SPECIAL APPLICATION NOTE
CyBi®-DROP 3D

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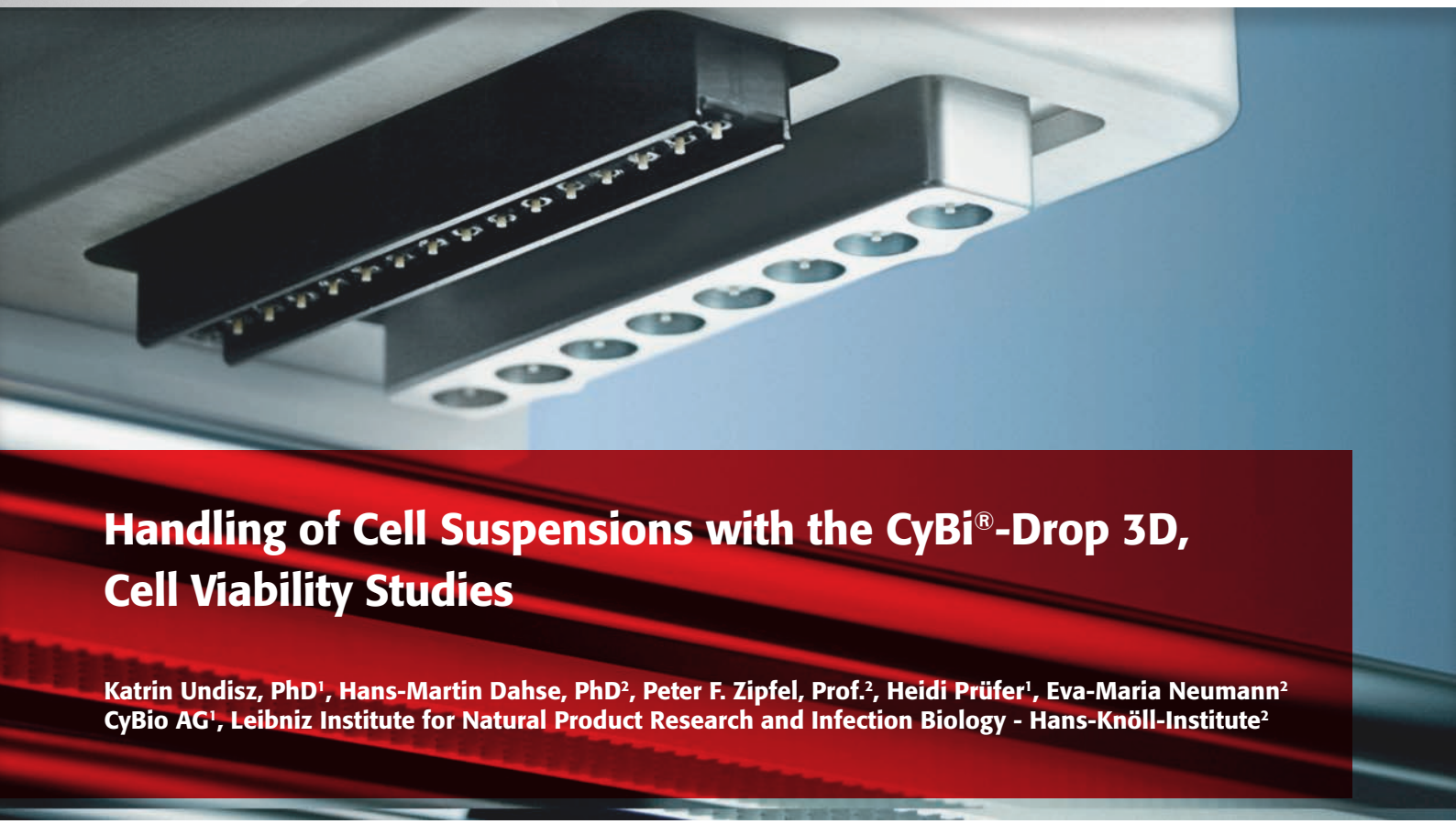

Handling of Cell Suspensions with the CyBi®-Drop 3D, Cell Viability Studies

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KEY WORDS

CyBi®-Drop 3D, K-562 Human Leukemia Cell Line, Homogenous Dispensing of Cell Suspensions with Recirculation, CASY® Cell Viability Control, CellTiter-Blue® Cell Viability Assay, Microscopic Examination

SUMMARY

The human leukemia cell line K-562 was used as model system to characterize the influence of the recirculation system and the dispensing pumps of the CyBi®-Drop 3D on the cell viability. Original cell suspensions from cell culture flasks were analyzed in comparison with cell suspensions following multiple recirculation and dispensing with the CyBi®-Drop 3D. The comparing cell viability studies were performed with the CASY® Cell Counter and Analyzer system (Innovatis), the CellTiter-Blue® Cell Viability Assay (Promega) and microscopic examinations (LSM 510 META, Zeiss). The cell appearance and metabolic activity remained stable over the whole examination period of 10 recirculation cycles.

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INTRODUCTION

The human leukemia cell line K-562 is used for a variety of antitumor tests as well as for pharmaco-dynamics, cloning, cytotoxicity and cell biology studies. The cells are growing monocytic and non adherent which are the ideal preconditions for cell handling studies with suspension cells.

In cellular screening applications the homogenous distribution of cell suspensions into microplates is a challenging tasks because of the high probability for cell sedimentation effects in the associated tube systems. The automatic recirculation function of the CyBi®-Drop cell handling combs enables the real time priming of the tube system with homogenous cell suspension without losing cells immediately before the dispensing procedure is started. Fig. 1 shows a detail of the CyBi®-Drop 3D with two cell recirculation dispensing combs. The aim of this study was to examine the influence of the cell recirculation and cell dispensing procedure on the cell appearance and the cell viability.

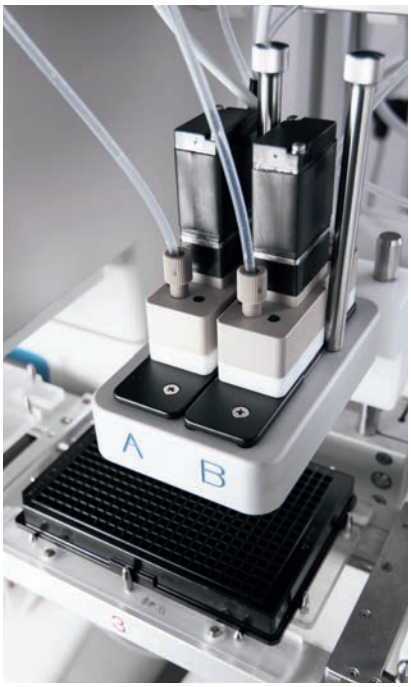


Fig. 1: : Detail of the CyBi®-Drop 3D with two cell recirculation dispensing combs, the recirculation valves on top of the combs allow a software controlled switching between pumping the cell suspension back into the storage flask or pumping it through the dispensing comb into the microplate.

INSTRUMENTS

- CyBi®-Drop 3D with 8-channel recirculation comb and PEEK nozzles controlled via CyBio Composer Software Version 1.2 (CyBio AG)
- CASY® Cell Counter+Analyzer System Model TT (Innovatis AG)
- Fluorescence Reader PolarStar (BMG Labtechnologies), filter set 544 nm (ex) and 590 nm (em)
- Laser Scanning Microscope LSM 510 META (Carl Zeiss AG)

MATERIALS AND REAGENTS

- K-562 (ACC 10)
- Flat bottom 96 well microplates PS (Greiner bio-one # 655 180)
- Cell culture flasks (NUNC, #156367)
- RPMI Cell culture medium (LONZA, #BE12-167F)
- Supplements:
- 10 mL / L Ultraglutamine 1 (LONZA, #BE17-605E/U1)
- 500 µL / L Gentamycin Sulfate (LONZA, #17-518Z)
- 10% heat inactivated fetal bovine serum (PAA, #A15-144)
- CellTiter-Blue® Cell Viability Assay (Promega, # G8080)

METHODS

Cell cultureK-562 suspension cells were cultivated in cell culture flasks, maintained at 0.1-0.5 x 106 cells / mL and split 1:3 to 1:5 every 3 days. The flasks were incubated at 37°C with 5% CO2.
The start cell concentration for the tests was adjusted with the CASY® to about 6 x 105 cells / mL.

Cell circulation and dispensing procedure
In our cell dispensing experiments, the volume of the connecting tube between the storage flask for the cell suspension and the recirculation valve of the dispensing comb was 2.5 mL. The liquid volume within the division system of the 8-channel comb was 0.35 mL. The working volume was adjusted to 5 µL per stroke and channel. To ensure a real time priming of the whole tube system 65 pump strokes were performed with the lowest possible pump speed of 20 rpm to handle the cells as careful as possible during the recirculation step. In the next step, 10 strokes were dispensed into the waste to refill the division system within the 8-channel comb with homogenous cell suspension and finally the dispensing into the 96-well microplate was started immediately (20 x 5 µL per well). For the dispensing steps, the pump speed was increased to 500 rpm to ensure a separation of the droplets. For every experimental condition, 2 columns of a 96-well microplate were dispensed to perform the CellTiter-Blue® cell viability assay and additionally a sample of 1 mL was taken for CASY® cell viability analysis and microscopic examination of the cell appearance. The cell suspension was analyzed following 1, 3, 5 and 10 recirculation cycles. The original cell suspension was used as control.
CASY® cell viability analysis
The CASY®-technology is a well accepted method for cell counting and cell viability analysis (1). In our experiments, the particle size distribution of each cell suspension sample was analyzed in the range from 0 µm to 30 µm. The mean diameter of intact K-562 cells is about 16 µm. Unspecific particles and cell debris usually have a mean diameter of about 5 µm. If cells are destroyed, the remaining nuclei have a mean diameter of about 10 µm. The cell damage was simulated by addition of 20 % ethanol and the characteristic profile was measured as control. If cell suspensions after multiple circulation cycles still show a comparable particle size distribution, this would indicate an vital cell population. Cell damage would result in an increase of the nuclear fraction with a peak at about 10 µm.

CellTiter-Blue® cell viability analysis
The CellTiter-Blue® cell viability assay provides a homogenous, fluorometric method for estimating the number of viable cells present in microplates. Viable cells retain the ability to reduce the indicator dye resazurin into the highly fluorescent resorufin. Nonviable cells rapidly lose this metabolic capacity and thus do not generate a fluorescent signal (2). Following the instructions of the CellTiter-Blue® cell viability assay protocol 20 µL CellTiter-Blue® reagent were added to each well of the sample microplate and the fluorescence signal was measured after 4 h incubation at 37°C with 5% CO2. The fluorescence signal of the original cell suspension was used as control (100%).

Microscopic estimation of the cell appearance
The cell suspension was analyzed microscopically following 1, 3, 5 and 10 recirculation cycles. The original cell suspension was used as control.

RESULTS AND DISCUSSION

Fig. 2 shows the CASY® 2D overlay of the particle size distribution of the original cell suspension and the ethanol treated cell suspension. The mean diameter of K-562 cells was about 16 µm.

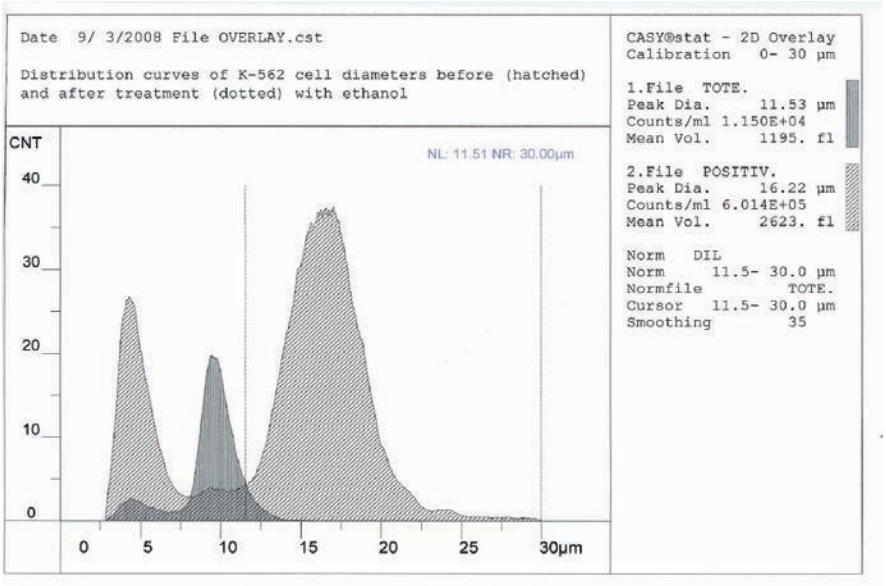


Fig 2: CASY® 2D overlay of the particle size distribution of the original K-562 cell suspension (hatched) and the ethanol treated cell suspension (dotted).

Treatment with 20% ethanol resulted in a cell bursting, the main particle fraction disappeared and the new main peak with a mean diameter of about 10 µm represents the cell nuclei.

In Fig. 3, the CASY® 2D overlay of the particle size distribution following 1 and 10 recirculation cycles is shown.
Both particle size profiles are comparable, no additional peak in the range of 10 µm appeared in the cell suspension after 10 recirculation cycles which would indicate damaged cells. It has to be pointed out, that the cell recirculation was performed with the lowest possible pump speed of 20 rpm which on the one hand ensures the priming of the tube system with homogenous cell suspension and on the other hand prevents cell damage.

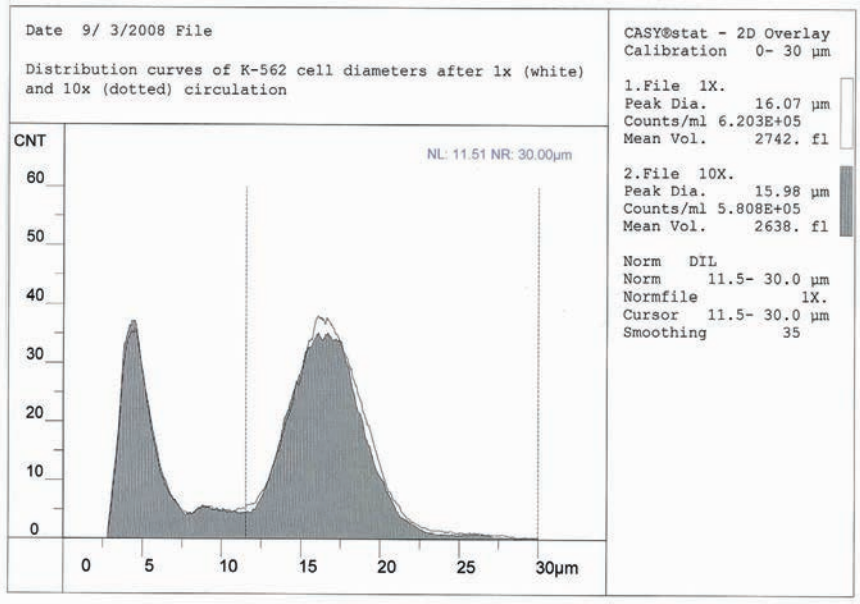


Fig 3: CASY® 2D overlay of the particle size distribution of the K-562 cell suspension following 1 (white) and 10 (dotted) recirculation cycles.

Furthermore, the viability of the cell suspension was analyzed following 1, 3, 5 and 10 recirculation cycles with the CellTiter-Blue® assay. The fluorescence signal of the original cell suspension was used as control (100%), increasing numbers of nonviable cells would result in a decrease of the fluorescence signal. The results are summarized in Fig. 4. The metabolic activity of the K-562 cells remained stable over the whole examination period of 10 recirculation cycles.

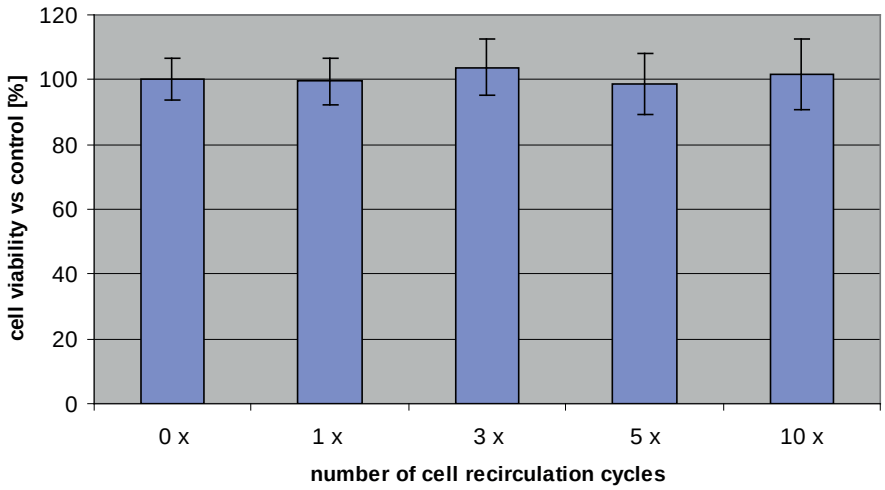
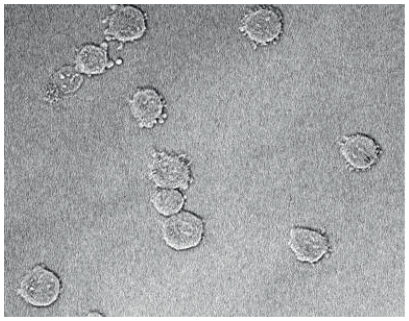


Fig 4: Cell viability of the K-562 cell suspension following different numbers of recirculation cycles with the CyBi®-Drop 3D, CellTiter-Blue® assay, 4h incubation at 37 °C and 5% CO2, mean fluorescence signal of the original cell suspension (0x) minus medium blank = control (100%), n=16.

Additionally the cell suspension was analyzed microscopically following 1, 3, 5 and 10 recirculation cycles in comparison to the original cell suspension. Fig. 5 shows a microscopic picture of the K-562 cell suspension after 10 recirculation cycles. No changes in the cell appearance could be observed in comparison to the original cell suspension.



These results demonstrate, that the cell appearance and metabolic activity of K-562 suspension cells remained stable over the whole examination period of 10 recirculation cycles and subsequent dispensing with the CyBi®-Drop 3D. The flexible adjustment possibility of the pump speed allows a most careful recirculation and homogenous dispensing of cell suspensions.

Fig 5: K-562 cell suspension following 10 recirculation cycles with the CyBi®-Drop 3D, pump speed = 20 rpm, mean cell diameter = 16 µm, LSM 510 META.

REFERENCE

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