

## Supplementary Information

### An immunomodulatory encapsulation system to deliver human iPSC-derived dopaminergic neuron progenitors for Parkinson's disease treatment

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##### References

## Materials

**Table S1: Details of materials, suppliers and catalogue numbers.**

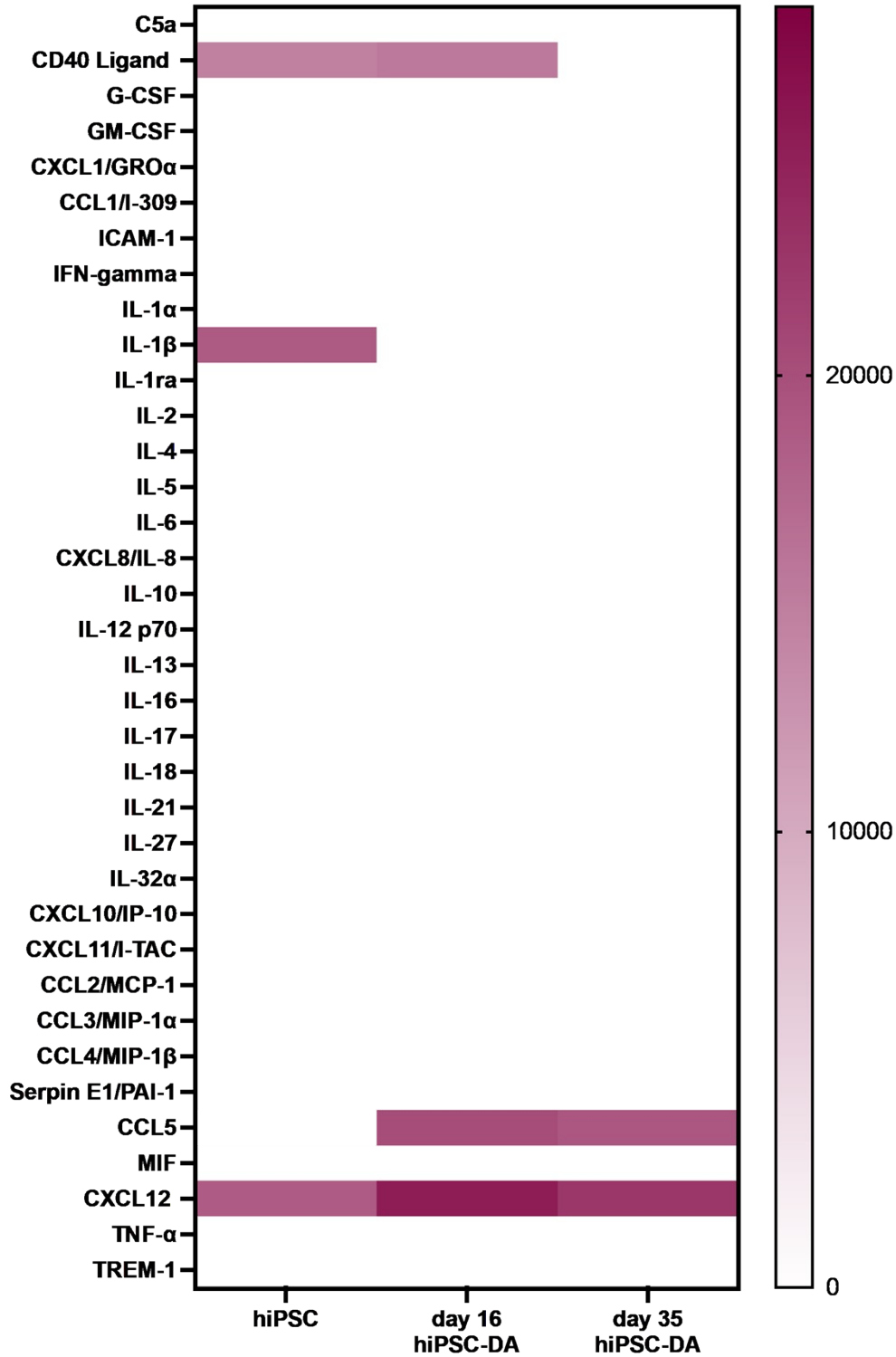
<b>Cells/reagents</b>	<b>Supplier</b>	<b>Catalogue Number</b>
Human induced pluripotent stem cells (hiPSC, CGT-RCiB-10)	Cell and Gene Therapy Catapult, UK	N/A
B-27 supplement (50X) serum free	ThermoFisher Scientific	17504044
Bovine Serum Albumin (BSA)	Sigma Aldrich	A9418
Brain derived neurotrophic factor (BDNF, human)	Cambridge Bioscience	Z03208-5
Calcium chloride	Fisher Scientific	15607730
CellTrace™ CFSE Cell Proliferation Kit	ThermoFisher Scientific	C34554
CHIR99021	Cambridge Bioscience	SM13-1
CryoStor™	Sigma Aldrich	C2874
Dopamine ELISA	Elabscience®	E-EL-0046
DAPT	Cambridge Bioscience	SM15-2
Dulbecco's Modified Eagle Medium (DMEM)	ThermoFisherScientific	
DMEM/F12: Neurobasal (-glutamine)	ThermoFisher Scientific	21331020
DPBS	ThermoFisher Scientific	14190144
EasySep™ Human CD3 Positive Selection Kit II	STEMCELL™ Technologies	17851
EDTA (UltraPure 0.5M)	ThermoFisher Scientific	15575020
Essential 8 Basal Medium	ThermoFisher Scientific	A1517001
Essential 8 Basal Medium Supplement	ThermoFisher Scientific	A1517001
Foetal Bovine Serum (FBS)	Sigma Aldrich	F74524
Hoechst 33258	Sigma Aldrich	861405
Human cytokine array	R&D Systems	ARY005B
L-glutamine	Sigma Aldrich	G7513
Laminin I	Bio-Techne	3401-010-02
1x N2 Supplement	ThermoFisher Scientific	17502058
Noggin	Bio-Techne	3344-NG-050
Normal horse serum (NHS)	Vector Laboratories	S-2000
Normal goat serum (NGS)	Dako	X0907
Neurobasal medium	ThermoFisher Scientific	A1371201
Paraformaldehyde (PFA)	ThermoFisher Scientific	P/0840/53
Phosphate buffered saline	Sigma Aldrich	P4417-100TAB
Poly-L-Ornithine	Merck	P3655-10MG
SB431542	Miltenyi Biotech	130-106-275
SHH-C24II	Bio-Techne	1845-SH-025
Sodium Alginate (PROVONA SLM 100)	Merck	4202301
Sunflower oil	Waitrose	N/A
Tacrolimus (FK506)	Abcam	Ab120223
Triton X-100	Sigma Aldrich	T9284
Trypsin-EDTA (1X)	Sigma Aldrich	T3924
VECTASHIELD antifade mounting medium	Vector Laboratories	H-1000-10
Vitronectin (VTN-N)	Thermo Fisher	A14700
Y-27632 (ROCK Inhibitor)	Cambridge Bioscience	10005583-1mg-CAY

**Table S2: Details of antibodies used, suppliers and catalogue numbers.**

<b>Primary Antibodies</b>				
<b>Antibody</b>	<b>Species Raised In</b>	<b>Dilution</b>	<b>Supplier</b>	<b>Catalogue Number</b>
$\beta$ -III-tubulin	Rabbit	1:300	Sigma Aldrich	T2200-200 $\mu$ L
$\beta$ -III-tubulin	Mouse	1:300	Sigma Aldrich	T8660-2mL
LMX1A	Rabbit	1:400	Abcam	Ab139726
Nestin	Mouse	1:400	Millipore	MAB5326
OCT3/4	Goat	1:100	Santa Cruz	J0809
Tyrosine Hydroxylase	Mouse	1:500	Sigma	T-2928-100 $\mu$ L
<b>Secondary Antibodies</b>				
Dylight 488 horse anti-mouse IgG		1:400	Vector Biolabs	DI-2488
Dylight 594 goat anti-rabbit IgG		1:400	Vector Biolabs	DI-1549
Dylight 594 horse anti-mouse IgG		1:400	Vector Biolabs	DI-2549
Dylight 594 horse anti-goat IgG		1:200	Vector Biolabs	DI-3094
Goat anti-mouse Alexa Fluor Plus 488		1:200	ThermoFisher Scientific	A32723
Goat anti-rabbit Alexa Fluor Plus 594		1:200	ThermoFisher Scientific	A32740

## Results

**Figure S1:** Extended heatmap showing the detection of cytokines in the secretome of cells throughout differentiation towards dopaminergic neurons. Data expressed as the mean pixel density of the two spots on the array for each cytokine (n=1).



**Equations S1-4:** Tate's law implies a limit to droplet size based on the force balance between the droplet's gravity G (1) and the capillary force F (2), where D is droplet diameter, g is the acceleration due to gravity,  $\rho$  is the density of the liquid,  $\sigma$  is the surface tension and d is the outer diameter of the needle.<sup>1</sup> For a droplet to form, gravity must overcome the capillary force, and therefore (3) can be used to infer the minimum droplet diameter, with (4) taking swelling into account to give the final diameter Df.

$$1) G = \frac{\pi\rho g D^3}{6}$$

$$2) F = \pi\sigma d$$

$$3) D = \frac{(6\sigma d/\rho g)^{1/3}}$$

$$4) Df = D \times 140$$

**Table 3:** Assumptions made when calculating the minimum droplet diameter.

Parameter	Assumption	Reference
Surface tension of 2% alginate	56 mN/m	2
Density of 2% alginate	1.01 g/cm <sup>3</sup>	2
Gravitational acceleration	9.80665 m/s <sup>2</sup>	
Minimum needle diameter used	147 $\mu$ m	
Swelling of 2% alginate at neutral pH	140x	3

## References

1. T. Tate, *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*, 1864, **27**, 176-180.
2. P. Del Gaudio, P. Colombo, G. Colombo, P. Russo and F. Sonvico, *Int J Pharm*, 2005, **302**, 1-9.
3. K. Guruviah, P. Kumar and K. Kumar, *Desalination and Water Treatment*, 2016, **57**, 1-14.