CONTINOUS MONITORING OF CELL TRANSFECTION EFFICIENCY ON MICROPATTERNED SUBSTRATES

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INTRODUCTION

Conventional cell cultures lack control over cell interactions and usually require detachment of the cells for the quantification of gene transfection efficiency.

In order to study the effect of cell-cell contact on the efficiency of transfection, we applied micropatterning of mesenchymal stromal cells to generate two different cell-cell contact scenarios, one of single cells and one of small cell-colonies, Figure 1, through microcontact printing in order to study the effect of cell-cell contact on the transfection efficiency [1].

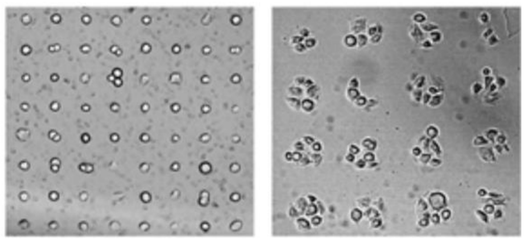

Figure 1: Patterns of single (left) and small colonies of Mesenchymal Stromal Cells

RESULTS

The lack of cell-cell contact influenced the transfection efficiency obtained, Figure 2 A. Whereas small cell-colonies (D100) presented a similar transfection efficiency (22 %) and peak transfection time (24 h after transfection) to that performed on conventional cell cultures, single cells (D20) presented an increment of the transfection efficiency (28 %) and a displacement of the peak of maximum transfection time 6 h earlier. D20 patterns were monitored over the course of 42 hours (Figure 2 B), allowing the monitoring of each individual cell over the course of the whole assay.

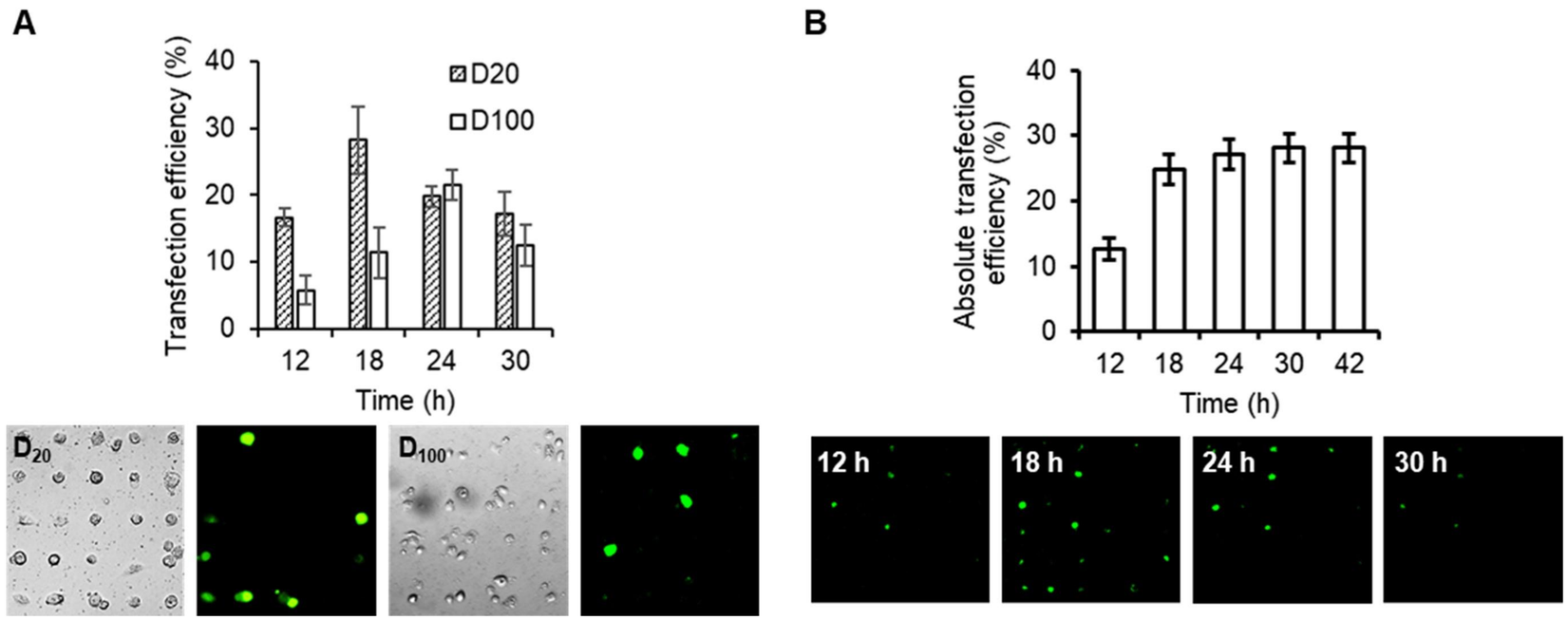

Figure 2: A) Plot of the transfection efficiency at different times for D20 and D100 (top) and microscope images of patterned cells and GFP expressing cells (bottom). B) Plot of the absolute transfection efficiency (top) and fluorescence microscopy images of the same GFP expressing cells at different times.

CONCLUSIONS

Micropatterning allowed the generation of cell patterns with different cell-cell contact. Unlike conventional cell cultures, this method enlabled to address the effect of cell-cell contact on transfection efficiency, implicating that our methodology allows more controllable monitoring of cell transfection. Furthermore, this methodology allowed to observe for how long each cell expressed GFP, as well as to calculate the absolute transfection efficiency counting all transfected cells independently of what time they expressed the protein.

1. Azuaje-Hualde, E.; Rosique, M.; Calatayud-Sanchez, A.; Benito-Lopez, F.; de Pancorbo, M. M.; Basabe-Desmonts, L. Continuous Monitoring of Cell Transfection Efficiency with Micropatterned Substrates. Biotechnol. Bioeng. 2021, bit.27783. https://doi.org/10.1002/bit.27783.

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