Neurophysiology and Electrophysiology of Human and Murine Dental Pulp Stem Cells

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February 2014

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

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Manuscript accepted for publication 10 February 2014:

Neuronal differentiation of murine dental pulp stem cells in vitro. Stem Cell Research & Therapy, 2014

Kylie Ellis

Date

Acknowledgements

The development and preparation of this thesis has been an extremely challenging yet highly rewarding journey. I have no doubt it would not exist were it not for the intellectual and emotional support of many people around me, some of whom I would now like to take the opportunity to thank.

Firstly to my supervisors David O'Carroll, Simon Koblar and Martin Lewis who helped me tackle an unknown world between academic disciplines with scientific eyes. Thanks also to Keiichi Torimitsu for the opportunity to work in Japan.

To my lab mates across the years, thank you for the laughs, the games and the beers. It's been great to have good friends amongst my colleagues and to count on you for your time, help and extra reagents when need be. I would particularly like to thank my friend and colleague Elizabeth Harford-Wright for keeping me sane throughout the final stages of this journey. Your record timing thesis feedback was invaluable, but insignificant compared to your general support.

My family has always been my biggest cheer squad. Thank you all for believing in me with unwavering support and understanding. I'm proud that you are all now well versed in neurophysiology terms. You must have been listening!

Finally, to my partner JR, you have kept me grounded with a sense of perspective throughout many dire days. Thanks for being wonderfully supportive and reminding me of what everyday life is about when that information was pushed out of my brain to make way for other, stem cell-related things.

Abbreviations

α-MEM	Alpha-Modified Eagles Medium
Ba^{2+}	Barium
BDNF	Brain-Derived Neurotrophic Factor
BMSC	Bone Marrow Stromal Cell
Ca^{2+}	Calcium
ChABC	Chondroitinase ABC
ChAT	Choline Acetyltransferase
Cl^-	Chlorine
CM	Conditioned Medium
CNS	Central Nervous System
CO_2	Carbon Dioxide
CSPG	Chondroitin Sulfate Proteoglycan
Cx43	Connexin 43
DAPI	4',6-diamidino-2-phenylindole
DIV	Days *in vitro*
DMEM	Dulbecco's Modified Eagles Medium
DPSC	Dental Pulp Stem Cell
ECM	Extracellular Matrix
ECoG	Electrocorticography
EEG	Electroencephalography
EM	Electron Microscopy
ER	Epigenetic Reprogramming
ESC	Embryonic Stem Cell
FBS	Fetal Bovine Serum
FES	Functional Electronic Stimulation
FGF	Fibroblast Growth Factor
GAD65/67	Glutamic Acid Decarboxylase 65/67
GAG	Glycosaminoglycan
GFAP	Glial Fibrillary Acidic Protein
H_2O_2	Hydrogen Peroxide
HAS	Hyaluronin Synthase

hDPSC	Human Dental Pulp Stem Cell
hFF	Human Foreskin Fibroblasts
IBMX	3-Isobutyl-1-methylxanthine
IHC	Immunohistochemistry
Interferon-γ	IFN-γ
ITO	Indium Tin Oxide
K^+	Potassium
MAG	Myelin Associated Glycoprotein
MCS	Multi Channel Systems
mDPSC	Murine Dental Pulp Stem Cell
MEA	Microelectrode Array
MMP	Matrix Metalloproteinase
MT	Middle Temporal Area
Na^+	Sodium
NeuN	Neuronal Nuclei
ND	Neuronal Differentiation
NDS	Normal Donkey Serum
NFM	Neurofilament-Medium Chain
NGF	Nerve Growth Factor
NM	Neuronal Maturation
NPC	Neural Progenitor Cell
NSC	Neural Stem Cell
NSPC	Neural Stem/Progenitor Cell
NT-3	Neurotrophin 3
NTT	Nippon Telephone and Telecommunications
Omgp	Oligodendrocyte Myelin Glycoprotein
PBS	Phosphate Buffered Solution
PEI	Poly(ethyleneimine)
PFA	Paraformaldehyde
PLL	Poly-l-lysine
PLO	Poly-l-ornithine
PNN	Perineuronal Net
SD	Standard Deviation
SEM	Standard Error of Noise

SHED	Stem Cells from Human Exfoliated Deciduous Teeth
SiO2	Silicon Oxide
XT-1	Xylotransferase-1
TBI	Traumatic Brain Injury
TEM	Transmission Electron Microscopy
TH	Tyrosine Hydroxylase
TiN	Titanium Nitride
Tn-R	Tenascin-R
TPA	Phorbol 12-myristate 13-acetate
TTX	Tetrodotoxin
V1	Visual Area 1 (visual cortex)
V4	Visual Area 4
V5	Visual Area 5
vGlut2	Vesicular Glutamate Transporter 2
WFA	Wisteria Floribunda Agglutinin

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Abstract

The cortical brain-machine interface has the potential to improve the quality of life for millions of patients with sensory or motor loss, however a range of limitations currently exist that restrict their long term clinical application. Primary amongst these is the low biocompatibility between electrodes and brain tissue. Injury to the central nervous system (CNS) causes a recruitment of inflammatory factors that lead to the long term upregulation of the perineuronal net (PNN) and the development of a glial scar that restrict recovery by forming an inhibitory peri-injury region. We propose that a biological layer of dental pulp stem cells (DPSC) will render the interface more compatible with cortical tissue to allow more efficient signal transduction and promote long-term success. I have approached this interface challenge *in vitro* to determine how DPSC may actively improve the local environment to achieve long-term biocompatibility.

Microelectrode arrays (MEAs) approximate the brain-machine interface *in vitro*. In Chapter 3 I designed and fabricated a novel MEA with design features specific to our research goals. Initial characterisation of these MEAs in comparison with commercial MEAs demonstrated high biocompatibility with cortical cultures, however electrodes had high impedance leading to a low signal-to-noise ratio that ultimately rendered the MEAs unable to detect extracellular electrical activity from the cultured cortical neurons. Future modifications including the addition of electrode polymers on these MEAs will render them more appropriate for *in vitro* use. This directed us to utilise commercial MEAs for subsequent use within the studies of this thesis.

In Chapter 4, human-derived DPSC (hDPSC) were seeded onto commercial MEAs to determine their long-term biocompatibility throughout neuronal differentiation and to assess the development of electrical activity within the developing cultures. DPSC had intrinsically low biocompatibility with MEAs, however long term culture was achieved. Stimulation-induced events were detected in long-term cultures yet no spontaneous activity was measured.

A novel source of DPSC derived from murine incisors (mDPSC) were also characterised for their neuronal potential *in vitro* in Chapter 5. mDPSC developed a neuronal morphology and high expression of neuronal and glial markers identified through immunohistochemical analysis. Differentiated mDPSC networks supported electrophysiology reminiscent of early embryonic development with high expression of L-type voltage-gated Ca^{2+} channels, gap junction proteins and gamma frequency oscillatory activity following neural induction. The ability of mDPSC to

differentiate into neural-like cells supports their future use in a murine model of autologous cell transplantation.

The impact of DPSC on the endogenous inhibition of the brain was also investigated in Chapter 6. It was hypothesised that co-culturing DPSC with dissociated cortical neurons would downregulate the expression of the restrictive PNN around neurons. It was demonstrated that hDPSC co-culture reduced the proportion of neurons that expressed PNN in a time and dose-dependent manner. Moreover, hDPSC conditioned medium also decreased the proportion of PNN-expressing neurons, suggesting that paracrine factors released by the cells may be responsible for this effect.

In conclusion, the present studies have identified a novel ability for DPSC to reduce cortical PNN expression that could improve the long-term efficacy of a brain-machine interface. However, biocompatibility of DPSC with *in vitro* MEAs is low and requires modification to achieve a successful interaction at the interface. Moreover, the neuronal potential of DPSC isolated from murine incisors has been demonstrated for the first time. The multifaceted characteristics of DPSC may present a viable approach to cell-based therapeutics for a range of CNS disorders.