Application Note MMI-CC-002 – Living Cells

Selective Isolation of Living Cells for Re-cultivation

Nils Körber[1], Heide Marie Resch[1], Stefan Niehren[1]

[1]Molecular Machines & Industries GmbH, Eching, Germany

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Abstract

The separation and isolation of single cells is an essential pre-requisite for homogenous cell culture populations. Many research projects in cell biology, immunology, and stem cell research rely on pure and reproducible cell culture conditions.

In this study, we demonstrate how to easily apply the MMI CellCut system to isolate individual living cells directly into culture dishes for subsequent re-cultivation to obtain a highly homogenous cell population.

Figure 1: MMI CellCut system on the Nikon Ti2-E inverted microscope. The system is compatible with many microscope brands and models and can be combined with the MMI CellEctor microcapillary system to isolate cells from suspension.

Introduction

The principle of cell culture was established in the late 19th century with first experiments to keep cells from explants alive for several days [1-3]. In the 1940s and '50s, the methods were optimized for the development and production of vaccines, such as the vaccine against Polio [4]. To date, countless research projects worldwide have been conducted using cell culture.

In recent years, many methods to isolate single cells have been developed for tissue sections and for cells in suspension. However, most of these methods lack the ability to select specific individual cells. In addition, most technologies are limited to dead or fixed cells, whereas methods to gently isolate living cells under sterile conditions from (primary) cell culture are still rare. Many procedures to generate homogeneous cell cultures from selected cells are laborious and difficult to perform under physiological and sterile conditions. Cells are therefore less likely to grow and divide into new cells and the risk of contamination makes these processes unreliable.

The MMI CellCut was initially developed for the selective isolation of single cells or cell clusters from tissue sections using laser microdissection (Figure 1). Its functionality and proficiency have been validated in

numerous publications using fresh frozen or FFPE material in various research areas such as oncology, pathology, immunology, forensics, and crop science [5-9].

Here, we demonstrate that the MMI CellCut, in combination with the MMI LiveCell Chamber, can efficiently be employed to isolate single living cells to initiate a homogeneous cell population. This study displays the semi-automated workflow to isolate single cells from the MMI Membrane Ring into a MMI Isolation Dish in a sterile and contamination-free way. During this process, the cells will fully remain in physiological cell culture conditions.

Material and Methods

HeLa cells were cultivated in Dulbecco's Modified Eagle's medium (DMEM), supplemented with FBS, non-essential amino acids, L-Glutamine and Penicillin-Streptomycin at 37 °C and 10 % CO_2. 2 ml of a cell suspension with 1x10^4 Cells/ml were transferred into the membrane ring of the MMI LiveCell Chamber (Product Number 50301) and grown for 48 h.

For single cell isolation, 1.5 ml of medium were removed from each well under a laminar flow hood. The MMI Membrane Ring was placed into the MMI Isolation Dish which is coated with optically clear silicone to ensure adhesion of the membrane to the bottom of the petri dish and sealed. This set-up was then transferred from the hood onto the microscope stage.

Single cells were selected, cut, and isolated using the MMI CellCut mounted on an inverted microscope. The MMI CellCut was equipped with the MMI PicoCut Universal laser for precise laser cutting.

After cutting, the sealed MMI LiveCell Chamber is opened under sterile conditions in the fume hood to carefully remove the MMI Membrane Ring with a tweezer. The MMI Isolation Dish contains the individual selected cells whereas the MMI Membrane Ring retains the non-selected cells and can be discarded.

Figure 2: MMI LiveCell Chamber. A) MMI Membrane Ring with petri dish: the cells can be grown on the membrane for subsequent laser microdissection experiments. B) MMI Isolation Dish: the bottom of the chamber is coated with an optically clear adhesive silicone enabling capture of microdissected cells. C) MMI Membrane Ring inside MMI Isolation Dish: the membrane ring with the cells is placed into the isolation dish and transferred to the laser microdissection system for selection and isolation of single living cells.

Results

The MMI LiveCell Chamber is specially designed for live cell laser microdissection (Figure 2). The MMI Membrane Ring consists of a metal or plastic ring spanned with the PEN-membrane traditionally used for standard MMI Membrane Slides. To ensure good cell growth conditions, the membrane can also be pre-treated with poly-L-Lysine or similar substances.

The membrane rings hold a volume of about 2-3 ml to allow cells to be in culture for several days.

To obtain precise and repeatable results, a MMI LiveCell Chamber filled only with medium was used to optimize laser focus, speed, and power within the MMI CellTools software. The same settings were then used for subsequent experiments.

Figure 3: HeLa cell being selected using the interactive pen screen on the MMI CellCut.

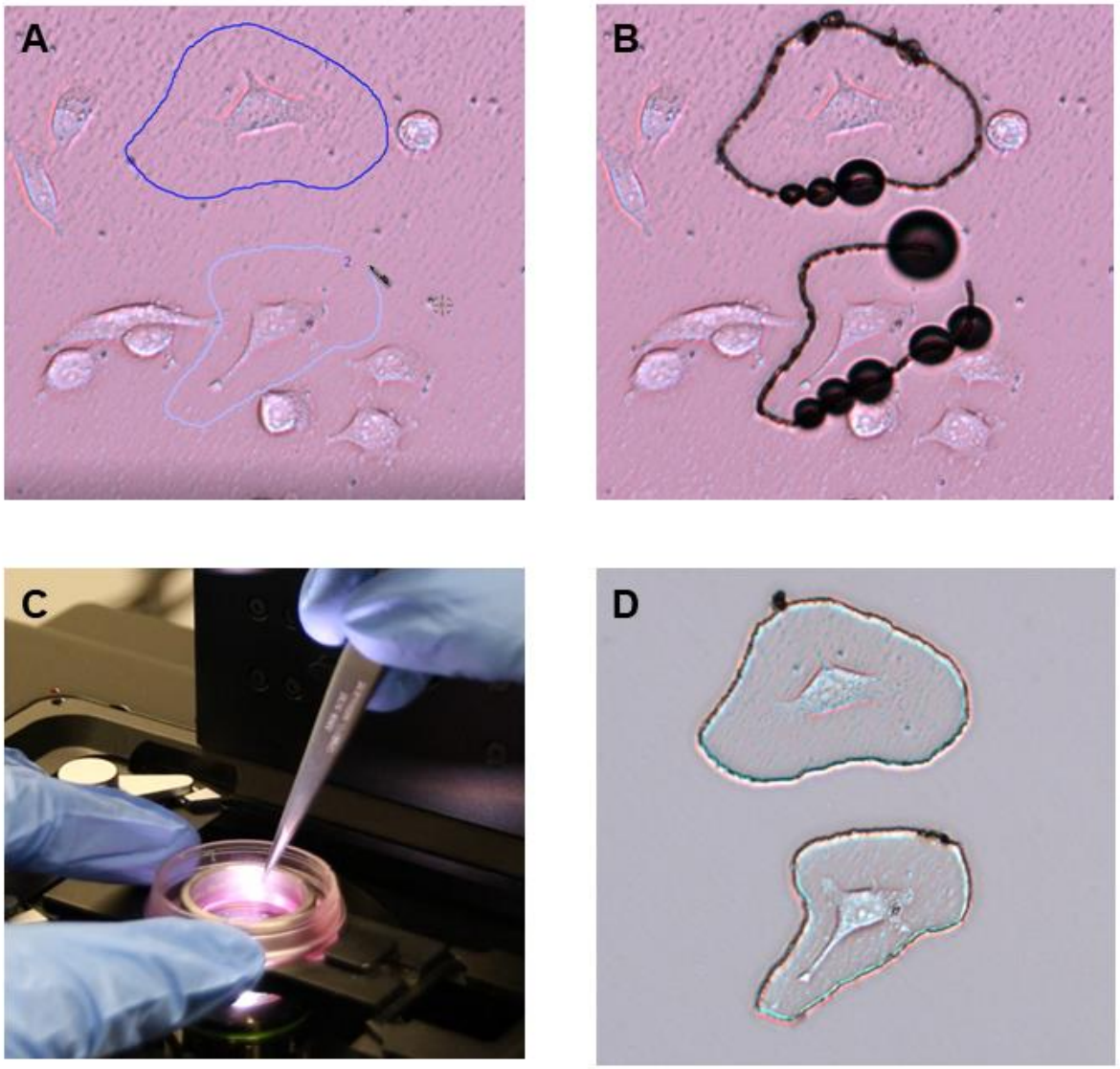

Figure 4: Workflow isolating a single living cell. A) Single cells are marked. B) The laser precisely cuts the membrane around each cell along the marked line. C) The MMI Membrane Ring is lifted with a tweezer. D) The dissected cells adhere to the MMI Isolation Dish and can be inspected under the microscope.

Before cutting, the membrane ring is inserted into a sterile MMI Isolation Dish in a cell culture hood to maintain sterility. The closed MMI LiveCell Chamber then goes onto the microscope stage of the MMI CellCut system.

Employing the powerful and easy to use MMI CellTools software, the sample can be inspected and searched for the target cells to be isolated (Figure 3).

Within the software, single cells can be marked and the “PicoCut” laser precisely cuts the membrane around each cell of interest (Figure 4). The cutting process is not compromised by liquid being present in the dish, thus enabling cell isolation in physiological medium conditions. Therefore, the cells are gently treated and the cells of interest can be re-grown after the isolation procedure.

After cutting, the MMI LiveCell Chamber is transferred to the fume hood. Using tweezers, the membrane ring is removed from the isolation dish and fresh medium is added to the dish. The dishes are placed back into the incubator or alternatively to a live cell imaging system including incubation chamber to control environmental conditions such as temperature, CO_2 and humidity.

Discussion

In this study, we demonstrate that the MMI CellCut system is able to isolate living cells maintained in cell culture conditions. The isolated cells can then be kept in the incubator to form a new homogenous cell culture or they can be subjected to live cell imaging experiments.

With this new cell isolation procedure, the MMI CellCut expands its application range beyond dissection of tissue sections to include research based on primary cell cultures as well as co-cultured cells.

For more information on the workflow, watch the short video here:

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