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Abstract

Gene expression patterns intimately connected to the interaction of cells with their environment or micro-environment. This includes contact with the extra-cellular matrix, exposure to growth factors, integrity of the membrane, pH and ion concentration of the medium. Numerous studies on stem cell sustainment or differentiation rely on the precise expression of markers that are sensitive to the exposure of cells to growth factors. Traditional technologies include a step of cell preparation involving cell detachment or lysis. It is well known that both these steps can be associated with dramatic changes in the gene expression pattern. In order to minimize this phenomenon we have developed AmpliCell, an innovative microculture slide chamber where cells can be grown and subsequently analyzed by PCR based methods without the need of sample preparation. The convenient workflow of AmpliCell results in reliable information on gene expression patterns without the risk of alterations during sample preparation. Taking advantage of this new platform, we analysed tumour stem cells which are assumed to be the relevant cells responsible for metastasis formation. It is known that they can either self renew or form differentiated daughter cells, dependent on different growth stimuli. Isolation of such cells from the circulating tumour cells is possible and addition of different growth factors to the culture medium induces different gene expression patterns assayed by qPCR. This approach helps to clarify which factors are responsible for differentiation pathways and which ones make the tumour stem cells able to settle and grow invasively.

Platform

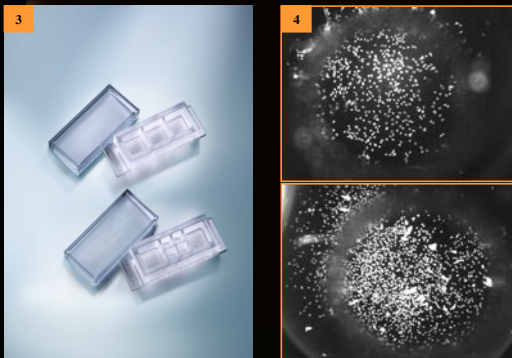
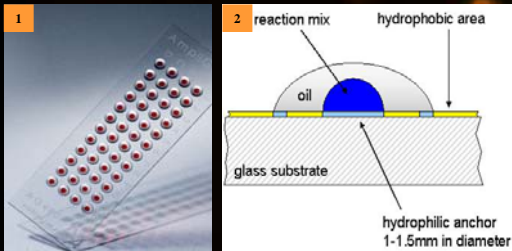


Figure 1: AmpliGrid and AmpliCell platforms

- 1 AmpliGrid platform with 48 reaction sites, suitable for 1µl PCR reaction
- 2 Hydrophobic and hydrophilic structure of the AmpliGrid reaction site
- 3 AmpliCell, the microculture slide chamber derived from AmpliGrid
- 4 Cells are seeded and grown in the microchambers under standard cell culture conditions and subsequently analyzed by PCR

Workflow

After erythrocyte lysis, cells are stained with a fluorochrome-labeled anti-epithelial antibody (EpCam) and directly transferred by micro-manipulation on AmpliCell for cell expansion followed by PCR analysis or on AmpliGrid for single cell PCR.

Cells are seeded on top of the reaction sites in AmpliCell and let grown till the desired confluency is reached. Thanks to the multichamber format, different growing media can be used simultaneously.

Once the cellular growth is finished, the microchamber is removed and the cells are analysed by PCR directly on the slide without any need for sample preparation.

Gene expression of EpCam and Her-2 (human epidermal growth factor receptor) is analysed by qRT-PCR on cells from breast cancer patients.

Results

CETCs from breast cancer patients isolated by EpCam antibody

Cells which are EpCam (green) or EpCam and stem cell antigen labeled (green and orange) and Hoechst (blue dye) positive are chosen for RT-PCR analysis

qRT-PCR results

qRT-PCR results showing gene expression of EpCam (blue) and Her2 (green) compared to a housekeeping gene (dark blue) on a single CETC

Conventional PCR results

Defined bands of EpCam Her2 and a housekeeping gene on a single CETC

Conclusion

Individual EpCam and stem cell antigen positive cells detected in peripheral blood of breast cancer patients can be micromanipulated onto AmpliGrid or AmpliCell platforms and either directly or after cell culture successfully amplified for gene expression of defined genes.