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Applying 3D constructs for gene therapy

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1. Introduction

The main objective of three-dimensional (3D) bioprinting technology is the development of 3D constructs combining biomaterials and cells to replicate tissues and organs. Using this technique, different kinds of epitheliums can be produced in order to imitate the in vivo environment with more precision than cell cultures [1]. Such scaffolds are usually characterized prior to evaluate cellular response upon the addition of different drugs and genetic materials [2].

The aim of this study was to make use of 3D bioprinting technology to develop scaffolds that mimic the in vivo lung environment to evaluate the capacity of niosomes (non-viral vectors) to deliver genetic material for the treatment of cystic fibrosis (CF) genetic disorder [3].

2. Material and methods

2.1. 3D bioprinting

The scaffolds were obtained in a extrusion-based 3D Cellink® BioX bioprinter equipment using bioink with biocompatible components such as hyaluronic acid, alginate, gelatin, fibrin and A-proteinin combined with human airway epithelial cells (CuFi-1) being homozygous for F508del (mutation responsible of 70 % of CF patients).

2.2. Scaffolds characterization

Assays were carried out at days 1, 3, 7, 14 and 21 after bioink printing. Live/dead™ staining was used for tracking cell status. Cell counting kit 8 (CCK-8) and lactate dehydrogenase (LDH) activity assays were performed to monitor metabolism activity and cell cytotoxicity, respectively.

2.3. Cellular transfections

Two different transfection approaches were evaluated. The first one consists in the addition of nioplexes in the scaffolds´ medium (2.5 µg DNA/scaffold) every 72 h (4 times). In the second one, nioplexes were added inside the bioink, using 3 different doses (7.5; 15 y 22.5 µg DNA/scaffold). The genetic material used in both approaches was the EGFP reporter plasmid at a cationic lipid/DNA mass ratio of 5/1. Transfection efficiency was evaluated in a Cytation™ 1 equipment during 13 days.

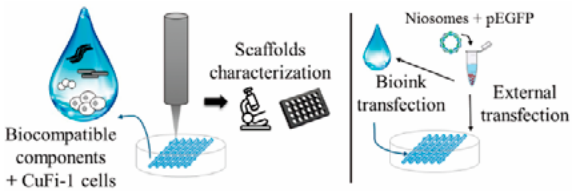

Fig. 1. General scheme of 3D bioprinting process, scaffolds characterization and transfection approaches.

3. Results and discussion

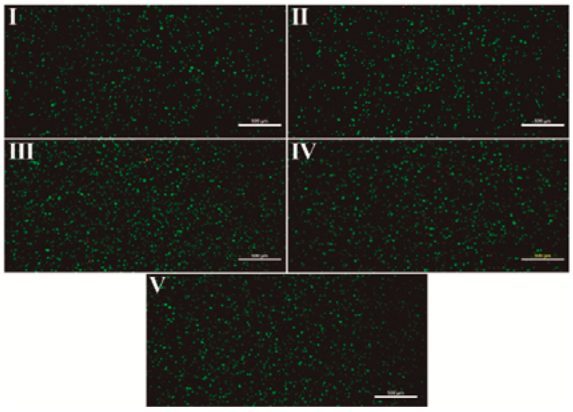

Fig. 2. Representative fluorescence images of Live/dead™ stained scaffolds at I) day 1; II) day 3; III) day 7; IV) day 14 and V) day 21. Scale bar: 500 μm.

Fluorescence images (Fig. 2) show live cells in green, which contain a high number of cells inside each scaffold, and dead cells in red, which scarcely appear in all pictures. The number of live cells was higher as the days go by and the number of dead ones was highest on days 7 and 14.

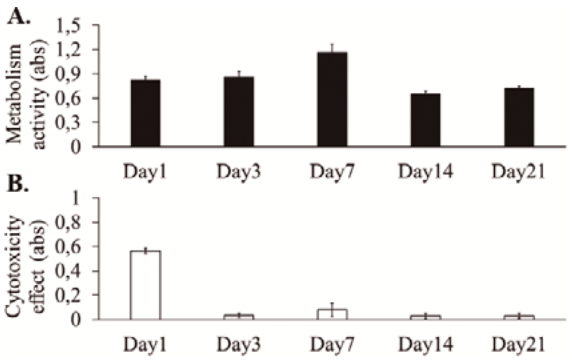

Fig. 3. Evolution of A. metabolism activity and B. cell cytotoxicity of scaffolds. Statistical significance *** $p<0.001$.

Metabolism activity (Fig. 3A) was similar on all days except in day 7 that it was higher, without statistical significance. Regarding the cytotoxicity effect (Fig. 3B), the first day reported the highest toxic value, showing stable and minimum values during next days.

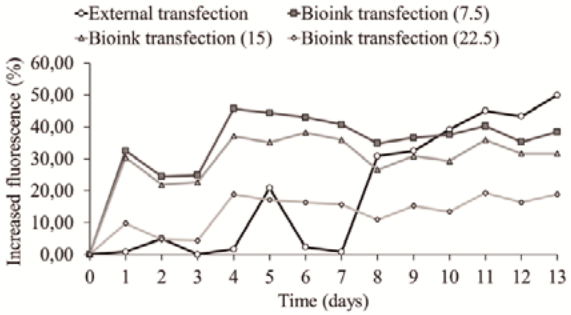

Fig. 4. Scaffold fluorescence increases using different transfection approaches.

External transfection (Fig. 4) had the lower values until day 7 when the fluorescence began to increase. This approach has 4 doses because nioplexes have more barriers to overcome and need more time to reach the cells. Bioink transfection (Fig. 4) has unique dose being 3 groups depending on the dose (7.5, 15 and 22.5 μg DNA/scaffold). The same pattern was followed by each dose: increased fluorescence raised until day 4 and the next days was stable; 7.5 was the dose with more increased fluorescence, following by 15 and finishing by 22.5 (the highest dose, the lowest fluorescence). At final point, external transfection showed more value of increased fluorescence than 7.5.

4. Conclusions

The main findings were the following ones: after 21 days in culture, cells into the scaffolds still remain viable and metabolically active; cells were more affected the first day after bioprinting due to the technique employed but then recovered their values; external multi-dose transfection has higher values of fluorescence than the bioink transfection.

Acknowledgements

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