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DIAGNOSTIC PLATFORM FOR AUTOMATED PERSONALIZED CHEMOSENSITIVITY ASSAYS

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MOTIVATION

Among other cases of cancer in women, the most common type is breast cancer with over 50,000 cases every year solely in Germany. Individualized cancer therapy as part of personalized medicine aims the objective to identify the most potent combination of chemotherapeutics for an optimal therapy of these patients.
In order to optimize the therapeutic treatment for breast cancer different diagnostic assays like Oncotype DX, Mammaprint, uPA/PAI-1 tests and ATP-TCA assays are used. Within these diagnostics a carcinoma biopsy from the patient is taken and processed for either genotype analysis or in case of in vitro assays like ATP/TCA for cell culture in the presence of chemotherapeutics to find the best combination for each individual patient.
Today, only a limited number of patients can benefit from these assays. The processing of carcinoma samples is performed manually causing high personnel costs and low reproducibility. Furthermore, yields of viable cells are insufficient and the signal-noise ratio is too low caused by non-tumor cells which remained in the cell suspension.
Automated process techniques facilitate a cost efficient alternative to manual processes while ensuring high yields and high quality of cells.

AIMS

For the efficient use of chemosensitivity assays a fully automated modular DiagnoSYS platform technology was developed including

- integrated carcinoma tissue preparation, including handling of tumor tissue and single cell suspension
- automated enrichment of tumor cells
- automated assay for cell viability by using an ATP/TCA luminescence assay

TECHNICAL APPROACH

The DiagnoSYS platform includes (figure 1):

- fully automated mechanical and enzymatic dissociation of the tumor tissue
- automated magnetic bead based tumor cell enrichment
- luminescence based cell vitality measurement (ATP/TCA)
- one-channel pipetting based on disposable tips with liquid level detection for high accuracy (4-channel pipetting optional)
- barcode reading for reagent/sample tracking and RFID technology for total assay control

The platform is integrated in a class II laminar flow to maintain sterility and for safety of the operating personnel.
The platform technology is based on the internationally accepted SBS-format. Therefore, all processing steps can be exchanged fast and easily and will allow to process different assay approaches on the same platform. Due to modular programming, further processing steps can be implemented easily.

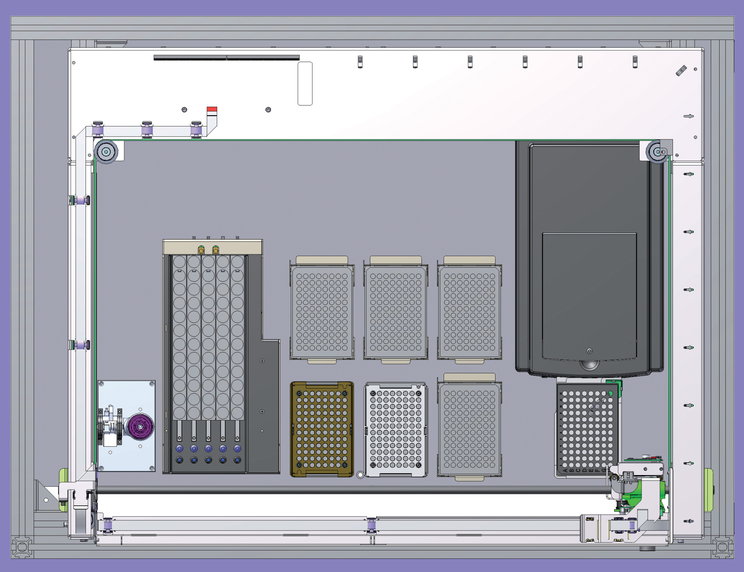

Figure 1: Platform layout for automated personalized chemosensitivity assaysed

RESULTS

The dissociation of tumor tissue is performed with a TissueProcessor developed at Fraunhofer IPA using gentleMACS™ C Tubes (Miltenyi Biotec GmbH, Germany), which ensures an automated preparation of a cell suspension while inline-temperature control is implemented (figure 2). Since cancerous tissue is inhomogeneous the dissociation can be influenced by stirring speed, incubation time and temperature. In first experiments a yield of $1.7x10^6 \pm 0.3x10^6$ viable cells with a cell vitality of 97 ± 2% were obtained. In comparison, in the manual process an average of $1.6x10^6 \pm 0.2x10^6$ viable cells with a cell vitality of 92 ± 6% are isolated.

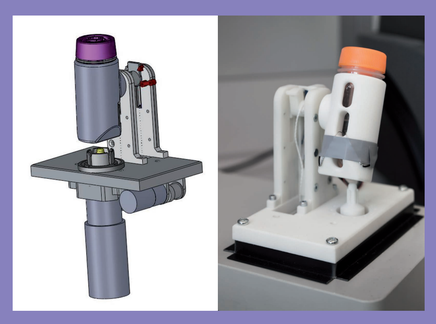

Figure 2: TissueProcessor for mechanical dissociation of tumor tissue

After cell extraction the depletion of enzymes and the simultaneous enrichment of tumor cells are performed by using magnetic bead technology against the epithelial cell adhesion molecule (EpCAM, figure 3). First experiments using the human breast adenocarcinoma cell line MCF-7 demonstrated the highest cell yield of 66% by the addition of 28 µl EpCAM Microbeads to $2.5x10^6$ MCF7 cells. Further experiments with primary tumor cells are ongoing.

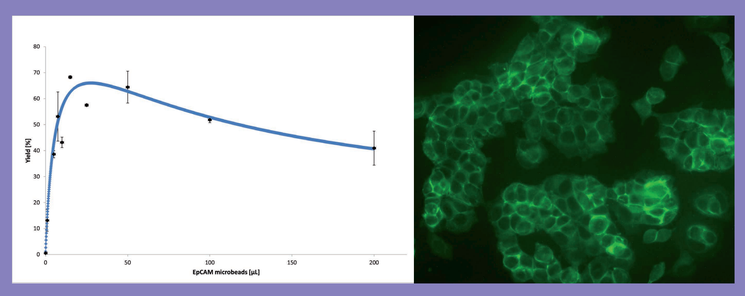

Figure 3: Enrichment of tumor cells by using EpCAM microbeads: (A) optimization of EpCAM microbead concentration and (B) EpCAM staining on selected cells

CONCLUSION

This platform facilitates the optimization and standardization of personalized assays and reduces costs. Due to the integrated tumor cell enrichment the significance of diagnostic chemosensitivity assays can be enhanced while automated processing ensures a high degree of reproducibility.