Change log for the dataset:

Neurothreads: development of supportive carriers for mature dopaminergic neuron differentiation and implantation

by

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Content

History

This change log describes the changes from version v1 (at https://doi.org/10.5281/zenodo.3608207) to version v2 (at https://doi.org/10.5281/zenodo.4008523), and then to version v3 (at https://doi.org/10.5281/zenodo.4441090).

Changes from v1 to v2 reflect the changes and data additions corresponding to the peer review process. Manon Locatelli was added as a new author for her contribution in protocol development of embryonic stem cell differentiation. There were a series of major changes in data evaluation, and addition of new data as listed below.

Changes from v2 to v3 reflect minor completion of supplementary data and documentation of the clustering analysis underpinning aggregation of technical replicates within biological experiments.

Change log v1 to v2

Figure 2

In figure 2, we evaluate relative efficiency of coating of carboxymethylcellulose cryogels with different extracellular matrix proteins.

Originally, we reported this in relation to the theoretically possible maximum amount of protein that can be absorbed. However, as the assumptions and especially the calibration underlying such absolute calculation are not fully warranted, we now only report estimated amounts of protein. This means that we removed the reaction efficiency calculation from the Excel, Graphpad and pdf files for 2b and 2c. This change affects all values proportionally, and so the trends for different pH and different EDC concentrations as well as all statistical evaluations are exactly the same as in version v1.

A run with more carefully controlled calibration (i.e. autoclaved, and with the presence of protein reactant) was added, this corresponds to new supplementary 7.

Figure 3

In figure 3, we evaluate the influence of protein coating parameters on neurite spread with LUHMES model cells.

Previously, we evaluated neurite scores with a nearly fully automated method as the number of beta-III tubulin objects detected per DAPI object. Once the threshold is set, this method is objective, but it has the drawback that fragmented neurites may contribute a large amount of objects while neurite bundles contribute only one object, while they actually correspond to many neurites.

Upon reviewer suggestion, we changed for semi-manual evaluation by ImageJ's simple neurite tracer (https://imagej.net/Simple_Neurite_Tracer), an approach which is likely

more standard in the field. While fragmented neurites are better controlled, the estimation of the number of elementary neurites per bundle still remains extremely challenging, and so the numbers probably remain underestimated and reflects tendencies more than exact values.

In any case, this change causes changes in all the values, and also statistical evaluation, although the global tendencies remain and main conclusions remain unchanged. The Excel, Graphpad and pdf files for Fig. 3c and Fig. 3e are therefore new in this version.

We also added the complete set of raw confocal stacks underlying Fig. 3c and Fig. 3e.

Figure 4

In figure 4, we compare 2D and 3D dopaminergic differentiation of the LUHMES midbrain model cell line.

In the review process, we changed the following pictures upon reviewer request:

4a, 2D, SOX2 and SOX2/Dapi: Due to the secondary antibody, we have occasional foreign particles in these images, and we thought that this wouldn't matter for a representative image (complying with the idea to show data with whatever defaults it has). But it turned out that these particles were easily mistaken for positive cells, which is misleading. This is a particular problem in the small size format of Fig. 4a. For this reason, we replaced the SOX2 and SOX2/Dapi image with one without a bright foreign particle.

4a, 2D, Ki67 and Ki67/DAPI: Same issue, the image shown had a foreign particle (and no positive cells, which is the case for the majority of the raw images). To make it clear how the few positive cells look and avoid confusion with foreign secondary antibody particles (recognizable by green but not blue staining), we replaced the image with one without foreign particles but a green, Ki-67 positive cell.

4a, 3D cryogel: In the review process, a remark was that the cryogel image looks like there is more TH than the graph in Fig 4b indicates, such that there arises a conflict between the visual impression indicating more TH response in the cryogels and the quantitative data indicating no statistical difference. We went through the raw data and looked for a 3D stack that during quantification had shown a percentage of TH positive cells close to the mean portrayed in Fig. 4b and replaced the 3D cryogel part for BIII-Tubulin and TH with this new image. The visual disparity however arises mostly from the 3D aspect: visually, it is difficult to appreciate how many more cells there are in 3D and so despite rather minor difference in proportion, it looks like there is much more TH in 3D than in 2D.

For Fig. 4b, we now provide also the raw images in addition to the counts.

Figure 5

In figure 5, we analyze the effect of injection on the cell viability of differentiated Luhmes cells. For the 2D conditions, this implies detachment to form a cell suspension, for the 3D scaffolds, this implies handling an injection of the intact neurothreads.

We added EDTA detachment, before we only had trypsin. This is to rule out that trypsin has a specifically harsh effect that could easily be fixed by using EDTA instead.

We also included a large body of raw images for completeness.

Supplementary 6

We added a new supplementary 6 describing tensile mechanical characterization of the cryogels.

Supplementary 7

We added a new supplementary 7 describing Langmuir isotherm adsorption of laminin at pH 6 to the cryogels. This is to understand at what adsorband concentration one would expect to reach saturation.

Supplementary 8

We added a new supplementary 8 comparing neurite spreading in LUHMES cells on cryogels coating with fibronectin at pH 6 in addition to the data present in version v1 at pH 4. The idea is to verify whether by avoiding potential protein denaturation at pH 4, neurite spreading cannot be improved; the answer is that if anything, it is even worse at pH 6, showing that protein denaturation is not the only limiting factor with fibronectin coating.

Supplementary 9

We added a supplementary 9 providing neurite spreading data for neural cells obtained by directed differentiation of human embryonic stem cells.
We also provide the raw images for this quantification.

Supplementary 10

We added raw data for supplementary 10, characterizing neurofilament and stem121 (human) marker expression in the cryogel and control 2D transplanted cells.

In this revision, we added image raw data for Fig. S10-2 and S10-3.

Supplementary 14

We added raw data for supplementary 14, characterizing expression of BIII-tubulin and Syn1 (neural identity and maturity markers) after *in-vitro* differentiation.

Change log v2 to v3

Version v3 consist of mostly minor completion without further impact on the substance of the manuscript.

Supplementary 8

Corrected minor error: In Supplementary_Fig_S8.xlsx, sheet "FN ph6 spread", by mistake Excel automatically numbered the experiment for the pH 6 coating with consecutive dates rather than invariably "07.06.2019 pH6".

Supplementary 10

We added the quantitative data (Excel and Graphpad).

Supplementary 11

We added raw data for supplementary 11, regarding Ki-67 expression in the grafts.

Supplementary 14

Minor corrections in "Supplementary_Fig_S14_2_and_3.xlsx" (clarification of some column headers in Sheet "Counting BIII Syn pos. Cells").

Clustering analysis

We added clustering analysis (in the Moulton framework[1]) documenting the rationale for our data aggregation strategy. This includes a summary document ("cluster_analysis.pdf") as well as the underpinning data analysis (R scripts, in "cluster_analysis_scripts.zip").

The R scripts make use of a custom R library "moultonTools", which for the purpose of appropriate versioning and facilitated installation is provided on a Github repository (i.e. https://github.com/tbgitoo/moultonTools). See "cluster_analysis.pdf" for details. For reference, the Github version of moultonTools used to calculate the Moulton factors reported in "cluster_analysis.pdf" corresponds to commit 2b6346d (commit page at https://github.com/tbgitoo/moultonTools/commit/2b6346d7866790743b6d0218be7192df25e2c5a2); the full commit hash is: 2b6346d7866790743b6d0218be7192df25e2c5a2.

References

1. Angrist, J. & Pischke, J.-S. *Mostly harmless econometrics: An empiricist's companion*. (Princeton University Press, 2009).