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Diagnostic Platform for Personalized Chemosensitivity Assays - Robust Results via Process Automation

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Abstract

Individualized cancer therapy, as part of personalized medicine, aims for an optimal treatment with minimal side effects. In chemotherapy, one approach is to screen for the most potent combination of chemotherapeutics (chemotherapeutic scheme) to improve quality of life and achieve a high therapy efficacy. Due to high personnel costs associated with the first generation of manual chemosensitivity assays most health insurance providers do not cover this type of therapy. Also, the outcome of such personalized assays has to be proven first. The fully automated DiagnoSYS platform technology is a system effectively using the potential of chemosensitivity assays. It was shown that with an adequate degree of automation, higher reproducibility was achieved. An ATP/TCA assay was used as the first demonstrator assay. Integrated tissue preparation, based on precise regulation of a Miltenyi Biotec M- or C-Tube, combined with magnetic cell enrichment and depletion via EpCAM and CD90 labeling and luminescence based cell vitality measurements, made it possible to enhance the signal to noise ratio of luminescence readings.
The platform technology is based on the internationally accepted SBS-format. Therefore, all processing steps, from tissue preparation to luminescence or fluorescence readings, could rapidly and easily be exchanged, and allows for processing different assay approaches, such as ATP/TCA or prognostic biomarkers as uPA/PAI-1, on the same platform. As a result of modular programming, further processing steps could also be implemented without difficulty. Besides the optimization and standardization of personalized assays, cost reduction, which goes hand in hand with automation, will make the platform affordable for research groups and clinical personnel, amplifying the acceptance of personalized medicine approaches in the future.

1 Personalization is a key topic in tumor analysis and therapy

Malign tumors are highly heterogeneous; thus the analysis of the patient specific tumor provides helpful information to the therapy selection. For years, oncologists have aimed to individualize cancer therapy and to find patient specific chemotherapy treatments that are highly effective or exclude non potent cytostatic drugs [1]. Even if such personalized medication can bring high benefits to cancer patients, so-called chemosensitivity assays are not often performed in cancer treatment [2]. Currently there are a lot of well documented assays on the market, but due to high labor costs of performing those assays, none are part of clinical routine.

1.1 State of the art in chemotherapy

The state of the art in clinical routine treatment of cancer patients is a standard combination of different cytostatic drugs based on consensus guidelines for the various tumor entities [3, 4]. Bearing in mind the heterogeneity of tumors, the optimal benefit to the patient is not guaranteed, and these treatments are often accompanied by strong side effects. Thus, relapse rates appear to be high and alternative cytostatic treatment becomes noteworthy. The patient's quality of life is limited and the unknown efficacy of the treatment may result in the patient's death [5, 6]. Presently, *ex vivo* chemosensitivity screening is a proven scientific method [1]: It can verifiably evaluate the efficacy of cytostatic drug combinations [7]. One method of pretherapeutic analysis is to measure the ATP status of a cell culture after *in vitro* treatment with different combinations of cytostatic drugs [8]. However, even if the patient benefits seem high, procedures for such individualized therapies are often complicated to perform or require complex and expensive technologies, and are not always available to clinicians. Additionally, preparation of tumor tissue and execution of the assays is, if done manually, very error-prone. In ATP status measurements, the influence of cell culture composition is high, resulting in a low signal-to-noise ratio of the tumor specific response.

To overcome these problems of individualized treatment, the key steps of chemosensitivity testing should be automated with affordable process solutions. This was achieved with DiagnoSYS, a personalized chemosensitivity assay platform. The goal was to develop a platform that could complete the key steps of the assay preparation and deliver robust response signals.

2 Materials and Methods

First, the various interfaces were determined. The DiagnoSYS platform was housed by a laminar air flow class II safety cabinet to ensure sterile working conditions and to prevent personnel from coming in contact with cytostatic drug aerosols. The loading of the platform (Image 1) was done via the front of the cabinet. DiagnoSYS includes multiple spaces on the working surface based on the standard ANSI-SBS microwell plate format. To prepare an ATP/TCA assay, the system required a tissue processing unit, a cell separation unit, an one-channel pipetting arm, a rack for loading chemicals, a luminescence plate reader and various disposables, i.e. 1 ml tips, luminescence plates and cell culture plates. The biopsies were placed in M-Tubes (Miltenyi Biotec) readily filled with tissue preparation solution. The preparation solution consisted of 12.5 mg Collagenase, 20 mg Albumin and 5 mg Hyaluronidase in 5 ml buffer. The tube was then mounted to the tissue processing unit. To ensure identification of all materials used, a barcode and a RFID system were operating simultaneously on DiagnoSYS. All chemicals were purchased from SIGMA-ALDRICH if not stated otherwise.

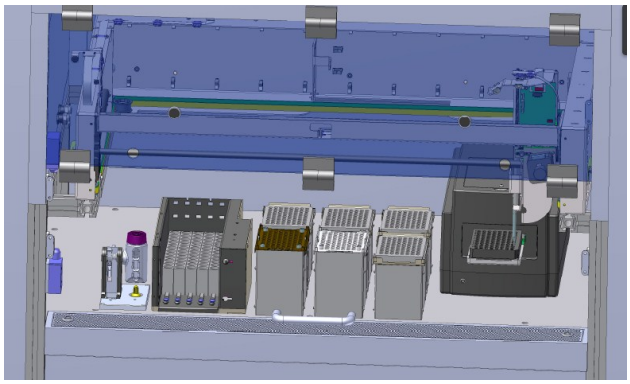

Image 1 DiagnoSYS platform for personalized chemosensitivity assays (CAD front view)

The first process step was to execute tissue homogenization. Therefore, the M-tube was rotated in the upside down position and the integrated cutter was actuated by a hub, which is directly connected to a motor. The cutting speed and direction for ovarian cancer biopsies for one cutting cycle had been set to 500 rpm for 30 s in each direction. Three cutting cycles were performed in total and with intermittent cycles of gentle stirring at 50 rpm. The total processing time was 4 hours at 37° C (Image 2). After generating a single cell suspension, the tube was rotated again and the cell suspension could be transferred to the cell separation unit. This automated process could be compared to the manual process of mechanical dissociation with a scalpel and using an enzymatic dissociation in the incubator with gentle mixing every 30 min. Other dissociation parameters remained the same.

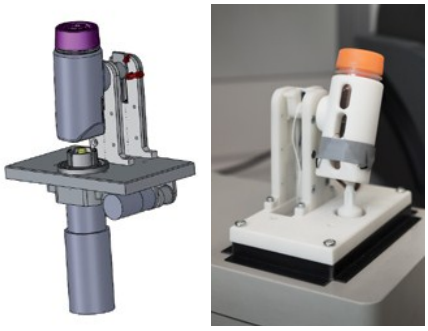

Image 2 First prototype of tissue processing unit with integrated heating device

Magnetic bead cell separation was executed in a module consisting of a modified LS-column (Miltenyi Biotec) using EpCAM positive and FSA negative selection. The cells had been checked for growth rates after marking them with beads using cells w/o beads as a control (Image 3). Hence, a seven day incubation of cells in a standard incubator (37° C, 5% CO_2, 95% rh) was chosen. After separation, cells were washed with CAM medium (DCS) and collected to determine the cell number. To make the platform affordable, a luminescence counter (Image 3) was integrated to count the ATP luminescence signal of cells rather than using a coulter counter or an optical system. Subsequently, the luminescence unit was calibrated with ATP yield signals of Collagenase treated and separated cells with a known concentration.

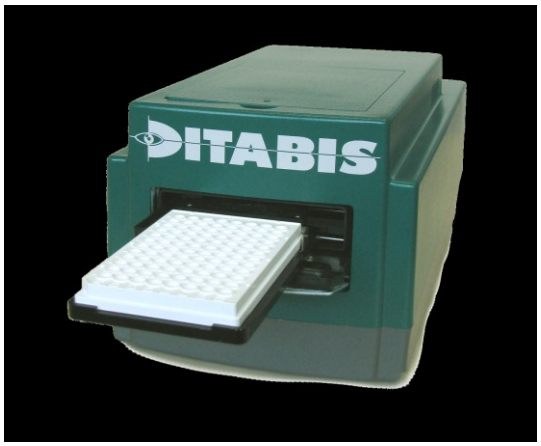

Image 3 Integrated luminescence plate reader

After determining cell numbers, 7500 cells per well were cultured in standard microwell-plates (Greiner Bio-One) in an external incubator (37° C, 5% CO_2, 95% rh) for 7 days in CAM medium (DCS). The luminescence signals of the incubated and purified cells were compared to cells that were cultured without magnetic bead separation.

3 Results

The automated DiagnoSYS process shows much improved results over manually performed chemosensitivity assays. Cell numbers after processing biopsies with the integrated

tissue processing module yields significantly higher numbers (Image 4).

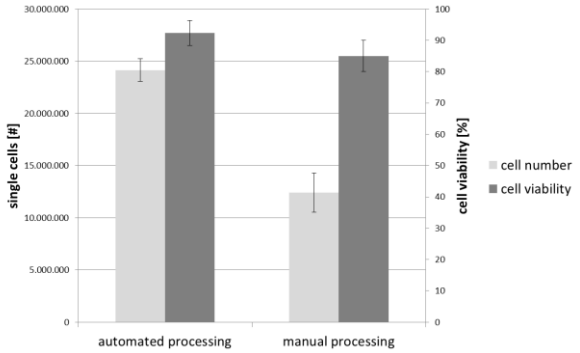

Image 4 Comparison of cells generated by automated and manual processing

3.1 Automated magnetic bead separation

Before performing the bead separation, the expression of EpCAM and FSP on primary ovarian fibroblasts from a non-cancer patient had been checked using EpCAM-FITC and FSP-FITC antibodies with the same antibody fragment used in EpCAM-Beads (Image 5). To determine the optimum volume of EpCAM-Beads for generating at least $2.16x10^6$ cells a polynomial fit of the concentration curve was used (Image 6). The optimum EpCAM-Bead volume is 25 µl: this volume was used to clean up cell suspensions from the automated tissue processing.

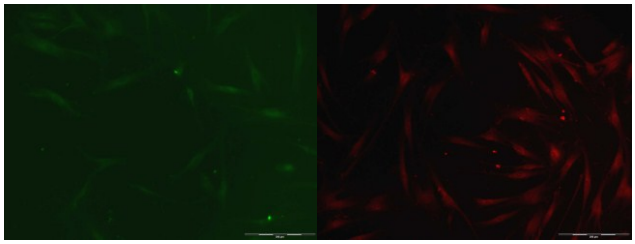

Image 5 Primary fibroblasts from ovarian cancer biopsies show low EpCAM (left) and higher FSP (right) expression

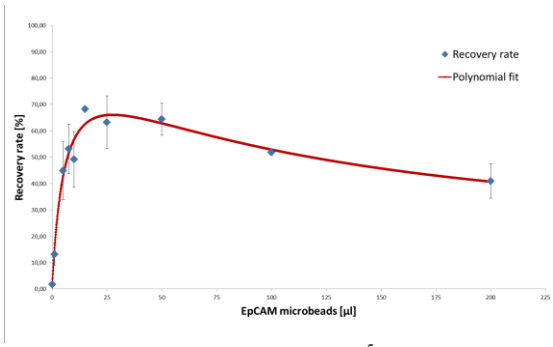

Image 6 Recovery rates of $5x10^5$ cells with EpCAM microbeads give an optimal bead volume of 25 µl

3.2 Luminescence based cell counting

The automated calculation of cell numbers after tissue processing and magnetic bead separation, the ATP status measurement exhibits more reliable results with kinetic analysis rather than end point measurement. End point measurement shows a high drift of RLU over time, and the luminescence signal is not as stable as it was stated in the chemosensitivity assay protocol (DCS) (Image 7). The kinetic analysis (Image 8) of the cell number calibration curve was used to determine the cell number of processed cells. Trypan blue staining was used to determine the viability of the cells. After tissue preparation and bead separation, a total cell number of $10x10^6$ cells and cell viabilities of 82% can be generated from a 1 g biopsy.

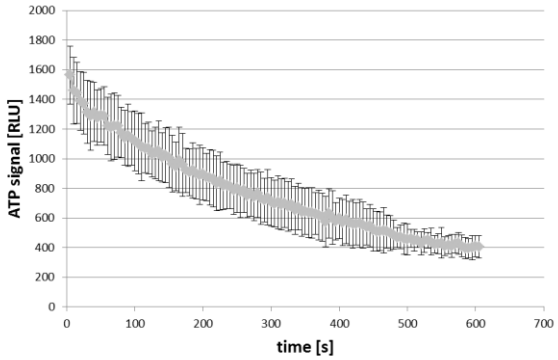

Image 7 Drift of ATP luminescence signal in cell number determination

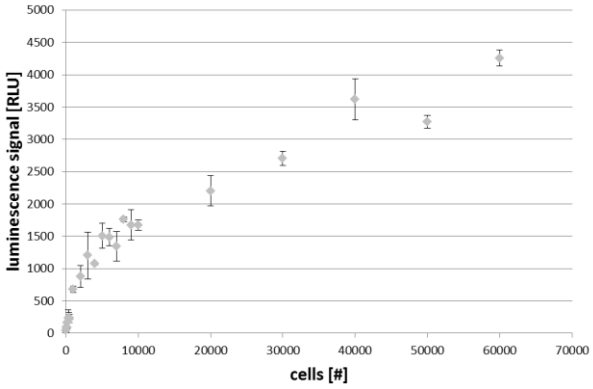

Image 8 Cell numbers could be determined by luminescence reading of ATP signal and kinetic analysis of data

3.3 ATP status measurement after incubation

To detect the signal to noise ratio of a manually prepared cell culture and the culture from the DiagnoSYS process, an ATP luminescence assay using Luciferin/Luciferase (DCS) was used. The cell cultures of pure cultures shows a very different ATP status compared to cultures that were prepared manually without bead separation. This increased noise derives from the higher standard deviation in non-purified cultures (Image 9). This result is also supported by the results from mixed cultures (MCF-7/primary fibroblasts) in 60/40 and 70/30 relation cultivated over 7 days.

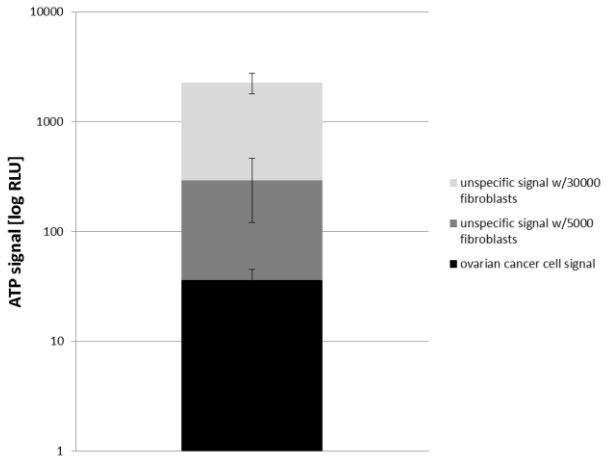

Image 9 Increasing numbers of fibroblasts in assay cause higher signal to noise ratios regarding the cancer signal

4 Discussion

It was shown, that automated tissue preparation resulted in higher yields of cell count as well as cell viability compared to the manual process. This was an important result, because more cells at the starting point of the assay increase the opportunities to test a large variety of cytostatic drug combinations. The magnetic bead separation used in DiagnoSYS had two advantages: unlike the manual process, it did not require centrifugation, making the system more affordable and the cells were purified, thus reducing the measurement noise. The latter is an important advantage, as the results from different cell culture mixtures showed that the noise was mainly caused by fibroblasts that are still alive in CAM medium after 7 days.

Even though there are no results from patients treated with drugs based on an automatically generated chemosensitivity assay, the signal to noise ratio minimization demonstrated that chemosensitivity testing could be more robust. This assay robustness may also be extended to other prognostic tests such as the uPA/PAI-1 assay.
For those assays, the DiagnoSYS system can simply be adapted using new modules.

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