

Lab-on-Chip for the Isolation and Characterization of Rare Cells (CTCs) from Whole Blood

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Introduction

With cancer being one of the most prominent health concerns, the development of new technologies for cancer diagnostics and therapy monitoring is of enormous interest. While traditional therapeutic approaches target primary tumor characteristics, tumor cell dissemination is the most critical aspect in respect of prognosis. To reflect the molecular characteristics of tumor cells, including their potential for metastasis development, tumor recurrence, and prognosis, disseminated tumor cells (DTCs) in bone marrow or circulating tumor cells (CTCs) in peripheral blood are discussed as relevant markers. CTCs and DTCs are cells that disseminate from the tumor and may lead to the formation of metastases. Although demonstrated to be of prognostic relevance, the necessity of invasive sampling procedures for DTC analysis hinders this approach from becoming established for therapy of solid tumors. In that respect, circulating tumor cell analysis in peripheral blood resembles a promising alternative. For metastasized patients with breast cancer the prognostic relevance of CTC detection in peripheral blood was already demonstrated. The advantage of a blood test compared to invasive methods is founded in the safety and potential frequency of examinations.

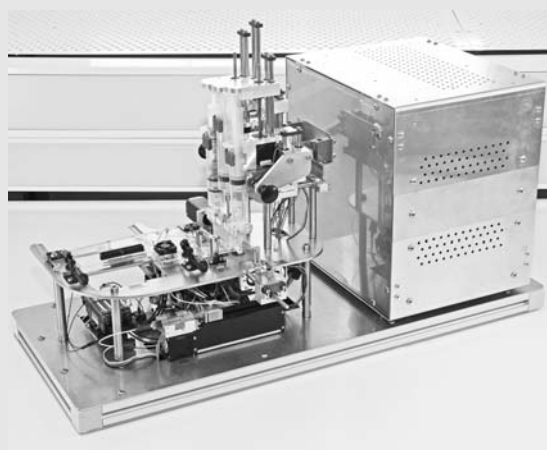


Fig. 1 System setup

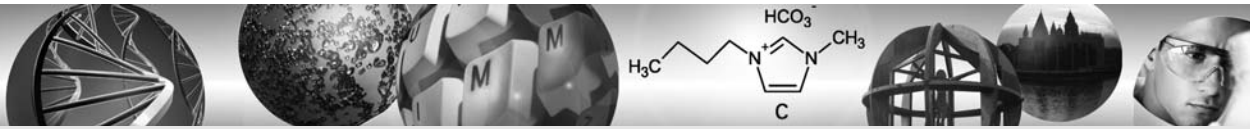
Against this background, the control of therapy responses through monitoring of CTC presence and numbers in peripheral blood before, during, and after therapy is highly interesting. Moreover, additional molecular characterization of the isolated CTCs might open significant insights into new aspects of tumor biology and metastasis, contribute to the interpretation of treatment responses, and support the development of new targeted therapies.

Due to their low abundance in peripheral blood, the isolation and analysis of CTCs is very challenging (down to one cell per ml blood). Current cytometric approaches to CTC analysis involve enrichment procedures based on physical properties (e.g. size, density, charge), immunomagnetic separation based on cell surface antigens (e.g. EpCAM) or density gradient methodologies, followed by immunocytochemical or flow cytometry analysis for quantification. However, the systems in the market still do not integrate the option of molecular CTC characterization. To address this unresolved option, the presented project focused on the development of a modular, microfluidic system for the isolation, enrichment, and molecular characterization of CTCs from peripheral blood.

Competences

Within the presented project IMM took the responsibility for the development of a modular, microfluidic system including instrumentation for the immunomagnetic isolation and enrichment of rare cells (e.g. CTCs) from peripheral blood and their preparation for molecular characterization and quantification via MLPA. This encompassed the design and integration of modules for a multitude of complex, multistep analytical protocols like e.g. cell enrichment, mRNA isolation, Reverse Transcriptase Multiplex Ligation-dependent Probe Amplification (RT-MLPA), and electrochemical analysis. Protocol simplification for on chip implementation was realized in tight co-

operation with the assay partners (e.g. MRC-Holland for MLPA). Moreover, the required periphery including actuation and fluidic control components as well as the electronic circuit were realized by IMM. The overall challenge was not only founded in the complexity of technologies to be translated and integrated into sequentially connected microfluidic modules but also in the realization of a macro-to-micro interface that enables fully automated sample analysis with sample volumes of 5 to 7.5 ml. The generic character of the platform allows its use for a diversity of applications that require rare cell (e.g. tumor, fetal, or stem cell) isolation, and enrichment, and molecular characterization of the isolates.



Setup



Fig. 2 Incubation module including paddle wheel mixer (1). Separation module with macro-to-micro interface and lysis chamber (2). Integrated unit for cell isolation, enrichment and lysis (3). Amplification module for Reverse Transcriptase MLPA (4) and electrochemical detection module (5).

The project outcome enfold a series of modules for immunomagnetic cell isolation and enrichment from peripheral blood (1), cell separation from the flow and lysis (2), Reverse Transcription, pre-amplification, and MLPA (4), and electrochemical amplicon detection (5). To allow for thorough characterization of the isolated cells, a panel of 21 mRNA markers was chosen for gene expression analysis via Reverse Transcriptase MLPA, which also inhibits quantification capability. Only requiring sample and buffer insertion, the integrated modular system is able to perform automatically without extra manual operation.

The sample analysis starts with the incubation (cell isolation) module that performs mixing, bead attraction, exchange of buffers, and transfer of cell/bead-complexes and free beads to the subsequently

connected microsystem. The macro-to-micro interface is realized with a slide valve. After transfer to the microsystem the cell/bead-complexes and free beads are collected by applying an external magnetic field. Once accumulated within the lysis chamber of the separation module, the isolated cells are lysed to release mRNA. Next, cDNA is generated and pre-amplified within the amplification module, followed by MLPA. This multistep amplification procedure, consisting of RT, multiplex pre-PCR, hybridization, ligation, and the final multiplex amplification step, became simplified and miniaturized to allow for automated performance on chip within a single amplification chamber. Within the final detection module, the pattern of expressed genes (bulk analysis of isolated cells) is determined by amplicon analysis on an array of electrochemical sensors.

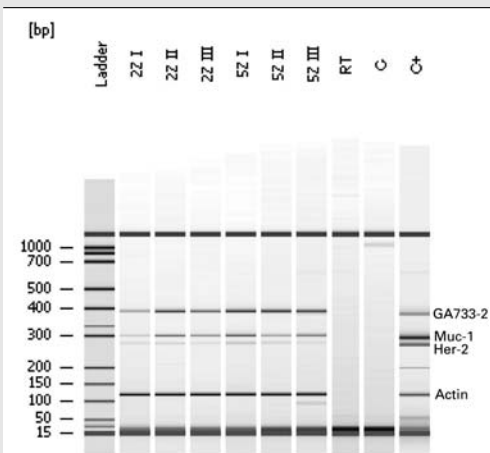


Fig. 3 Analysis of breast cancer-associated gene expression after cell isolation experiments performed within the incubation module (Adna BreastCancerSelect reagents, done by AdnaGen AG, Langenhagen). 5 ml of blood were spiked with 2 (lane 2-3) respectively 5 MCF-7 cells (lane 5-7). The isolate was prepared according to the Adna BreastCancerDetect protocol and analyzed on an Agilent 2100 Bioanalyzer. RT: RT control; C-: negative control; C+: positive control.

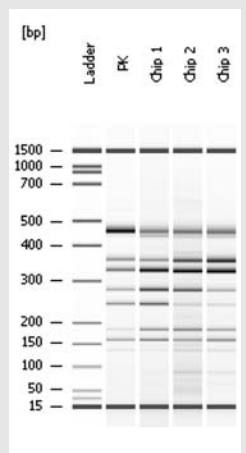


Fig. 4 Electrophoretic analysis of amplicons. PK: Reverse Transcriptase MLPA control performed in benchtop cyclor starting with purified total RNA from MCF-7 cells. Chip 1: On chip RT/pre-PCR, subsequent steps performed in benchtop cyclor. Chip 2: On chip Reverse Transcriptase MLPA (single amplification chamber). Chip 3: On chip MLPA using pre-amplified cDNA generated on chip as template (separate amplification chambers).

Summary

The presented modular platform enables immunomagnetic cell isolation, enrichment, and characterization starting from macroscopic sample volumes of 5 to 7.5 ml, enabling its use for rare cell applications. The lab demonstrator consists of a basic platform including the control units for fluidic actuation and signal reception and a set of disposable polymer modules. Its generic character facilitates usage of the platform for a diversity of cell-based diagnostic

approaches and research. All modules were successfully demonstrated with the respective assay sub-steps, like e.g.:

- Target cell enrichment from 7.5 ml whole blood down to 10 μ l
- Cell recovery demonstrated down to 2 spiked cancer cells (MCF-7 cells) per 5 ml of whole blood sample (Fig. 3)
- On chip implementation of Reverse Transcriptase MLPA for gene expression profiling based on 11 mRNA markers (Fig. 4)