APPLICATION NOTE

Handling of Adherent Cells with the CyBi®-Drop 3D, Example Data

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Key words

CyBi®-Drop 3D, Non-contact Dispensing, Dispensing on Adherent Cell Monolayers in Microplates

Introduction

The CyBi®-Drop 3D offers fast and accurate non-contact dispensing of up to four reagents to microplates. Especially designed dispensing combs with 8 or 16 channels made from stainless steel (nozzle diameter 250 µm) or PEEK (nozzle diameter 380 µm) are available for different applications (Fig.1).

In cellular assays, the dispensing of assay reagents or media into the wells of an assay plate with an adherent cell monolayer on the well bottom is often required. The complete preservation of the cell monolayer is an essential precondition to ensure the correct evaluation of the assay results.

Both CyBio® Software versions CyBio® Composer 2.13 as well as CyBio® Control 3.60 enable the flexible adjustment of the pump speed, the dispensing volume per stroke and the stroke number per well.

In our experiments we defined the optimal dispensing conditions which prevented the disruption of a CHO cell monolayer in 96 well microplates via optimizing these parameters and subsequent stereo microscopic analysis.

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Figure 1: CyBi®-Drop 3D with one 16 channel comb (A) and one 8 channel comb (B).

Instruments and Materials

- CyBi®-Drop 3D with 8 channel comb, stainless steel nozzles
- Stereo Microscope ASKANIA MZM 1 (Askania Mikroskoptechnik Rathenow)
- CHO-K1 cell line
- Flat bottom 96 well microplates PS transparent (Greiner bio-one # 655 180)
- Cell culture flasks 75 cm^2 (Fischer Scientific # 430641)
- DMEM/F-12 Cell Culture medium (Fischer Scientific # 31331-028)

  Supplements
  - Penicillin-Streptomycin-Solution (Fischer Scientific # 15140-122), 1%
  - Fetal Bovine Serum (Sigma # F 9665), 10%
- Trypsin-EDTA (1x) (Gibco # 25300-054)
- Phosphate Buffered Saline, pH 7.4 (PBS, Sigma # P3813-10PAK)

Methods

Cell culture

CHO-K1 cells were cultivated in cell culture flasks at 37°C and 5% CO_2. The adherent cells were split 1:4 every 2 or 3 days. The cell number for seeding into a microplate was adjusted to about 10^5 cells/ml and the microplates were inoculated with this cell suspension (100 µl per well). The microplates were incubated at 37°C and 5% CO_2 for 24 hours. The confluency of the monolayer was checked microscopically as precondition for the dispensing experiments.

Dispensing on adherent cells

The purpose of this experiment was to optimize the dispensing parameters for the addition of further assay reagents with the CyBi®-Drop 3D into wells of a 96 well microplate plate containing 100 µl cell culture medium and an adherent monolayer of CHO-K1 cells, without disrupting the cell monolayer (e.g. scattering the cells to the sides of the well). Several dispense volumes (10 µl, 5 µl and 2 µl) and shot numbers per well (1, 2 and 5) were tested with a moderate pump speed (300 rpm) and 3 microplate colums per experimental condition. The evaluation of the preservation state of the cell monolayer after the dispensing was done microscopically.

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Results and Discussion

The stereomicroscopic pictures following the addition of cell medium with the CyBi®-Drop 3D with different dispensing parameters are shown in Figure 2 A - D.

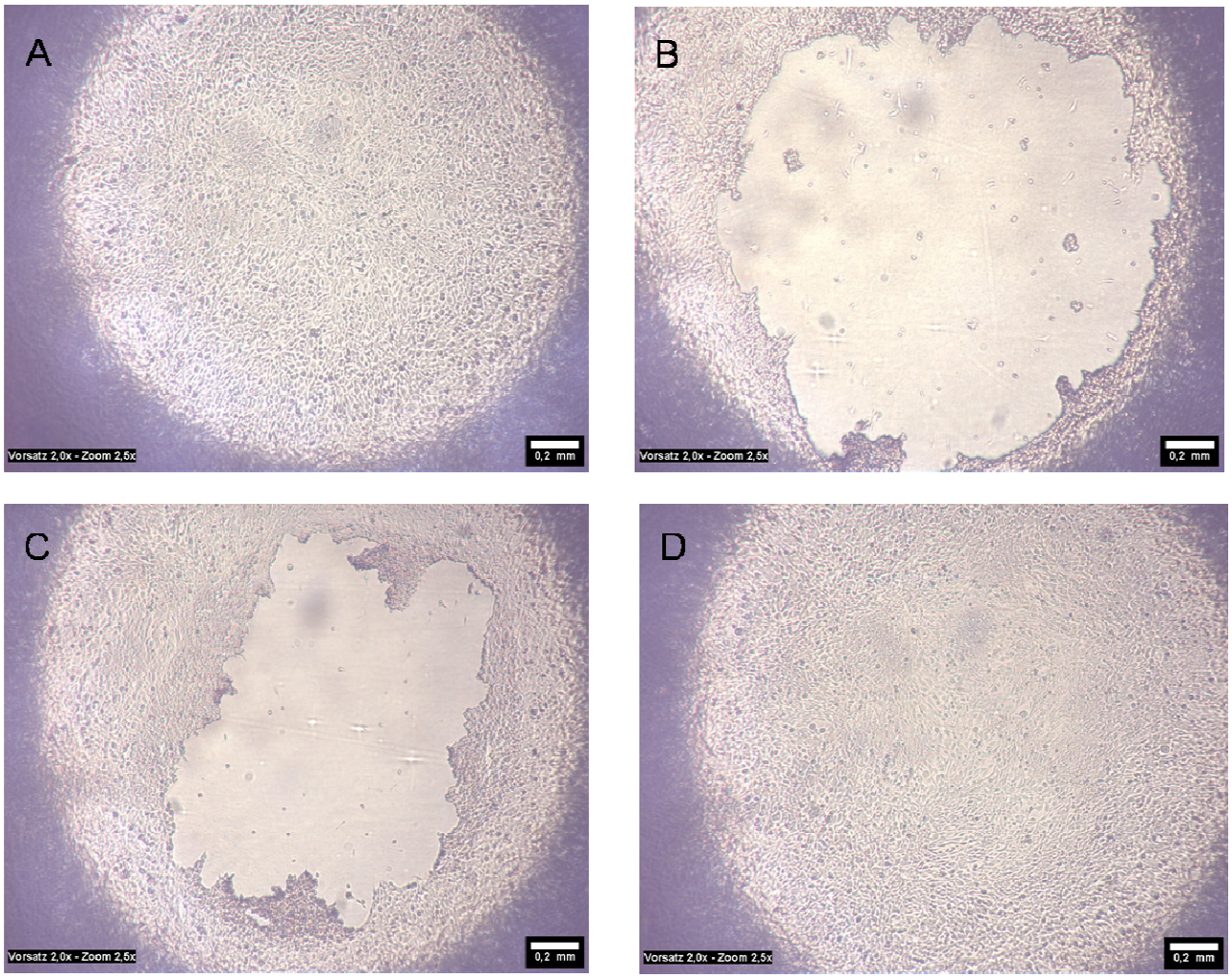

Figure 2: Stereomicroscopic pictures following the addition of cell medium with the CyBi®-Drop 3D (pump speed 300 rpm)

A: Confluent cell monolayer after a cultivation time of 24 h before dispensing

B: Cell monolayer following dispensing of 10 µl in one shot

C: Cell monolayer following dispensing of 2 x 5 µl

D: Cell monolayer following dispensing of 5 x 2 µl

Considering the CyBi®-Drop 3D evaluation results, which were obtained in comparable dispensing experiments on HEK293 cell monolayers in 384 well plates in the HTS Solutions Laboratory from Invitrogen (1), our dispensing experiments generally were done with a moderate pump speed setting of 300 rpm. For the dispensing on very sensitive adherent cell monolayers the pump speed could be further reduced to 200 rpm.

While dispensing 10 µL cell medium in one stroke into the wells of a 96 well microplate containing 100 µl medium and an adherent monolayer of CHO-K1 cells, the cell monolayer could not be preserved completely (see Figure 2 B). Following dispensing two strokes of 5 µl medium the disruption area at the well bottom became smaller (see Figure 2 C). Following dispensing 5 times 2 µl medium the cell monolayer remained completely preserved (see Figure 2 D).

These results agree with CyBi®-Drop 3D evaluation data, which were obtained in comparable dispensing experiments on HEK293 cell monolayers in 384 well plates in the HTS Solutions Laboratory from Invitrogen (1).

Generally, dispensing tasks on adherent cell monolayers with the CyBi®-Drop 3D should be performed with low pump speed and multiple shots of low dispensing volume units. Both CyBio® Software versions CyBio® Composer 2.13 as well as CyBio® Control 3.60 allow the flexible adaptation of all these dispensing parameters to the customer specific application.

Further the use of dispensing combs with 380 µm PEEK nozzles may contribute to an additional reduction of the mechanical stress on the cell monolayer during dispensing, which would be recommended for very sensitive adherent cell applications.

References:

1. Lasky, D., Grevelis, H., and Ahrweiler, P., "Customer Evaluation of the CyBi®-Drop 3D for Screening Purposes", Application Note CyBio AG, www.cybio-ag.com