Standard Operating Procedure

TU/e

SOP details

Title	Cell seeding onto the TopoChip
Description	This SOP describes how cells are seeded onto the TopoChip
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SOP number	2.2
Version number	1

	Name	Date	Signature
Prepared	Phani Krishna Sudarsanam	07-05-2020	
Reviewed	Jan de Boer	18-3-2021	
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Version changes

Version	Name	Date	Changes made
1	Phani Krishna Sudarsanam	07-05-2020	Made in TU/e
2	Jan de Boer	16-4-21	Track changes
3			
4			
5			

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1 Purpose

This SOP describes how to seed cells and achieve homogeneous distribution of them on TopoChips.

2 Principle

TopoChip seeding is performed by immersing a TopoChip in medium in a 6-well plate. When cells are seeded in a normal well plate, they will sink to the bottom of the well by gravity, but cells tend to cluster in the middle of the well. Moreover, not only the top but also the bottom of the TopoChip can be colonized by cells and thus interfere in image analysis, To obtain equal distribution of cells across the whole chip and cells only on the patterned side, TopoChips are placed in the well of a 6-well plate immersed in medium overnight without air seeping beneath to avoid floating before seeding the cells. Cells are seeded with the TopoShake protocol to achieve homogeneous cell distribution across the chip.

3 Before You Start

This SOP can be used in principle for any kind of TopoChip screen. Before using this SOP, one should be familiar on how to perform cell culture experiments such as expansion of cells in culture flasks or well plates, and should know how to optimize the cell seeding densities as this may differ from cell line to cell line. Furthermore, the seeding protocol needs to consider the number of cells/cm^2 based on the specifics of the screen to be performed. It may vary depending on the bioassay chosen for the screen. For TopoChip screenings, 5,000 cells /cm^2 is a good starting point to obtain a sub confluent cell population across the TopoChip.

4 Required materials

4.1 Workplace

This SOP can be performed in the Cell lab (Gemini-Zuid 4.01 & 4.02). Follow the safety protocols instructed by the lab managers and perform the experiment accordingly in allocated locations in the lab.

4.2 Equipment and disposables

- Class II-biological safety cabinet
- Incubator with 5% CO_2 and set to 37 °C
- Conical flask for waste with chlorin tablets
- Biohazard waste bag and standard
- Paper tissues
- 6 well plate (Gibo, Cat. No: 662160)
- Micropipettes
- Sterile 1.5 ml Eppendorf tubes
- Sterile pipette tips (1000 µl)

4.3 Reagents

70% EtOH for sterilization
Phosphate Buffered Saline (Sigma, Cat. No: D8537)

5 Procedure

5.1 Working procedure

The surface area of a 6-well plate is 9.6 cm^2. The volume of medium used for seeding in each well is 2 ml. Cell seeding is done after sterilization of the TopoChips, as described in SOP 2.1.

Seeding

1. After sterilization, put the TopoChip in a well and add 2 ml of the corresponding cell culture medium. The TopoChip may float up due to air bubbles in the medium, and sometimes because of the chemistry of the chips.
2. Using a sterile pipette tip, the TopoChip is slowly pressed down on the edges, while avoiding to touch the patterned areas, to the bottom of the well plate thus letting the air escape beneath the Topochip. Air trapped beneath the TopoChip will lead to floating.
3. The well plate is labelled and kept in the incubator and not disturbed overnight to let the Topochip settle down to the bottom. This step is important to make sure the cells are not seeping beneath the TopoChips upon seeding.
4. Next day, 500 µl of the medium is aspirated out of the well from the 6-well plate which was incubated overnight with the TopoChip. This is done to not let any air get into the medium as it will unsettle the TopoChips in the wells.
5. 500 µl of medium containing the required number of cells is prepared in a sterile 1.5 ml Eppendorf tube.
6. Just prior to seeding, the cell suspension from the tube is homogenized by carefully pipetting three times up and down slowly.
7. Now the cell suspension is pipetted at an angle of 45° from the top right corner of the well onto the right corner of the TopoChip for an even distribution. This is done to avoid having clusters of cells attached in the middle of the TopoChip
8. Next comes the TopoShake, the movement with which you achieve a more or less homogeneous distribution of cells on the TopoChip. The well plate is slowly tilted in clockwise direction 360° from top left corner to bottom left corner in a continuous motion for three times. This movement is meant to avoid clustering of cells in the middle or on edges of the TopoChip and to get even distribution of cells across the well.
9. The well plate is now carefully transported into the incubator until further use.

Tips : During the whole process, it important to make sure the TopoChips do not float as it may hamper the even distribution of cell across the surfaces of the TopoChip. To avoid this, the TopoChips are

plasma oxygen treated after the fabrication (SOP 1.2). Once TopoChips are submerged in medium, medium should never be completely aspirated because that will lead to air bubbles between the well surface and Topochip.

5.2 Safety.

Work in the Cell lab in Gemini-Zuid according the safety regulations. Follow the instructions given in the lab introduction by the lab managers

6 Waste

When working in the cell lab, handle waste according to guidelines which are labelled at the waste disposal based on its categories as given below in the table 1

7 References

SOPnr	Title
2.1	Sterilization of Topochip

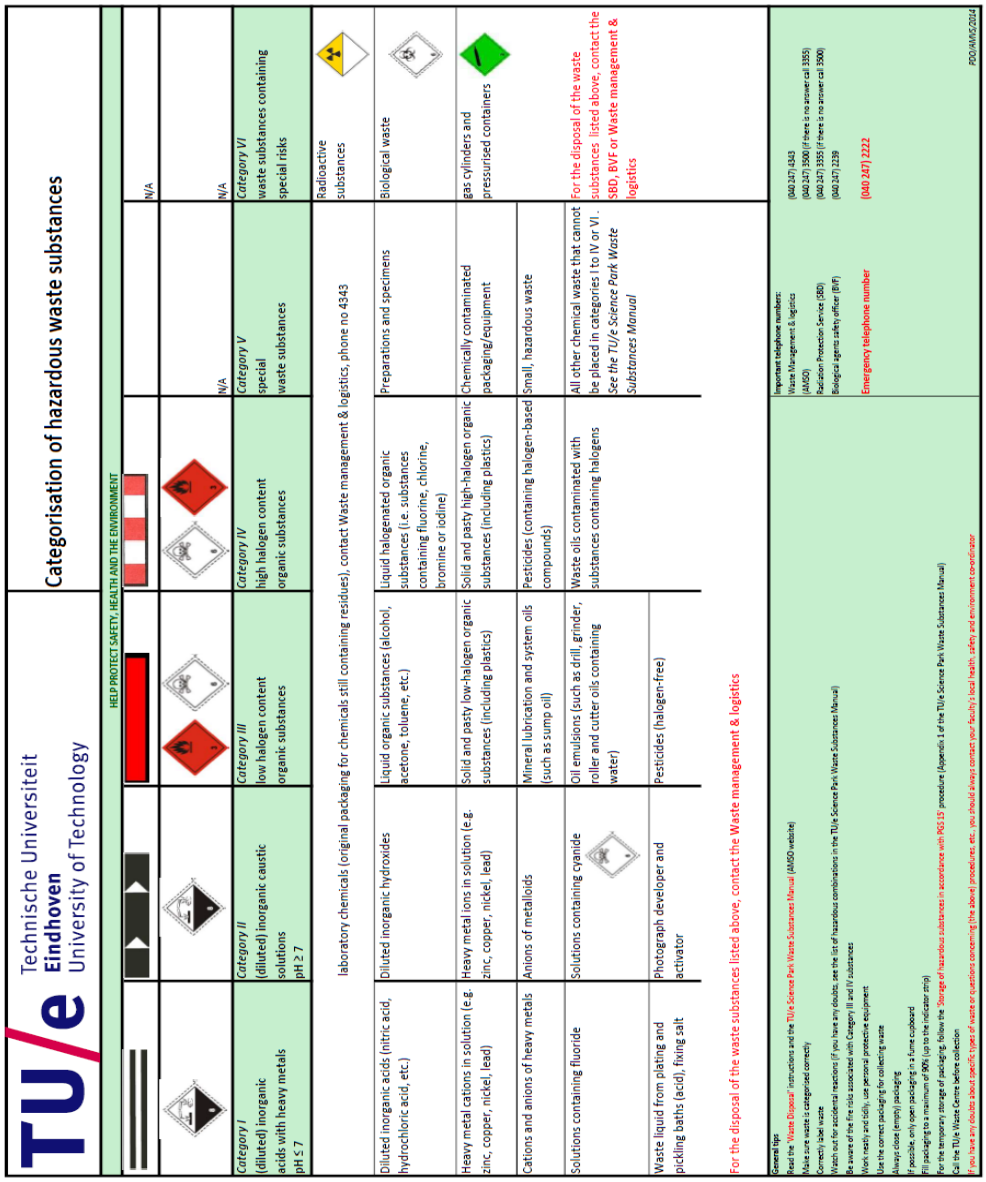

Table 1: Categories of hazardous liquid waste