GMP-COMPLIANT MICROCHIP BASED CELL SORTING OF IPSCS-DERIVED DOPAMINERGIC PROGENITORS

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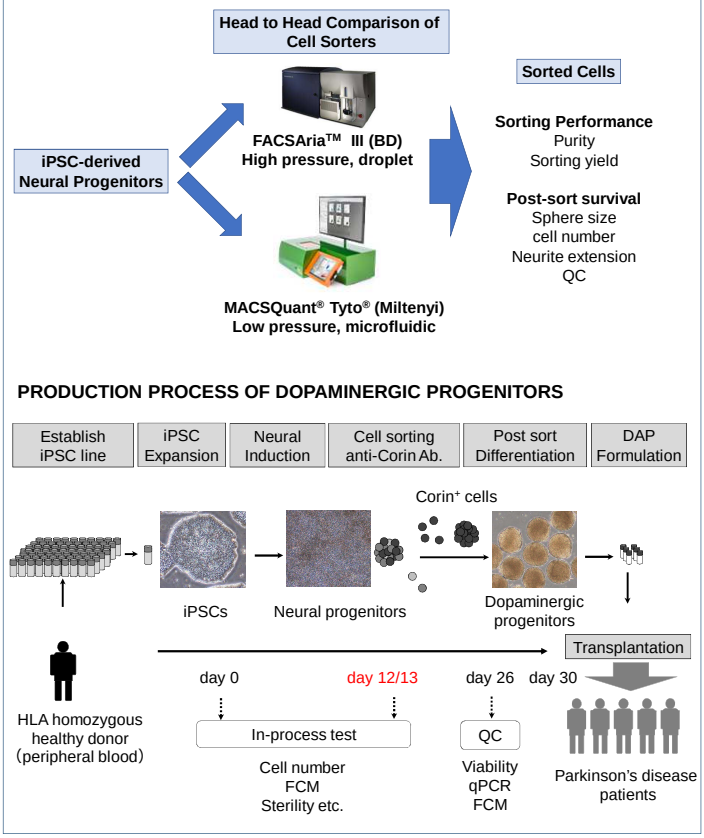
BACKGROUND

A first-in-human trial was initiated aiming at investigating the safety and efficiency of iPSC-derived dopaminergic cells for treatment of Parkinson's disease in Japan.
Manufacturing of the cell product comprises a flow-cytometry based cell sorting step at culture day 12 to 13 of differentiation. Corin+ progenitor cells are isolated and subsequently aggregated in spheres to ensure homogenous differentiation until day 30 when dopaminergic neurons with the desired midbrain phenotype are transplanted into the striatum of PD-patient.

Although a conventional high pressure droplet-based flow sorter (BD Influx) is sufficient to match the cell product specification by purity (>90%), cell viability is impacted by the procedure as shown by reduced re-plating efficiency of target cells leading to smaller sphere sizes after sort, and eventually low overall cell yields. For reaching desired cell numbers to conduct QC and surgery two days of cell sorting (d12 and 13) with constant operator interaction are currently needed.

MATERIALS AND METHODS

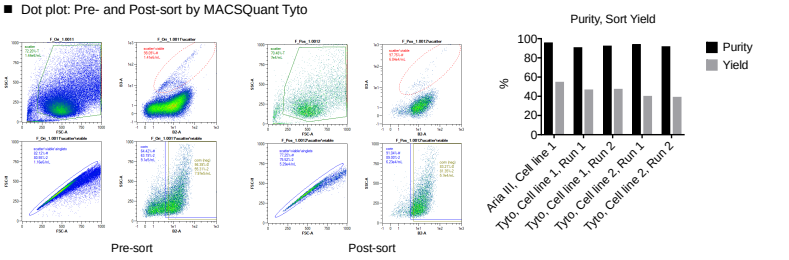


PRODUCTION PROCESS OF DOPAMINERGIC PROGENITORS

RESULTS

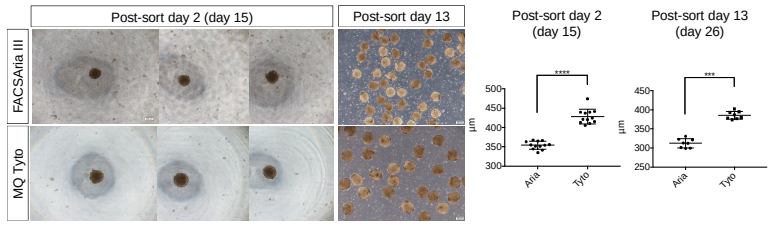
Sorting performances were comparable between BD FACSAria III and MACSQuant Tyto

- Dot plot: Pre- and Post-sort by MACSQuant Tyto

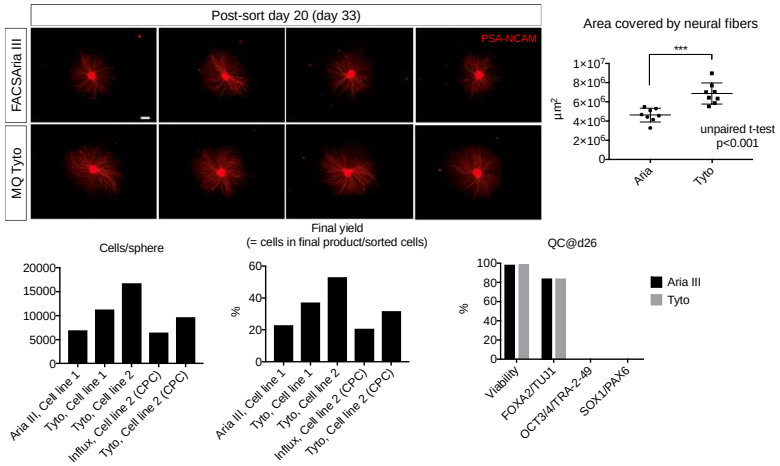


Post-sort survival and final yield increased by using MACSQuant Tyto

- Size of aggregate sphere



- Neurite extension: spheres were cultured on LN511-E8 fragment for 5 days



Cell sorter	Input target cell number (x 10^6)	Final cell number (day 26, x 10^6)	Sorting time	Final cell number / hr (x 10^4)	Sorting Time for one patient: 1.2 x 10^7 cells
Aria	46.1	2.60	4 hr	65	18.5 hr
Influx (CPC)	203.6	6.89	32 hr	21.5	55.8 hr
Tyto	42.5	3.98	4 hr	99.5	12 hr

SUMMARY

- **Sorting performances (= purity and yield) were comparable between BD FACSAria III and MACSQuant Tyto**
- **Post sort survival increased by using low-pressure sorting system, consequently contribute higher yield of final cell product.**
- **Estimated time of producing cells required for 1 patient is reduced by using MACSQuant Tyto**

CONCLUSION

- **Low pressure microfluidic sorting system can provide a solution for sorting the required cell number to generate a clinical patient sample.**

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