Non-Contact Cell Spotting in the Low Nano Liter Range Using a Novel Dispensing Technique

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Motivation

A very important issue of cellular biotechnology e. g. tissue engineering is the analyses of cell-cell interaction and a defined pooling of different cell types to mimic a certain environment like present in different tissue or a stem cell niche. Therefore, a local controlled deposition of living cells is required. Here, we present a method which enables a defined positioning of different cell lines using a dispensing technique (M2 Dispenser).

Dispensing Technique

The M2-Dispenser (M2MD) contains a shockwave generator and a modulator. The shockwave generator includes a special version of a miniaturized solenoid valve. The M2MD is actuated with a modified electrical pulse consisting of two sections with a different, but constant amplitude. The first part of the pulse is called “pulse duration” and the total pulse width is called “pulse duration hold”. The total pulse opens the solenoid valve for the specified pulse duration hold. If it is very short, the valve will only partially open with the consequence that the flow resistance is higher than compared to a fully open valve. Consequently, the pulse duration peak is an important parameter for tuning droplet ejection. The amplitude during the peak duration is 24 V. This voltage is required to open the valve. As depicted in figure 1, optimizing these parameters with the used fluid can result in droplets as little as 20 nL. The biggest advantage of this dispensing technique is the capability of dispensing liquids with high viscosities, without causing a lot of shearing stress. Therefore, using this non-contact dispensing method is the perfect fit for cell spotting applications.

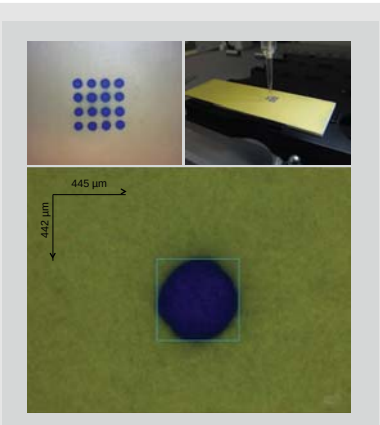

Fig. 1: Spotting with the M2-Dispenser

Droplet Formation:

The figure to the left illustrates the droplet formation on water sensitive paper. Using disposable tips with an orifice of 200 µm allows the deposition of droplets with a feature-to-feature distance of 700 µm or less. This distance largely depends on the surface characteristics of the used substrate and the physico-chemical characteristics of the used solution.

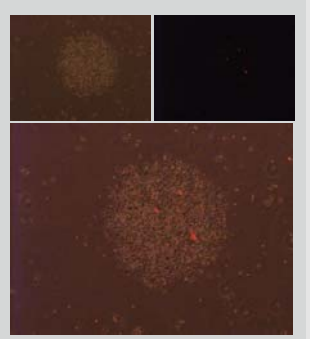

Fig. 2: Dead Staining (Propidium iodide) of CHO Cells

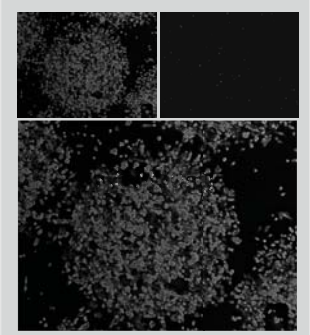

Fig. 3 Dead Staining (Propidium iodide) of MDA Cells

Spotting of Different Cell Lines

In order to test whether cells survive the dispensing process, we used CHO (Chinese hamster ovary) cells and spotted a cell suspension on a cell culture dish. Spotting of cells was conducted at standard conditions (RT, 40% humidity). The whole spotting process lasted 1.5 minutes for a 10x10 pattern. Due to the characteristics of the culture dish and the cell culture medium the spots have a diameter of 800-900 µm. This resulted in a feature-to-feature distance of 1000 µm. The generated spots on the surface were homogenous in shape, arranged in the spotting grate and contained cells. No further modifications to pulse duration or pulse duration hold were needed. The dish was incubated at 37 °C and 5% CO_2 for one hour for allowing the CHO to adhere to the surface of the cell culture dish. Subsequent the complete dish was filled with cell medium to ensure a sufficient nutrient supply. A dead staining using propidium iodide (PI) afterwards showed that more than 95% of the cells survived the spotting process (Fig. 2). The second tested cell line was the human mammary gland (MDA) cell line. Those cells were more delicate in handling, hence we were able to spot these cells with a survival rate of 95% (Fig. 3) as well.

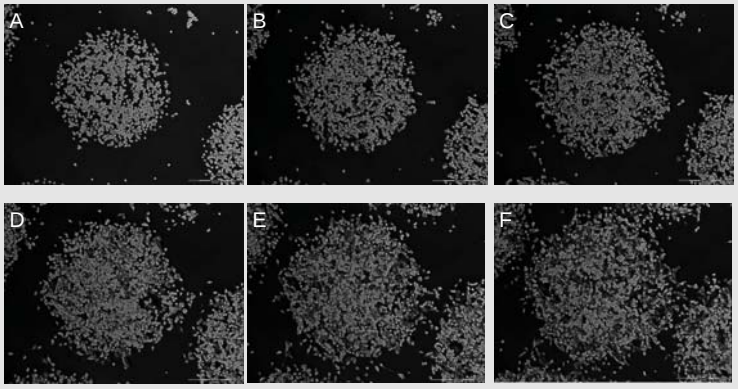

Fig. 4: The figure illustrate the cell growing process of the cells after spotting starting at t = 0h (A), 8h (B), 16h (C), 24h (D), 32h (E) and 40h (F) of cultivation.

Analysis of cell growth after spottting:

Furthermore, we investigated the cell behavior in the spots by microscopic observation for 40 hours (Fig. 4 A-F). Over time the cells migrate and proliferate until an interaction between the individual cells spots occurred. The linear increase in the spot diameter can be analyzed (Fig. 5). The slope of the diameter increase can be used as reference to compare different relevant chemicals which may influence the cell behavior or the invasion potential of different types.

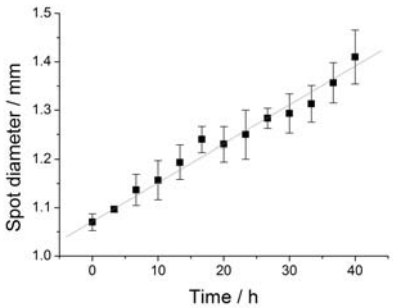

Fig. 5: Increase of the cell spot diameter in dependence of cell culture time.

Acknowledgements

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