INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Darilang Mawrie
Roll Number	:	10610601
Programme of Study	:	Ph.D.

Thesis Title: Isolation and Characterization of Mesenchymal Stem Cells from Ocular Adipose Tissue and Extra Ocular Muscle

Name of Thesis Supervisor(s)	:	Dr. Bithiah Grace Jaganathan
Thesis Submitted to the Department/ Center	:	Department of Biosciences & Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	25.01.2018
Key words for description of Thesis Work	:	Adult stem cells, cell therapy, MSC, multipotent, neuronal differentiation

SHORT ABSTRACT

Adult stem cells are cells capable to self-renew and are multipotent in nature. Mesenchymal stem cells can be isolated *in vitro* originally from the bone marrow but off-late they have also been isolated from various fetal and adult tissues such as the adipose, limbus, skin, intestinal tissue, various part of the eye, umbilical cord blood, umbilical cord, Wharton's jelly, and even placenta of the mother and the fetus. They expressed and are identified by an array of surface markers, can differentiate into mesodermal lineage owing to their origin and can on induction also differentiate into neural cells, especially into neuronal cells, and hepatic cells. They can escape the immunogenic effects of the host environment. Owing to these properties mesenchymal stem cells are proposed to be good candidates for cell therapy for neurological disorders.

OAT derived mesenchymal progenitors had been previously reported by (Korn, Kikkawa, & Hicok, 2009). We isolated OAT-MSC from central and medial orbital tissue of the upper eyelid. The MSC derived from both the tissues were spindle shaped and adherent in nature. They expressed mesenchymal markers on their cell surface and differentiated into adipocytes, osteocytes and chondrocytes. They were differentiated into neuronal cells and differentiation was confirmed by the expression of NGFR. We found out that central OAT-MSC had more capacity to differentiate into adipocytes and osteocytes compared to medial OAT-MSC.

We hypothesized that EOM have a high capacity to differentiate into neurons thereby we have isolated MSC from EOM for the first time and tested the *in vitro* neuronal differentiation potential. In the study, EOM tissue derived MSC were characterized and compared with bone marrow derived MSC. We found that EOM derived MSC proliferated as a monolayer, exhibited spindle shape, maintained a normal karyotype (2n = 46) in extended culture period, expressed important BM-MSC surface markers such as CD73, CD90, CD105 and HLA-2. They expressed high levels of embryonic transcription factors OCT4, SOX2, NANOG and NES in the undifferentiated state. They also expressed embryonic surface marker SSEA4 and their intracellular mitochondrial distribution pattern was similar to that of multipotent stem cells. Lipid droplets were formed after adipogenic differentiation, the osteocytes stained positive for ALP and alizarin red stain during osteogenic differentiation and micromass pellet was formed after chondrogenic differentiation. Genes expressed in mature cells were upregulated - ADIPONECTIN in adipocytes, OSTEOCALCIN in osteocytes and SOX9 in chondrocytes. The EOM-MSC differentiated more efficiently into neuroectodermal cells. They were able to generate neurosphere which were positive for GFAP and neurons were confirmed by the expression of MAP2b and NGFR. To further confirmed neuronal differentiation neuronal markers such as the NEUROD1, TUBB3, PAX6 and NESTIN were upregulated in MSC grown in neuronal media instead of growth media. Clones from single-celled EOM-MSC were selected and differentiated into neuroectodermal cells which was confirmed by NGFR expression.

The OAT derived MSC have been used in preclinical studies previously and controls the symptoms in few disorders but has never been tried in preclinical studies of neurodegenerative diseases. Thus, the EOM derived MSC and the OAT derived MSC could be good candidates for stem cell based therapies for treating neurodegenerative diseases.