

# Generation of PSC-derived neural organoids using an Alginate-based 3D scaffold.

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## ABSTRACT

Organoids are stem cell-derived microtissues composed of multiple cell types that are typically arranged with *in vivo*-like patterning and recapitulate organ function, *in vitro*. As such, these complex models are quickly becoming a favored system to study embryonic development and disease. However, producing sufficient quantities of these organoids remains a significant hurdle. Here we demonstrate the utility of an animal origin-free, chemically-defined, alginate-based 3D scaffold to generate increased numbers of neural organoids. For this approach we used pluripotent stem cells to form embryoid bodies seeded directly into a preformed alginate 3D scaffold followed by neural induction. Neurospheres subsequently isolated from this scaffold continued to grow and undergo self-directed neural differentiation. These neural organoids formed multiple rosette-like structures that express progenitor markers such as SOX1 and FOXG1, and later we detected strong expression of the cortical markers SATB2 and CTIP2 as well as other neuronal markers such as MAP2. Additional gene expression analyses also indicated these organoids expressed many neuronal and cell type-specific markers suggesting these organoids are composed of multiple neural cell types. In all, our data suggest that the application of 3D alginate scaffolds are an effective means to generate neural organoids and may help overcome the issue of scale.

## INTRODUCTION

Many strategies for generating neural organoids are based on seeding a single organoid per well in 96-well plates and successful differentiation is variable<sup>1</sup>. In addition, manually embedding samples into basement membrane extracts is a laborious effort and these samples are not efficiently retrieved for image based analysis. Together, this imposes a significant level of difficulty in generating large numbers of organoids for method optimization and for mid-high throughput analyses.

Alginate is a defined, bioinert material that has been successfully used to form various 3D cell models including hepatic spheroids<sup>2</sup>, retinal<sup>3</sup>, and neural<sup>4</sup> organoids. Previous demonstrations for neural organoids utilized hollow fiber alginate configuration in a microfluidic system to successfully form and culture these models<sup>4</sup>. Here we demonstrate the utility of alginate 'sponges' in static culture to generate larger numbers of phenotypically homogenous cortical neural organoids.

## MATERIALS AND METHODS

PSC cultures were maintained in in StemFlex™ and clump passaged every 3-4 days. Embryoid bodies (EB) were formed following the singularization of PSC cultures and seeding in non-adherent microwell 24-well plates. Lyophilized alginate scaffolds were hydrated and firmed in 10% firming buffer ~5 days before EB formation. Firmed alginate was kept in DMEM/F12 in the cell culture incubator and changed daily to allow the trapped air to dissipate. 1-2 day old EBs were then seeded into the alginate sponges prior to neural differentiation. At ~12-14 days of culture, the alginate was non-enzymatically dissolved to obtain intact cell masses where we continued culturing them in non-adherent cell culture plastics on an orbital shaker at 5% CO<sub>2</sub>, 37°C. At about day 26-30 of culture rosette like structures were prominently visible in most samples. Neural organoids were characterizations at various time points and cultured for up to 110 days.

## RESULTS

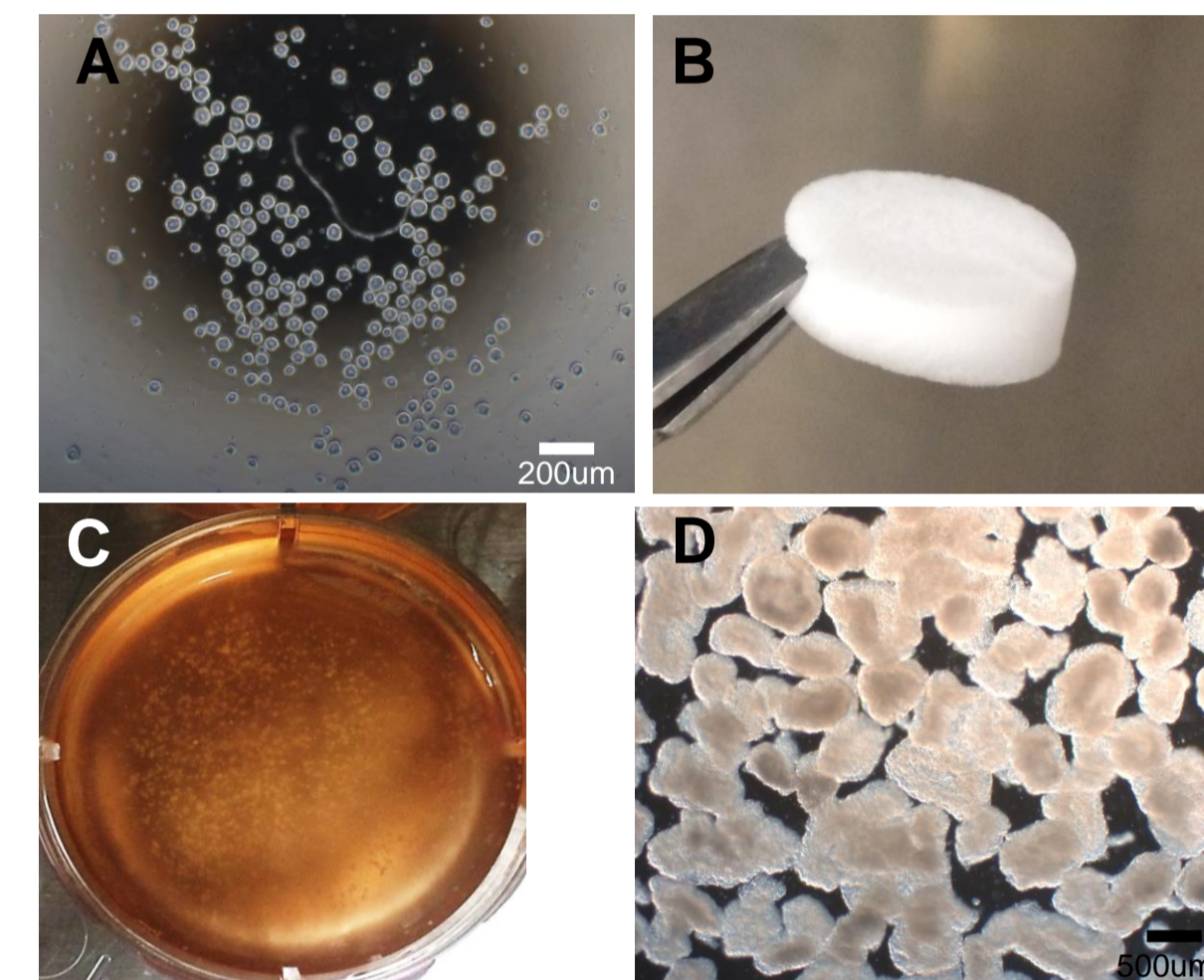


Figure 1. Culturing embryoid bodies in an alginate-based scaffold. A) Embryoid bodies approximately ~75µm in diameter were formed for 1-2 days prior to seeding in alginate. B) Example of an alginate sponge. C) Hundreds of embryoid bodies several days after re-seeding inside the alginate. D) Differentiating cell aggregates harvested from alginate after neural induction (~day 12-14 of culture).

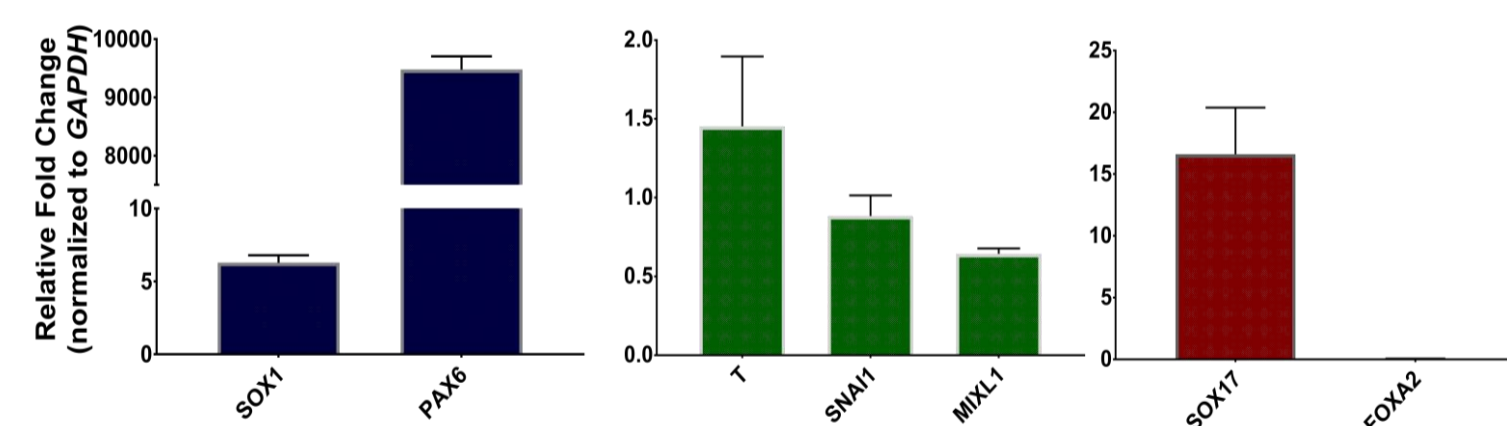


Figure 2. Evaluation of neural induction. Gene expression analysis indicates that embryoid bodies grown in alginate sponges are competent to neural induction. Levels shown are relative to undifferentiated PSCs. Note we did observe some endoderm differentiation but early mesoderm genes were not induced.



Figure 3. Examples of neural organoids. A) Brightfield image of an organoid illustrates the formation of numerous rosette-like structures. B) Immunofluorescent labelling demonstrates the rosette structures express neural progenitor markers such as SOX1 surrounded by early DCX-positive neurons. These representative images are of day 26-30 organoids.

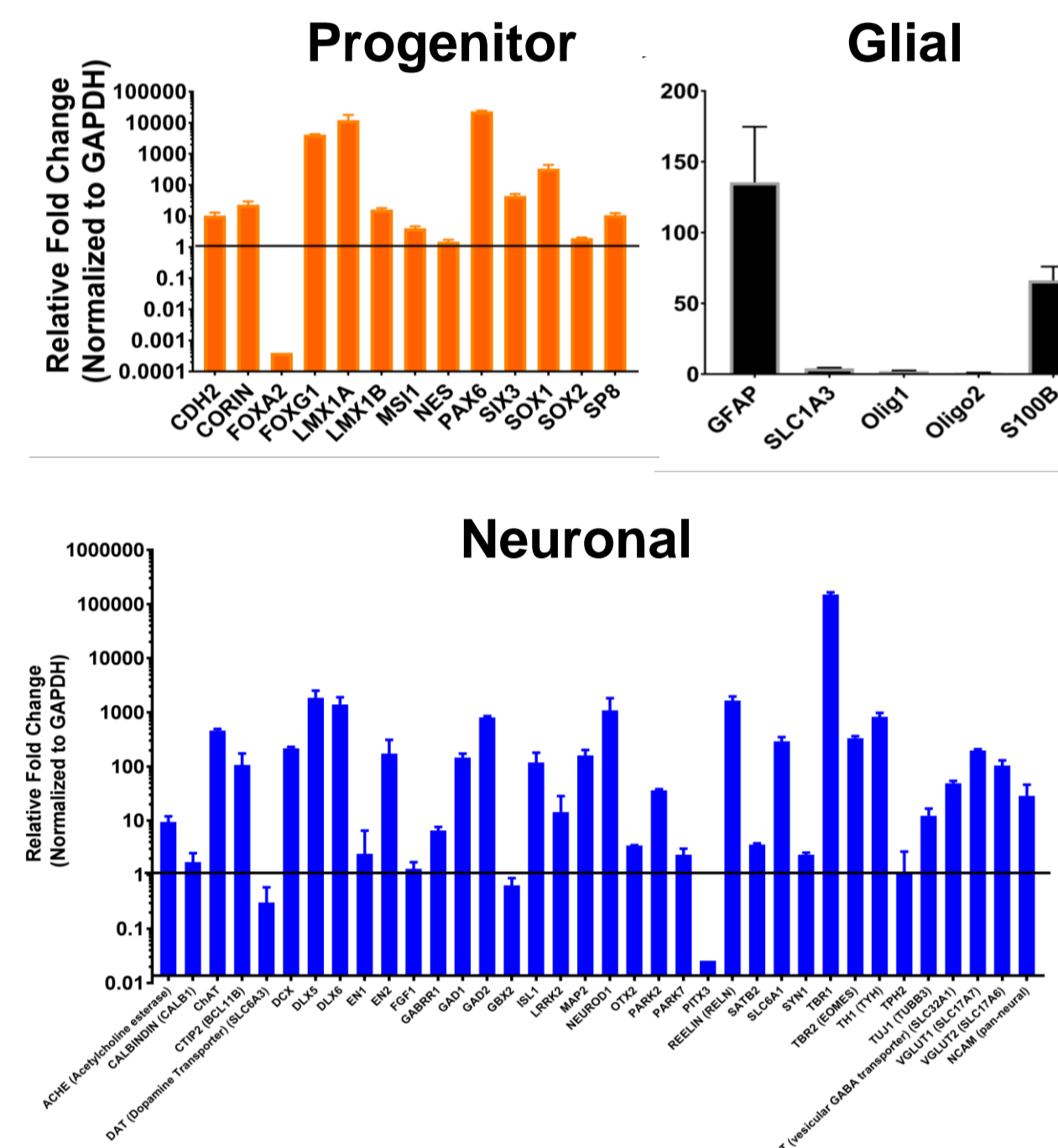


Figure 4. Gene expression analysis in day 61 neural organoids. Marker analysis indicates these organoids express many progenitor and early differentiation markers as well as markers of astrocytes (ie. *GFAP* and *S100B*) but not oligodendrocytes. Many neuronal differentiation markers were also expressed including many associated with corticogenesis. Note the different scales on the y-axes.

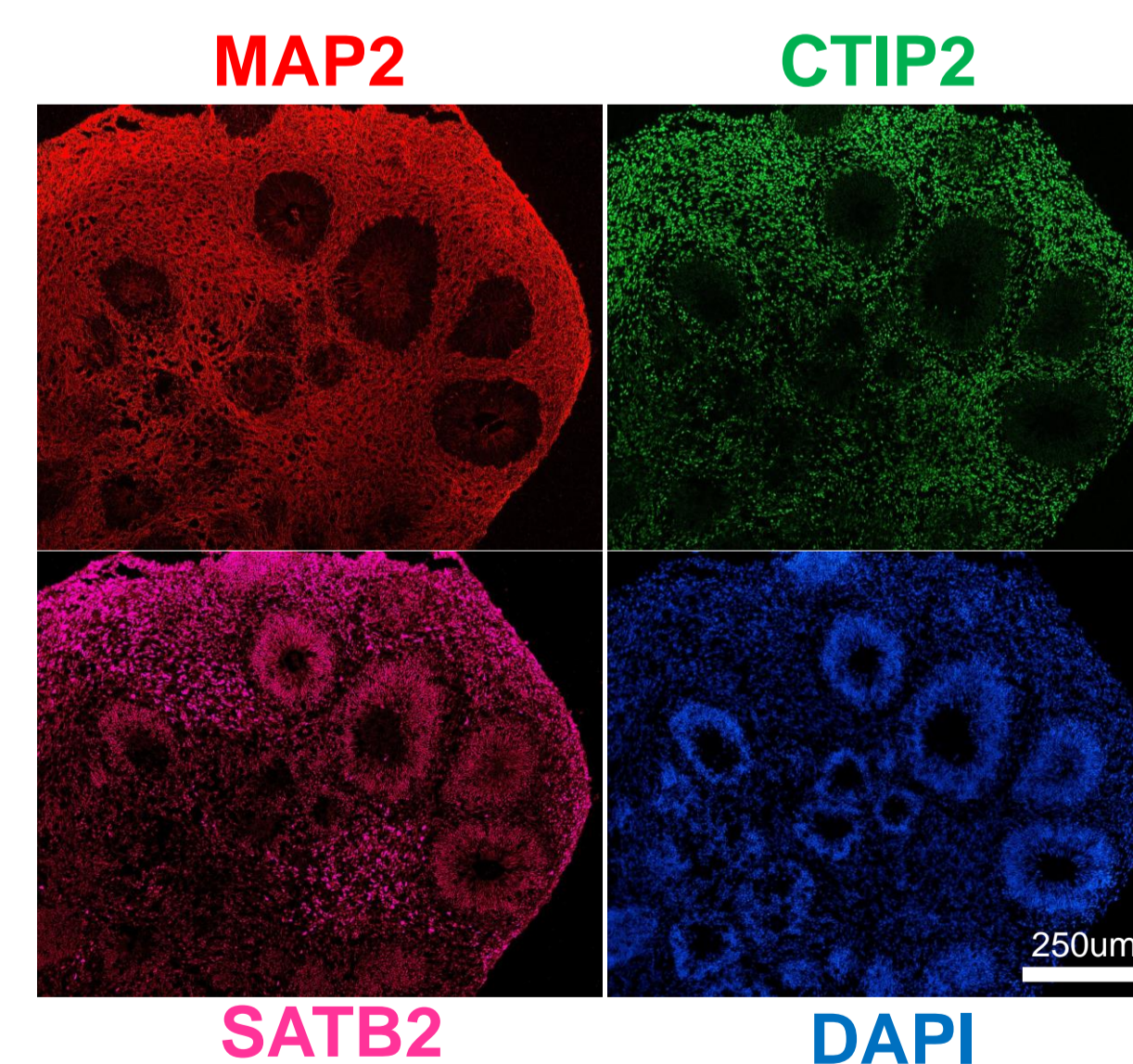


Figure 5. Characterizing marker organization in day 61 neural organoids. Immunofluorescent labeling of 12µm thick cryosections confirmed the expression of the cortical markers SATB2 and CTIP2 as well as the pan-neural marker MAP2. Interestingly the expression of SATB2 and CTIP2 were significantly overlapping but SATB2 was also detected in the rosette structure whereas CTIP2 was not.

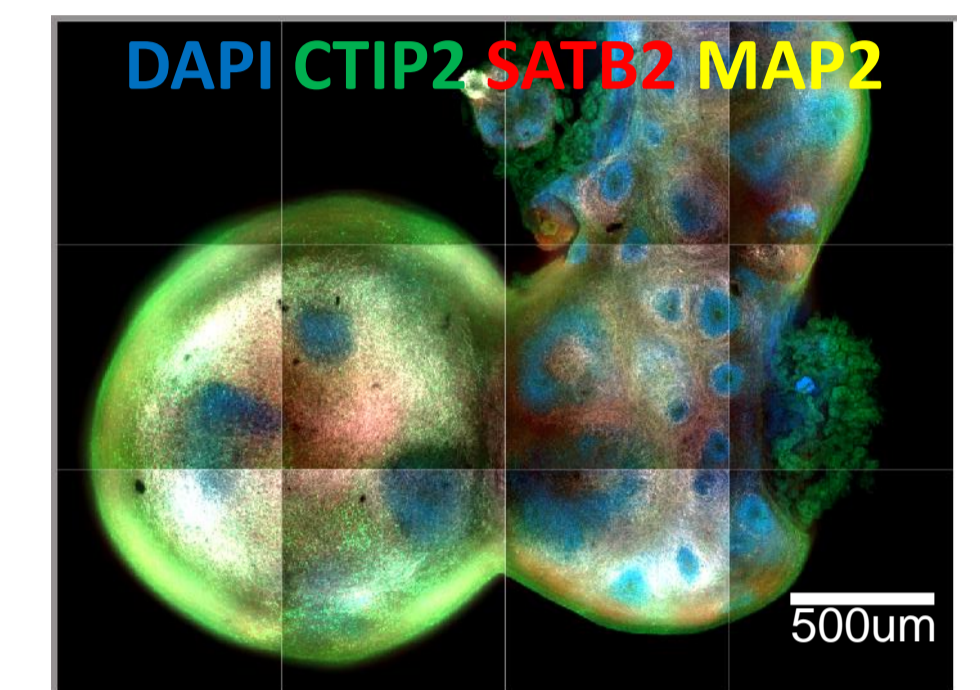


Figure 6. Whole mount immuno-labeling of day 89 neural organoids. Organoids were immunostained and imaged using a high content screening confocal imaging-based platform. This approach demonstrated the intactness of the markers as observed in cryosections in Figure 5 indicating the utility of this platform for high-throughput characterization of large microtissues.

## CONCLUSIONS

Our studies demonstrate the utility of prefabrication alginate scaffolds as a means to enable the development of larger numbers of organoids in static culture. Based on our data we conclude that:

- 3D alginate scaffolds are compatible with neural induction and organoid development
- Alginate-derived neural organoids were phenotypically homogenous and did not require the use of basement membrane extract for post-neural induction development
- Alginate sponges could be seeded with hundreds of EBs allowing the generation of much larger number of samples for study

## REFERENCES

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