Directed Differentiation of Oligodendrocyte Precursor Cells Using Rationally Designed Solid State Peptide Materials

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ABSTRACT

Oligodendrocytes are neuroglial cells whose function is to support and myelinate axons in the CNS. Oligodendrocytes have been found to arise from oligodendrocyte precursor cells (OPCs) during late embryogenesis and early post natal development. A single oligodendrocyte can myelinate as many as 40 or more different axons, wrapping the axon with between 20 and 200 layers of highly modified membrane processes[1]. The differentiation of OPCs into myelin-synthesizing oligodendrocytes is not well understood, and research suggests that cues for differentiation involve mechanical and chemical signaling from astrocytes and neurons. Many proteins are known to be involved in the migration, proliferation, survival, and differentiation of oligodendrocyte precursors, but their specific roles are not well defined or understood. A better understanding of the mechanism through which these proteins affect the differentiation of OPCs will allow us to more effectively differentiate OPCs to oligodendrocytes, allowing us to better assess the potential for using OPCs as a neurological therapy.

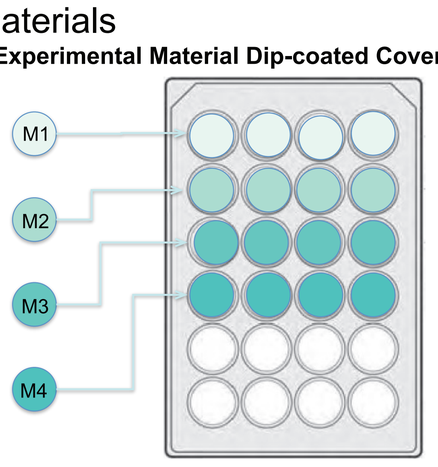
INTRODUCTION

The cells used in this study are CG4s, a bipotential glial cell line capable of differentiating into oligodendrocytes[2]. Various peptide materials are being used to enhance differentiation of CG4 OPCs into mature oligodendrocytes with myelinating capabilities as well as to support mature oligodendrocytes in culture for further study.

MATERIALS AND METHODS

Material Preparation: Dip-coated coverslips in silica-based, peptide sol-gel materials

Experimental Material Dip-coated Coverslips



Control Material Coverslips

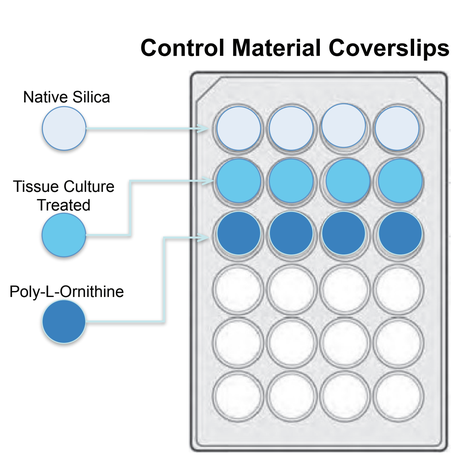


Table 1: Experimental Materials

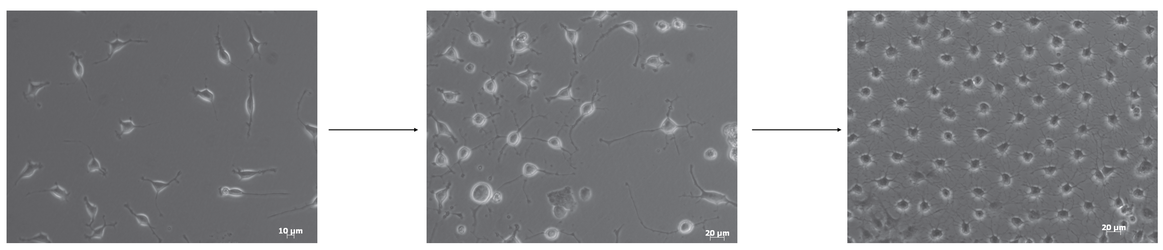
Control Materials	Materials	Peptides in Materials
Negative Control		
Native Silica	M1	YIG
Positive Control	M2	YIG, AGP
Poly-L-Ornithine	M3	YIG, AGP, SNR
Tissue Culture Treated glass	M4	YIG, AGP, SNR, NID

Table 2: Peptide Amino Acid Sequences

Peptide	Sequence
YIG	A C D P G Y I G S R G A
AGP	A G P H S R N A G A
SNR	A S L V R N R R V I T I Q G
NID	A N D N I D P N A V A
RGD	A Y A V T G R G D S P A S

Cell Culture: CG4 Cells

- Cells are cultured on PLO-coated plates supplemented with 10ng/mL bFGF and PDGF
- Cells are seeded on material coverslips, 10, 000 cells/cm^3, without growth factor supplements
- Cells fed every 2 days, removing and replacing only half of the media each time so that cells are exposed to the growth factors and signaling molecules they have secreted
- Cells cultured on materials 10-14 days before fixation



Undifferentiated CG4 cells

Day 3, CG4 differentiation on PLO, induced by growth factor removal

Day 7, Differentiated CG4 cells

Cell Analysis:
Immunocytochemistry used to analyze protein expression of CNPase, Myelin Basic Protein, Proteolipid Protein, Glial Fibrillary Acidic Protein, Actin, and Vinculin (focal adhesion protein)

OBJECTIVES

The objective of this study is to determine the effects of 2D silica-based materials containing bioactive extracellular matrix peptides on the differentiation of CG4 oligodendrocyte precursor cells, and to determine the ability of these synthetic peptides to enhance the expression of genes relevant to myelin production.

RESULTS

- **Proof of Concept**

Staining cells on PLO (CNPase and Myelin Basic Protein) show that cells cultured in the absence of growth factors express oligodendrocytic markers

Cultured with growth factors bFGF and PDGF

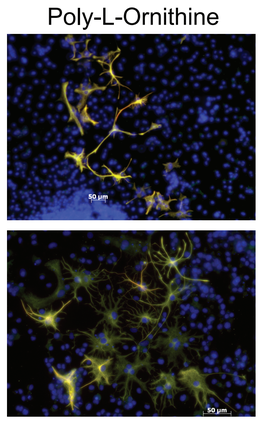
Cultured without growth factors

The images below are CG4 cells cultured on materials for 14 days. Stains for CNPase and Myelin Basic Protein (MBP) have been applied.

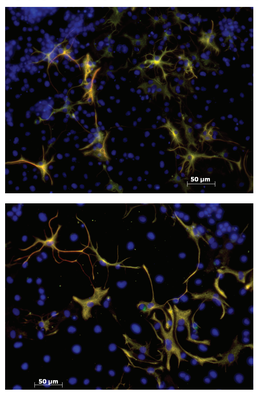
MBP = Red **CNPase = Green**

Positive Controls

Poly-L-Ornithine

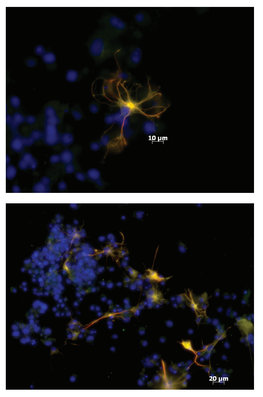


Tissue Culture Treated Glass



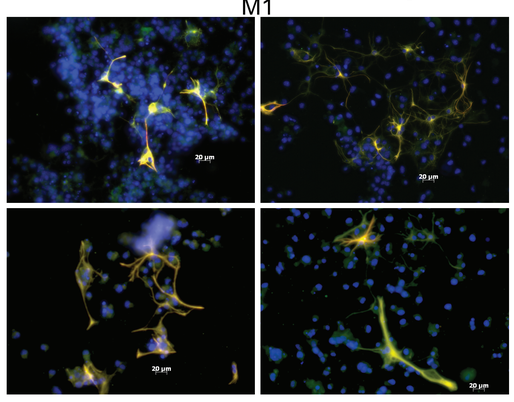
Negative Control

Native Silica

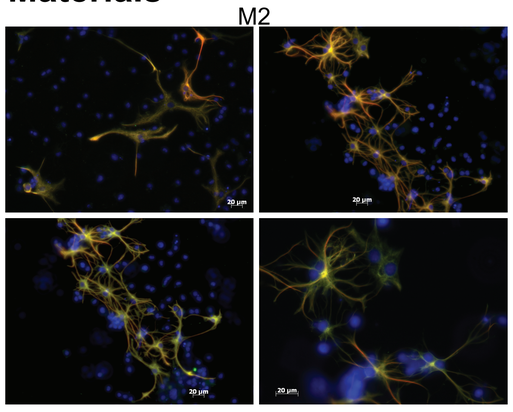


Experimental Materials

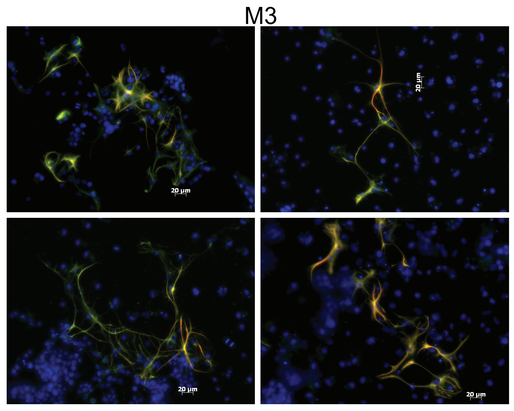
M1



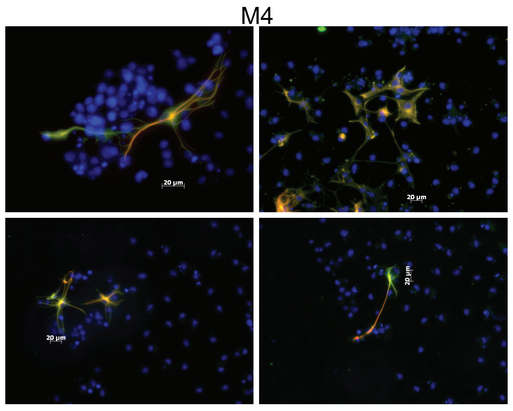
M2



M3



M4



CONCLUSIONS

- More cells positive for MBP on material 2, containing YIG and AGP, and on material 4, containing YIG, AGP, ANR, NID
- Indicates that these combinations of peptides may enhance oligodendrocyte differentiation

FUTURE WORK

- qt-PCR
- Repeat experiment using P19 cell line, another embryonic mouse cell line with a neural-oligodendrocytic potential

References

[1] Widmaier, Eric P., Hershel Raff, Kevin T. Strang. *Vander's Human Physiology The Mechanisms of Body Function*. New York: McGraw-Hill Science/Engineering/Math, 2007.

[2] Louis, J. C., E. Magal, M. Manthorpe, and S. Varon. "CG-4, A New Bipotential Glial Cell Line From Rat Brain, Is Capable of Differentiating In Vitro Into Either Mature Oligodendrocytes or Type-2 Astrocytes." J*ournal of Neuroscience Research* 31 (1992): 193-204.

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