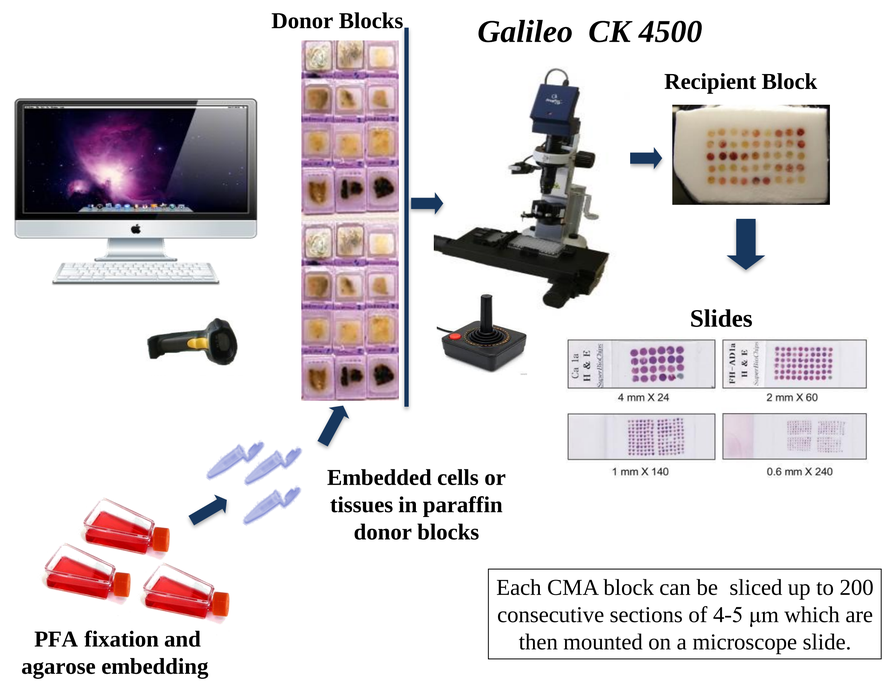
Conclusions

CMA technology applied to pluripotent stem cells allows to determine:
-pluripotency of each cell aggregate;
-spatial and cell specific sub-cellular localization of markers in each organoid;
-quick and simultaneous analysis of several clones as experimental conditions during pluripotency and differentiation ,without loss of the proper 3-D cellular morphology.

Cell MicroArray Technology

The Application of the CMA technology in stem cell research, and in particular in 3-D cultures during the differentiation of induced Pluripotent Stem Cells, is an alternative approach. The semi-automatic process (CMA platform), is a high-throughput analysis tool to rapidly and simultaneously characterize the hPSCs and could provide an opportunity to minimize work time and reagent cost.
Currently, hPSCs can be routinely expanded as cell suspensions in 3-D culture conditions. Traditional approaches to screen their pluripotent and differentiation potential are cumbersome. Thus, the CMA technology could streamline the whole process by analyzing hundreds of samples at the same time (La Spada A et al., 2014).

E ***Schematic overview of the CMA construction process***



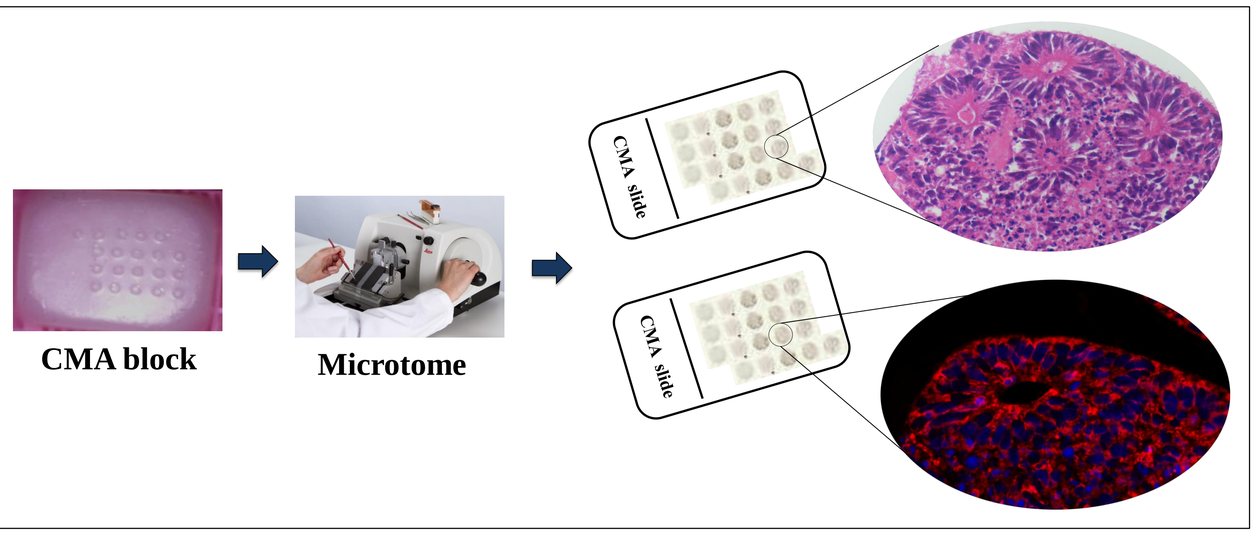
Each CMA block can be sliced up to 200 consecutive sections of 4-5 µm which are then mounted on a microscope slide.

Cell suspension are fixed in formalin and embedded in paraffin (E). The paraffin blocks are then used as donor blocks in the different CMA constructions and the slides obtained from the CMA are immunostained and the *bona fide* clones are then used for down the line needs.

Immunofluorescence and *H&E* staining in 3-D morphology

Artificial tissue block (CMA) was constructed using several hiPS cells that were cultured as 3-D cell aggregates: the CMA simultaneously contains different hiPS clones and culture conditions which are representative of the pluripotency and differentiative statuses. CMA blocks were sliced using a microtome and the slides were H&E and immunofluorescence (IF) stained. In details this methodological approach, combining, CMA & 3-D cultures preserves cell morphology and allows to better characterize protein expression and visualize the sub-cellular localization of the markers more comprehensively (Hewitt SM et al., 2012).

F



Here we show the Tuj1 (anti-beta-III-tubulin immunofluorescence, red) and Hoechst 33258 (blue) counterstaining of neural rosettes derived from human pluripotent stem cell differentiations grown in three dimensional (3-D) conditions (F). 3-D aggregates were processed following the Cell MicroArray technology (CMA) using the Galileo CK4500 (Integrated Systems Engineering) platform.

References: Elanzew A et al., *Biotechnol J.* 2015; Hewitt SM et al., *Methods Mol Biol.* 2012; La Spada A et al., *Mycroarrays*. 2014; Lancaster MA et al., *Nature.* 2013; Rodrigues GM et al., *Stem Cell Rev.* 2014; Vinci M et al., *BMC Biol.* 2012

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