NETRI's APP NOTE

SURFACE-TENSION-BASED CELL SEEDING IN NETRI MICROFLUIDIC DEVICES

- Increased reproducibility
- Increased homogeneity
- Increased efficiency (using fewer cells for the same final density)
- Better control of cell repartition within the device
- Independent of the substrate
- Prediction of the amount of cells needed to reach a specific density

OVERVIEW

One of the limiting factors to scale in vitro assays is cells' availabilities for ethical reasons (primary cells) or cost reasons (especially for human induced pluripotent stem cells). Miniaturization of assays (e.g use of 384/1536-wells plates) is often used to increase the number of replicates and conditions possible with one preparation or one vial of cells. Here, we changed methodology to achieve the same goal without comprising on the benefits of our compartimentalized architectures to model human physiology.

RESULTS

In this protocol we suggest a novel way to seed in NETRI microfluidic devices. The aim of the seeding step in a cell culture within microfluidic devices is to deposit a maximum of cells inside the channels, in a reproducible and homogeneous manner.

A basic way to replace the content of a channel in a microfluidic device can be to use hydrostatic pressure to generate liquid flow. By emptying the outlet and filling the inlet, a difference of volume hence a difference of pressure (ΔP) is created between the two ends, driving a flow from the inlet to the outlet until equilibrium. Therefore, the content of the channel will be replaced by the new solution. This method can be used to load fresh medium but also to seed cells inside the channel. The main disadvantage of this method when loading cells, is that the volume of solution used to apply hydrostatic pressure to a sub volume that will enter the channel is inherently lost inside the inlet reservoir (Figure 1A). Therefore, a high number of cells remains in the reservoir, outside of the region of interest, and in a higher density than in the channel which leads to a non-homogeneous culture.

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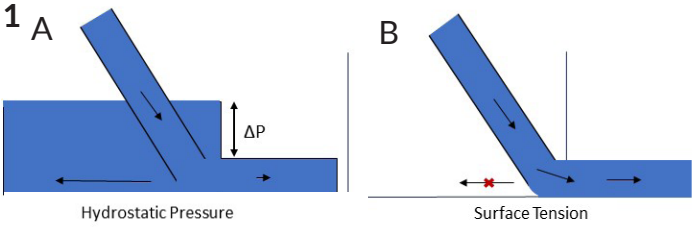

Figure 1: Scheme representing the physical concepts behind the two seeding methods. A) Hydrostatic-pressure-based seeding. B) Surface-tension-based seeding.

Here, we suggest a protocol using the surface tension of liquids coupled with the pressure applied by the pipette to bring cells directly inside the channel, hence greatly reducing the number of cells in the inlet reservoir. For this method to work the inlet reservoir must be dried without drying the channel or the outlet. On one hand, if the inlet reservoir is properly dried, the liquid from the channel will not flow back because the opening of the channel in the reservoir creates a capillary stop valve[1].

Then, when a droplet of cell suspension is deposited at the entrance of the channel, it will connect to the liquid within it, and will be drawn inside to minimize its surface energy (Figure 1B). On the other hand, the droplet deposition at the channel entrance will break the capillary stop valve. Therefore, the liquid remaining in the outlet reservoir must be kept to its minimum so the hydraulic resistance of the channel can prevent the liquid from flowing back to the inlet.

This new seeding method presents several advantages. First, as described earlier, it prevents the formation of cell aggregates in the inlet reservoir. Also, with the right volume injected, the cell solution will integrally pass through the channel without filling the outlet reservoir. This is a perfect method to control the deposition of cells, with a homogeneous repartition in the channel and no aggregate in the inlet and outlet reservoirs. Imaging of the device illustrates the low number of cells in the reservoirs after the seeding (Figure 2).

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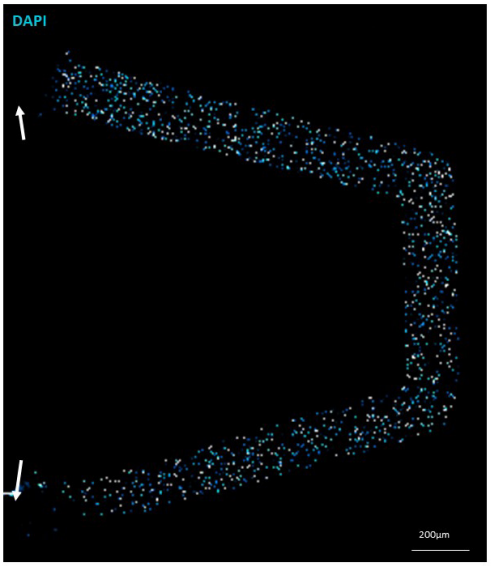

Figure 2 : Representative picture of the repartition of cells stained with nuclei marker DAPI in the channel with the surface-tension-based seeding. Arrows indicate reservoirs.

Another advantage coming from the well-controlled cell deposition is that less cells are needed to get the same density. If we consider a perfect seeding as every cell being in the channel, the efficiency of this method can be calculated. Here, since the repartition of cells is homogeneous across the channel, the evaluation of the cell density was performed in the target region (vertical part of the channel, Figure 3A), and brought to the total surface of the channel to calculate the efficiency. The surface-tension-based method yielded a 82% efficiency of cell deposition in the channel while the hydrostatic-pressure-based method yielded only 28% (Figure 3B). In other words, the same density can be achieved with a third of the number of cells.

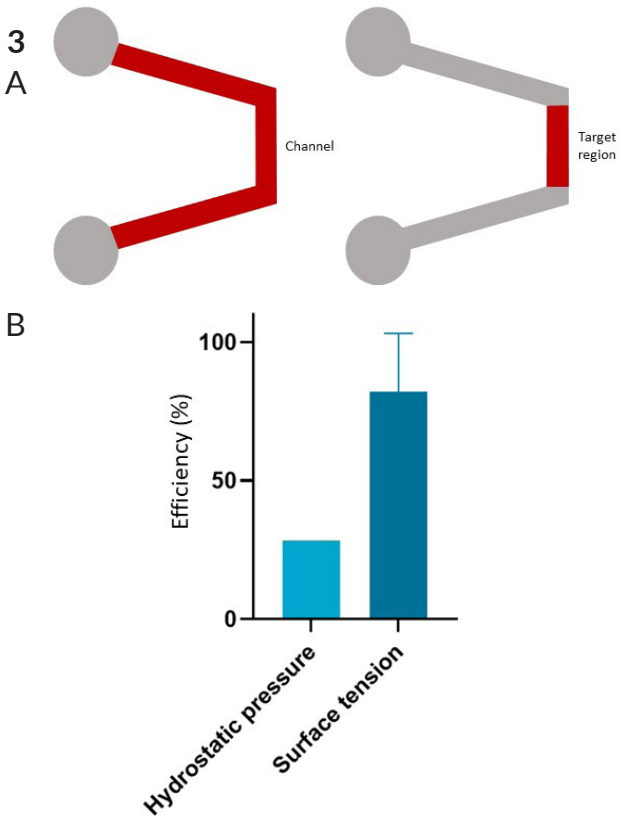

Figure 3 : A) Scheme representing the entire channel where cells are loaded, and the target region where cell density and homogeneity are assessed. B) Comparison of the efficiency of each method, representing their capacity to bring cells in the target region.

Finally, because this method is reproducible and the liquid almost integrally injected in the channel, a direct correlation between the number of cells injected and the obtained density can be calculated. Four different concentrations were used, and the cell nuclei where imaged after the injection for a quantification inside the target region (Figure 4A). Calculated density was, as expected, directly proportional to the number of injected cells, and the obtained ratio can be used to anticipate the number of cells required to reach a specific density (Figure 4B).

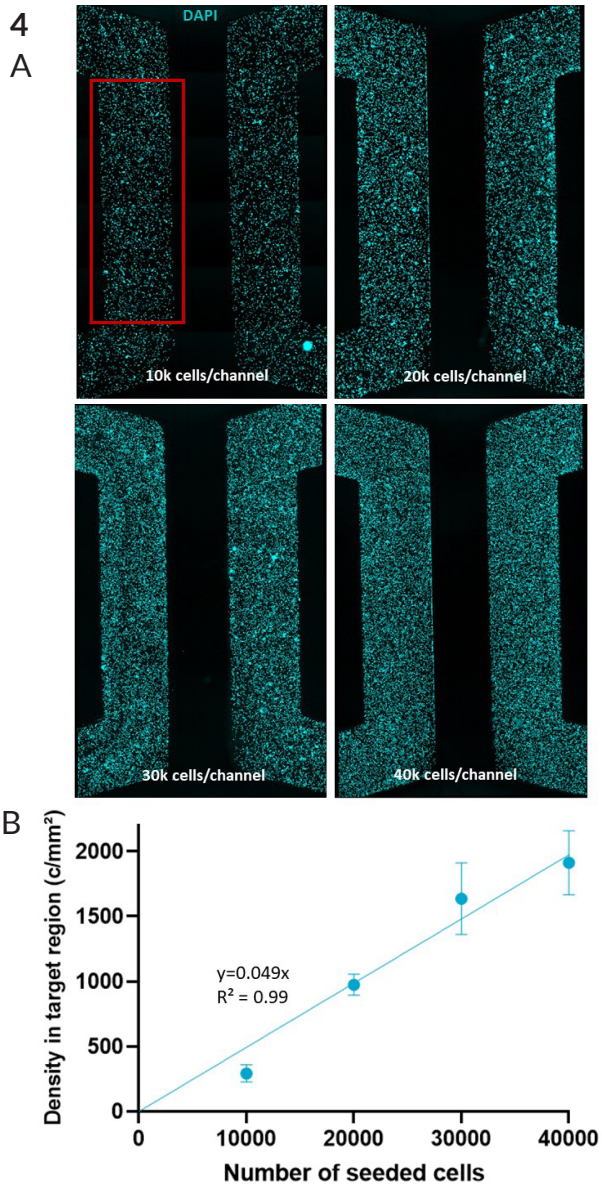

Figure 4 : A) Representative pictures of the channels with 10k, 20k, 30k and 40k cells stained with DAPI and injected with the surface-tension-based seeding. The red box indicates the target region where cell density was assessed. B) Quantification of the primary cell density in the target region.

This correlation has been established with rat cortical neurons. A fit line of slightly different steepness can be expected with a different cell type. Indeed, medium containing cells of different diameter is likely to behave differently. Also, it is still unclear at which point the density would reach a plateau, but cell diameter, cell behavior (like spreading or clustering) as well as available surface will define a maximum that is cell type dependent.

CONCLUSION

In summary, we illustrate here a novel way to handle liquids in NETRI microfluidic devices, that can be used to seed cells in a reproducible and homogeneous manner. The developed seeding protocol (cf. Operating Protocols) has been designed and perfected to precisely deposit cells only in channels, without cell aggregates in the reservoirs. The method is efficient as more than 80% of cells enter the region of interest, and reproducible since a prediction curve of the density could be established. The use of surface tension for injection is not limited to cell seeding and can be used in various situations implicating liquid replacement.

RESOURCES

Available upon request

- Operating Protocol from the NeuroFluidics™ Line

[1]P.F. Man, C.H. Mastrangelo, M.A. Burns, D.T. Burke, Microfabricated capillary driven stop valves and sample injector, in: MEMS Conference, Heidelberg, Germany, January 25–29, 1998.

Based on 10 years of scientific research, NETRI has developed a unique know-how in designing organs/organoids-on-Chip by integrating disruptive building blocks into the same microfluidic devices, while maintaining industrial production standards compatible with pharma industry equipments & requirements.

Thanks to our patented technologies, we are capable of manufacturing prototypes and validating their biological function using primary animal or human induced pluripotent stem cells differentiated in our chip. Our unique infrastructure allows us also to scale up chip production for mass production.

Need more information about NETRI's products and services or how Organs-on-Chip revolutionize pre-clinical trials and human *in-vitro* models? Contact us!

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