FluidFM BIO SERIES

CELL VIABILITY ASSESSMENT

Cytosurge AG, 12 November 2019

1. TABLE OF CONTENTS

1. TABLE OF CONTENTS 2
2. INTRODUCTION 3
3. MATERIALS 4
4. PROCEDURE 4
5. TROUBLESHOOTING 6

2. INTRODUCTION

Cells are an extremely well-balanced chemical system, in which subtle modification can rapidly lead to cell death. Breaking the membrane and disturbing cell homeostasis by bringing new compounds into it can become a high source of stress. Performing nano-injection is therefore not trivial if you wish to maintain cell viability at a high level.

To achieve high rate of successful nano-injection with high cell viability, several parameters need to be controlled:

Use appropriate medium: To maintain cell homeostatis, cell medium must be properly balanced and provide every essential nutrient. Using the wrong medium will lead to severe cell death, especially for long term observation in the FluidFM BOT. For HeLa cells and CHO cells, **Leibovitz medium** supplemented with 10% FBS and antibiotics is recommended to work in CO2-free conditions. Other cell lines might require another medium.

- Avoid contaminations. Using **antibiotics** in the medium is absolutely required as the FluidFM nanoinjection is not performed in a perfectly sterile environment. Every compound that is injected also need to be kept sterile and endotoxin-free. Therefore, working under a **laminar flow hood** when manipulating these compounds is highly recommended.

- Use buffer suitable for microinjection. HEPES or TE (10mM Tris-HCl, pH7.4, 0.25mM EDTA) buffer are the most suitable buffer. **Buffers need to be filtered through a 0.2um filter before use.** If preparing the buffer by yourself, make sure to use pure nuclease free water (for example Sigma Cat. no. W4502).

- Start with "good" cells. If the cell culture shows less than 90% of viable cells (before injection), the yield of successful injection will drastically decrease as this is a sign of an highly stressful environment.

- Phototoxicity. Exposing cells to light, especially when working with fluorescent compounds, can damage subcellular components and compromise cell viability. These damages are mediated by the production of ROS or are linked to specific components of the medium like riboflavin or tryptophan. To minimize phototoxicity, it is important to **illuminate the cell as little as possible.** This is particularly true when performing long term observation. An idle settings without illumination must be set

Here we propose a protocol to assess cell viability after injection. We use the property of the propidium iodide to stain DNA. Once added to the medium, propidium iodide will only be able to penetrate dead cells, as their membrane become porous. Living cells will not be stained. This protocol is proposed for HeLa cells cultivated in a 6-well plate.

3. MATERIALS

- FluidFM nanosyringe, 600nm aperture, 2N/m nominal stiffness, coated with Sigmacote.
- Coating protocol published in SOP.
- Lucifer Yellow solution (5mg/ml in HEPES or PBS or TE pH7.4 buffer), filtered through a 0.22um filter.
- PBS.
- Ethanol 70%.
- HELA cells, plated in 6-wells plate (Thermo Fischer, Nunc).
- CO2 independent growth medium, supplemented with 2nM L-glutamine, 10% FBS and penicillin/streptomycin.
- DMEM medium supplemented with FBS and penicillin-streptomycin (for cell culture in CO2 environment).
- Propidium iodide solution (5mg/ml in sterile PBS).

4. PROCEDURE

- The day before: seed cells in 2 wells (density doesn't matter), in DMEM medium. One of the well will be used as a control, the other one will be used for the nano-injection of lucifer yellow.

- On the day of the experiment, change the medium of both wells with 3ml of L-15 medium supplemented with 10%FBS and penicillin-Streptomycin.

- Add 2ul of propidium iodide to the well A2 (control).

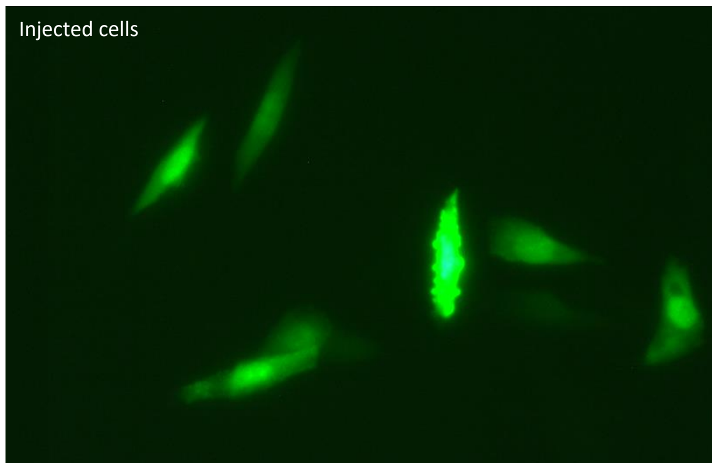
- Incubate for 10 minutes.

- Place the cells in the BOT.

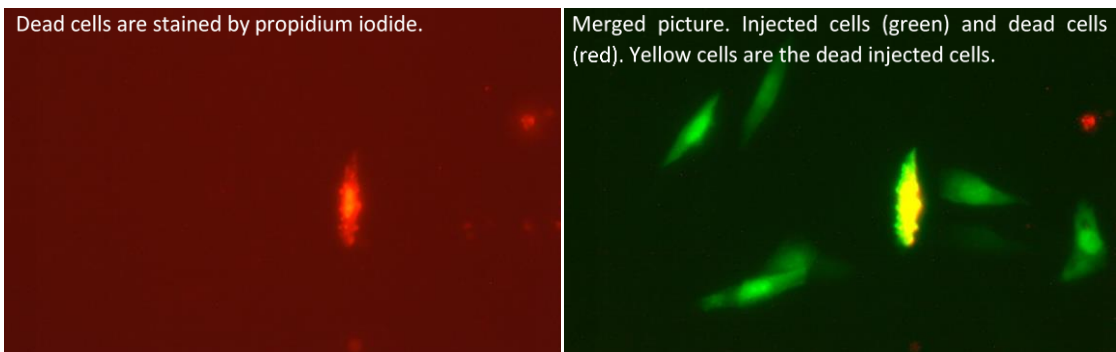
- Check cell viability in the control well (A2 - use RITC filter set). If too many cells are dead, prepare fresh cells (cf. Troubleshooting).

- Load the nanosyringe with lucifer yellow (prepared in a buffer suitable for microinjection)

- Perform the injection in A1:

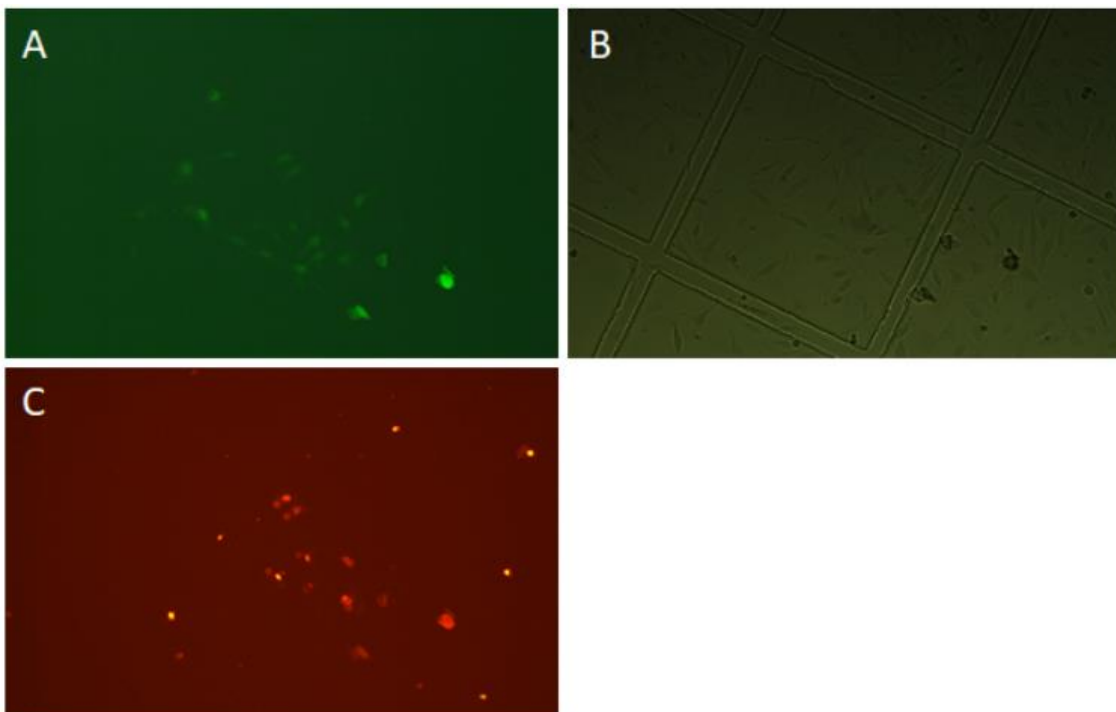


- Record the position of the injected cells.
- Take a picture.
- Inject 50 cells
- Incubate 30 minutes at 37°C.
- Move to the left port.
- Add 2 ul of propidium iodide to the medium. Mix gently the medium with 1 ml pipette. Avoid touching the plate while mixing, in order to save the exact position where the injection has been performed.
- Incubate for a minimum of 5 min.
- Observe the injected cells with RITC filter set. Propidium iodide stains dead cells as shown below. Observe as well the non-injected cells (control well in A2). Cell viability in this well should be the same as previously observed at the beginning of the experiment.



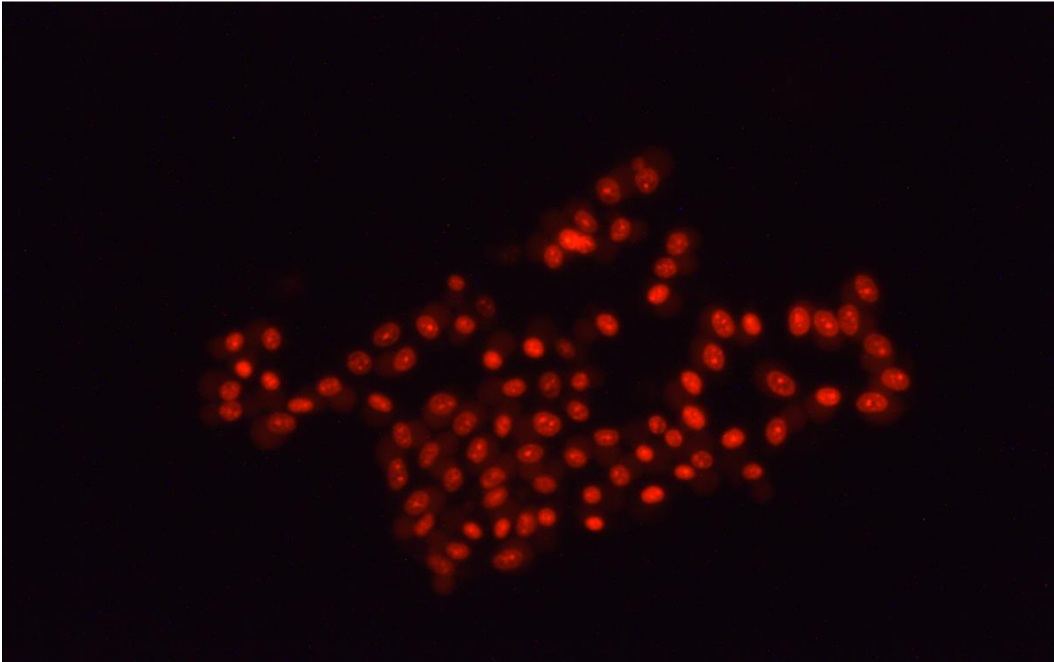
5. TROUBLESHOOTING

- Harmful injection conditions – mechanical stress. When selected injection parameters are too harmful for the cells, they cannot recover after the injection. As an example, in the picture below, HeLa cells were injected with a setpoint of 800 mV, with an approach speed of 100 um/s, with a pressure of 100 mbar and an injection time of 10 s. Cell viability was assessed with propidium iodide staining after 4 h of injection: around 35% of the injected cells were stained with PI, indicating that the chosen conditions for injection were too harmful for some cells.

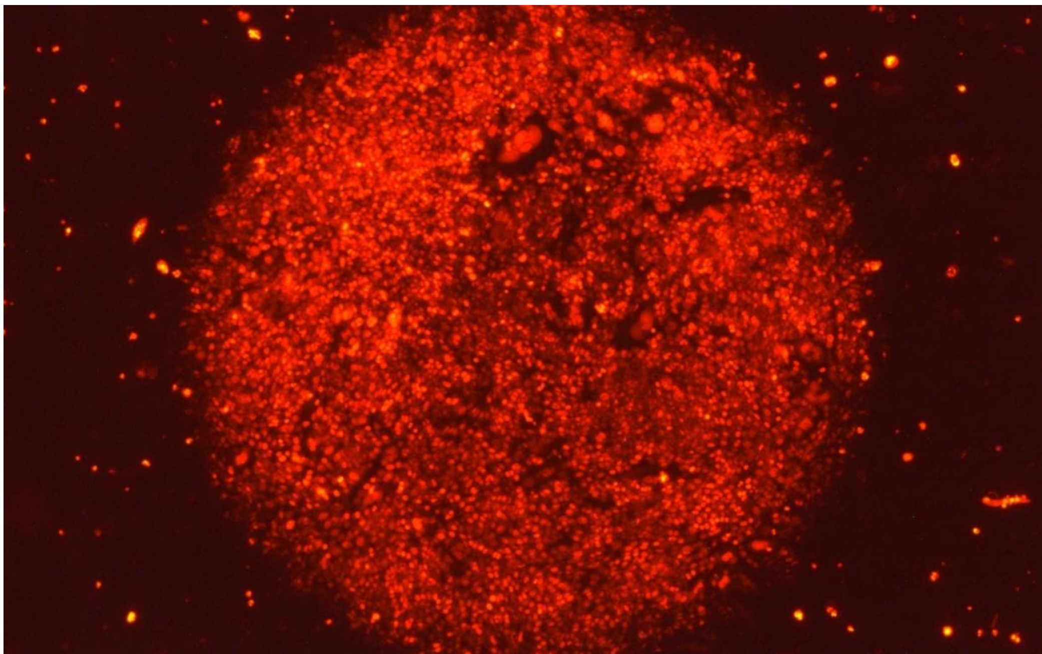


- Using the wrong buffer for injection – biochemical stress. In the image below, pure water has been injected into CHO cells. Injected cells are stained in red as this is very harmful to the cells. Always make sure to use appropriate buffers when performing an injection.

CYTOSURGE AG, SÄGEREISTRASSE 25, CH-8152 GLATTBRUGG, SWITZERLAND
PHONE +41 43 544 87 00, FAX +41 43 544 87 09, WWW.CYTOSURGE.COM

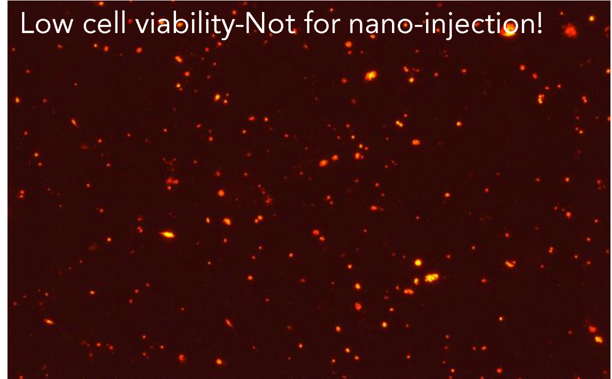


- Long fluorescence exposure – Phototoxicity. In this case, cells have been illuminated for 30 min with maximum intensity through the 20x objective. Phototoxicity is critical: all illuminated cells are dead (observation with a 5x objective).



- Controls for viability – only work with healthy cell cultures. Here there is an example of non-injected cells. On the left, viability is good enough and shows that cells can be used for injection. ON the right, we can observe too many dead cells, indicating a high stress level. These cells should not be used for nano-injection!

Good cell viability. Ok for nano-injection!


Low cell viability-Not for nano-injection!