

**A STUDY OF FETAL NEURONAL TISSUE GRAFT IN A HETEROTOPIC
TRANSPLANTATION SITE AND ITS IMPLICATIONS**

BY

BIMAL KRISHNA SAMANTA

**Thesis submitted for the degree of Doctor of Philosophy (Science)
Jadavpur University**

**Bijoygarh State General Hospital and Dept of Pharmaceutical Technology,
Jadavpur University, Kolkata 700032, India**

2008

**A Study of Fetal Neuronal Tissue Graft in a Heterotopic Transplantation
Site and its Implications**

By

Bimal Krishna Samanta

Supervisors :

Dr Niranjan Bhattacharya

D. Sc., MBBS, MD, MS, FACS (USA)

Prof Malay Chatterjee Ph.D., D.Sc.

**Thesis submitted for the degree of Doctor of Philosophy (Science)
Jadavpur University**

**Bijoygarh State Hospital, Jadavpur and Department of Pharmaceutical
Technology, Jadavpur University, Kolkata 700032, India
2008**

Certificate from the Supervisors

This is to certify that the thesis entitled “A Study of Fetal Neuronal Tissue Graft in a Heterotopic Transplantation Site and its Implications” submitted by Bimal Krishna Samanta, who got his name registered on 22.12.03 for the award of Ph.D. (Science) degree of Jadavpur University, is absolutely based upon his own work under the supervision of Dr Niranjan Bhattacharya and Prof Malay Chatterjee and that neither this thesis nor any part of its has been submitted for any degree / diploma or any other academic award anywhere before.

1.

2.

(Signature of the supervisors & date with official
seal)

Acknowledgement

My words fail to express my sincere gratitude to Dr Niranjan Bhattacharya, D.Sc., MBBS, MD, MS, FACS (USA), Former Superintendent, Bijoygarh State Hospital, my teacher, mentor and guide for his constant inspiration, valuable guidance and suggestions in my research career.

I also gratefully acknowledge the educational, scientific and moral support I have received from my Co-guide, Prof Malay Chatterjee, Ph. D., D. Sc., Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

I also express gratitude for the guidance and overall assistance I have received from the legendary Professor in Medicine, Prof M. K. Chhetri, and the outstanding Scientist, Professor Kanailal Mukherjee.

I also express my deep sense of appreciation and gratitude to Professor Sanjukta Bhattacharya of the Department of International Relations, Jadavpur University, for constant encouragement, unbiased suggestions regarding the overall objectivity and focus of the entire work.

I further express my appreciation for the overall support I have received from Prof Abha Sarkar of S. S. K. M. Hospital, Dr S. Upadhyay of A. M. R. I., Dr Nishikanto Halder of Vidyasagar Hospital, and Dr Mahua Bhattacharya, Dr Ranajit Nandi, , Dr A. B. Ghosh, Dr N. G. Bhattacharya, Dr Ujjwal Mani, Dr Banya Biswas, Dr Anita Chatterjee, Mrs Ananya Bhadra and Dr S. P. Das of Bijoygarh State Hospital, and for the technical support I have received from the experts of the Department of Instrumentation Science, Jadavpur University, in scanning electron microscopy.

I am also grateful to my wife, Mrs Aparajita Samanta, for her immense patience and support for my research work.

I wish to express my thanks to Mr Pinaki Nath and Mr Samir Majumdar for their assistance in preparing the manuscripts.

Lastly I am extremely grateful to the patients with advanced Parkinsonism who all supported my research work with dignity, patience and hope.

Kolkata, the , 2008.

(BIMAL KRISHNA SAMANTA)

DEDICATION

To

The memory of my departed father Late Lalit mohan Samanta.

List of Publications and Conferences Attended

PUBLICATIONS:

1. Samanta, B. K., Chandra, N. C., Ghosh, S. and Mukherjee, K. L. (1984). Aldose metabolism in developing human fetal brain and liver. *Experientia*. 40, 1420-1422.
2. Chandra, N. C., Bhattacharya, R., Samanta, B. K., Dutta, G. and Mukherjee, K. L. (1987). Accumulation of triacylglycerol in human foetal organs. *Ind. J. Med. Res.* 86, 401-409.
3. Samanta, B. K. and Mukherjee, K. L. (1989). Protein synthesis in human foetal brain and liver. *Ind. J. Med. Res.* 90, 69-76.
4. Ghosh, P. K. and Samanta, B. K. (1993). Studies on glycolysis and respiration on certain transplanted tumors in mice. *Ind. J. Physiol. & Allied Sci.* 47(1), 19-25.
5. Bhattacharya, N., Chhetri, M. K., Mukherjee, K. L., Ghosh, A. B., Samanta, B. K., Mitra, R., Bhattacharya, M., Bhattacharya, S. and Bandopadhyaya, T. (2002). Can human fetal cortical brain tissue transplant (upto 20 weeks) sustain its metabolic and oxygen requirements in a heterotopic site outside the brain? A study of 12 volunteers with Parkinson's disease. *Clin. Exp. Obst. & Gyn.* 29(4), 259-266.
6. Bhattacharya, N., Samanta, B. K., Bhattacharya, M. and Bhattacharya, S. (2004). Experience with human fetal cortical brain tissue transplant in adult neurodegenerative disorder. *Trends in Biomaterials & Artificial Organs. Proceedings of the National Conference of Biomedical Materials – 2002.* 17(2), 78-89.

CONFERENCES ATTENDED FOR THE LAST 11 YEARS
(poster presentation / oral presentation / as delegates):

1. XXIVth Annual Conference of the Association of Clinical Biochemists of India, 12-14 December, 1997, Science City, Kolkata.
2. 13th Annual Meeting of the Society of Biomaterials & Artificial Organs, India, 20-21 December, 2002, J.U., Kolkata.
3. 49th Annual Conference of the International College of Surgeons (Indian Section), 4-7 September, 2003, Chennai.
4. 63rd Annual Conference of the Association of Surgeons of India, 25-30 December, 2003, Pune.
5. 6th Annual Conference of the Association of Clinical Biochemists of India (West Bengal Chapter), 29th February, 2004, Institute of Child Health, Kolkata.
6. International Symposium on Advanced Materials and Processing, 6-8 December, 2004, IIT, Kharagpur.
7. 50th Annual Conference of the International College of Surgeons (Indian Section), 28-31 December, 2004, New Delhi.
8. Conference on Current Trends in Clinical Biochemistry and Workshop on Application of HPLC in the diagnosis of Thalassaemia and Haemoglobinopathies of Eastern Zone Association of Clinical Biochemists of India, 29-30 April and May 1, 2005, Vivekananda Institute of Medical Sciences, Kolkata.
9. Conference on Stress 2006 of National Referral Centre for the Prevention of Lead Absorption in India (West Bengal), 10-11 June, 2006, Vivekananda Institute of Medical Sciences, Kolkata.

Contents

	<u>Page No.</u>
Certificate from the supervisors	iii
Acknowledgement	iv
Dedication	vi
List of Publications and Conferences Attended	vii
Contents	ix
List of Figures	xii
List of Tables	xvi
Preface	xviii
CHAPTER – 1: Introduction	1 – 9
References	7
CHAPTER – 2: Materials and Methods	10 – 62
Methodology of the different tests done	12 – 57
HIV -1 and HIV -2 detection	12
Hepatitis B Antigen	14
Hepatitis C Antigen	17
VDRL Screening Test	18
Methods of Haemoglobin Determination	18
Determination of Total Counts and Hb Concentration	21
Determination of Erythrocyte Sedimentation Rate	22
ABO Blood Grouping	23
Rh Blood Typing	24
C – Reactive Protein Assay	24
Glucose Estimation	27
Urea Estimation	29
Creatinine Estimation	30
Bilirubin Estimation	32
Anti-ds DNA Estimation	34
Estimation of ANA	38
Ferritin Estimation	40

Histological Methods	43 – 51
Harri’s Haematoxylin and Eosin Method	45
Gomori Stain	47
PAS Technique	49
Giemsa Technique	51
Electron Microscopy	52
Scanning Electron Microscopy	52
References	58
CHAPTER – 3: Review of the Literature	63 - 107
Basics of Transplantation	63
Basic Stem Cell Biology	67
Embryonic Stem Cells	69
Fetal Stem Cells found in fetal tissue	72
Performance potential of fetal stem cells	74
Adult Stem Cells	74
Tests are used for identifying stem cells	75
Stem cells for the future treatment of Parkinson’s Disease	79
Transplantation of human embryonic dopamine neurons	79
References	98
CHAPTER – 4: Result and Analysis	108 – 205
Treatment protocol and results	111
Case studies and analysis	118
Histological Study	169 -179
Histological analysis	179
Scanning Electron Microscopy Photographs	180 - 205
Analysis of electron microscopy photographs	206
References	206
CHAPTER – 5: Discussion	207 –230

Secondary advantages of neuronal tissue transplantation in the present study	217
Improvement of aches and pain with fetal neuronal tissue transplantation	223
Weight gain and sense of wellbeing with fetal tissue transplantation	225
References	229
Summary and Conclusion	231
CHAPTER – 6: Bibliography	233 - 249
Appendix	250 - 253
Abbreviation used in the thesis	250
Unit abbreviation and prefixes used	252
Prefix used in international system of units	253
Departmental permission / Ethical committee / Animal prevention of cruelty permission and other ethical questions	254 - 279

List of Figures

<u>Figures</u>	<u>Page No.</u>
Figure – 2.1: An insect coated in gold, having been prepared for viewing with a scanning electron microscope	53
Figure – 2.2: Scanning electron microscope (JEOL JSM-5200).....	57
Figure – 3.1: Stem Cells	67
Figure – 3.2: Stimulation procedure of embryonic stem cells for differentiation	71
Figure – 3.3: Differentiation potentialities of adult stem cells	78

Figure – 3.4: Capability of stem cell transformation	80
Figure – 4.1: Post transplant (1 month) clinical improvement	118
Figure – 4.2: Sex-wise distribution of patients	119
Figure – 4.3: Age Group-wise distribution of patients	120
Figure – 4.4: Post-transplant (3 months) clinical improvement	125
Figure – 4.5: Post-transplant (6 months) clinical improvement	130
Figure – 4.6: Post-transplant (1 year) clinical improvement	139
Figure – 4.7: Post-transplant (2 years) clinical improvement (36 cases) ...	139
Figure – 4.8: L-dopa dosage in pre- and post-transplant (1 month) cases...	143

Figures

Page No.

Figure – 4.9: Dyskinesia rating (0-4) in pre-transplant and post-transplant (1 month) cases	143
Figure – 4.10: L-dopa dosage in pre-transplant and post-transplant (3 months) cases	46
Figure – 4.11: Dyskinesia rating (0-4) in pre-transplant and post-transplant (3 months) cases	146
Figure – 4.12: L-dopa dosage in pre-transplant and post-transplant	

(6 months) cases	149
Figure – 4.13: Dyskinesia rating (0-4) in pre-transplant and post-transplant (6 months) cases	149
Figure – 4.14: L-dopa dosage in pre-transplant and post-transplant (1 year) cases	152
Figure – 4.15: Dyskinesia rating (0-4) in pre-transplant and post-transplant (1 year) cases.....	152
Figure – 4.16: L-dopa dosage in pre-transplant and post-transplant (2 years) cases (36 cases)	156

<u>Figures</u>	<u>Page No.</u>
Figure – 4.17: Dyskinesia rating (0-4) in pre- and post-transplant (2 years) cases (36 cases)	156
Figure – 4.18: Leucocyte count in pre- and post-transplant (1 month) cases	161
Figure – 4.19: Blood urea level in pre- and post-transplant (1 month) cases	162

Figure – 4.20: Creatinine level in pre-transplant and post-transplant (1 month) cases	163
Figure – 4.21: Total bilirubin in pre-transplant and post-transplant (1 month) cases	164
Figure – 4.22: Fasting and PP blood glucose level in pre-transplant and post-transplant (1 month) cases	165
Figure – 4.23: Ferritin level in pre-transplant and post-transplant (1 month) cases	166
Figure – 4.24: CRP level in pre- and post-transplant (1 month) cases	166
Figure – 4.25: ANA level (index value) in pre-transplant and post- transplant (1 month) cases	167

Figures

Page No.

Figure – 4.26: Anti-ds DNA level in pre-transplant and post-transplant (1 month) cases	168
Histological micro-photographs (1-9)	170 - 178
Scanning Electron Microscopy Photographs (Figure – 4.27 to	

Figure – 4.52)	180 - 205
----------------------	-----------

List of Tables

<u>Tables</u>	<u>Page No.</u>
Table – 4.1: List of patients who took the fetal subcortical brain tissue transplant (48 cases) and post-transplant (1 month) follow-up...	113
Table – 4.2: Follow-up study after 3 months of transplantation (48 cases)	121
Table – 4.3: Follow-up study after 6 months of transplantation (48 cases)	126
Table – 4.4: Follow-up study after 1 year of transplantation (48 cases) ...	131
Table – 4.5: Follow-up study after 2 years of transplantation (36 cases) ...	135
Table – 4.6: Clinical impact of fetal subcortical brain tissue transplant after 1 month on dyskinesia rating scale and L-dopa dosage	140
Table – 4.7: Clinical impact of fetal subcortical brain tissue transplant after 3 months on dyskinesia rating scale and L-dopa dosage	144
Table - 4.8: Clinical impact of fetal subcortical brain tissue transplant after 6 months on dyskinesia rating scale and L-dopa dosage	147
Table – 4.9: Clinical impact of fetal subcortical brain tissue transplant after 1 year on dyskinesia rating scale and L-dopa dosage	150
Table – 4.10: Clinical impact of fetal subcortical brain tissue transplant after 2 years on dyskinesia rating scale and L-dopa dosage (36 cases)	153

Tables

Page No.

Table – 4.11: Bodyweight, haemoglobin content, total counts, urea, creatinine, total bilirubin content in pre- and post-transplant (1 month) cases (48 cases)157

Table – 4.12: Fasting blood glucose, PP blood glucose, C- reactive protein, anti nuclear antibody, anti-ds DNA, ferritin content in pre- and post-transplant (1 month) cases (48 cases) 159

Table – 5.1: Gain in weight and rise in haemoglobin value at 4 weeks from the date of transplant placement of patients who took the fetal subcortical brain tissue transplant (48 cases) 218

Preface

The most advanced research on the use of human fetal tissue has been done in Parkinson's disease, which affects about 1.5 million people in the U.S only. Patients with Parkinson's disease experience tremors, slurred speech and slowness of movement that eventually progresses to total paralysis. In this progressive, debilitating illness, the cells in a small part of the brain called the substantia nigra are destroyed, depriving the striatum (the part of the brain that controls movement) of a critical molecule called dopamine. Despite devastating loss of motor control, mental faculties in Parkinson's patients remain intact, and while the disease is in itself not fatal, patients often succumb to complications such as injuries from falls or pneumonia.

During brain development, one of the most important structures is the subventricular zone (SVZ), from which most neurons are generated. In adulthood the SVZ maintains a pool of progenitor cells that continuously replace neurons in the olfactory bulb. Neurodegenerative diseases induce a substantial upregulation or downregulation of SVZ progenitor cell proliferation, depending on the type of disorder. Far from being a dormant layer, the SVZ responds to neurodegenerative disease in a way that makes it a potential target for therapeutic intervention (1).

One of the most promising areas in medical research today is fetal tissue transplantation. At stake is a source for stem cells, progenitor cells harvested from human fetuses that can differentiate into any cell in the adult human body. This chameleon-like ability of stem cells makes them potentially useful in replacing critical cells in the adult human body that have been ravaged by injury or disease.

Fetal tissue transplants, in which such organ specific and nonspecific stem cells live in their natural environment, and are injected into the failing organs of patients, work on the premise that placed in the right environment, the

transplanted cells take their cues from their surroundings and develop into the needed tissue. Inject them into the brain, they become brain cells. Inject them into the pancreas, and they develop into pancreatic cells. Stem cells seem adaptable to such procedures, growing rapidly after transplantation, and secreting hormones and other chemicals that promote tissue growth. As an added bonus, these “master” cells are too undeveloped to be detected by the recipient’s immune system, and thus often avoid the rejection that plagues normal organ transplant procedures.

Fetal Tissue Used to Treat Diseases and Defects

Fetal tissue transplants are being investigated as treatments for a wide range of debilitating human conditions. Researchers hope to cure diabetes by regenerating insulin-producing pancreatic cells in diabetics, and blindness by regrowing retinal tissue in the eye. Scientists hope to develop better treatments for heart attack victims with fetal tissue used to regrow damaged heart muscle. Fetal tissue transplants also look promising for a variety of problems caused by destroyed nerve cells, such as Parkinson’s disease, Huntington’s Chorea, and even spinal cord injuries.

The crux of the method is the use of fetal stem cells to replace damaged tissue that the body itself cannot repair. For instance, paralysis is currently incurable because, once destroyed, the nerve cells of the spinal cord are not able to grow back. Researchers hope that stem cells can be used to bridge a spinal cord injury in much the same way as skin cells grow back to cover a cut. Although not ready to be tried in people, procedures that inject fetal tissue cells at spinal cord breaks have shown encouraging results in small animals, as in one study where scientists were able to get partially paralyzed cats to walk again. Similar experiments to regenerate nerve cells of the brain are also being investigated as cures for Huntington’s Chorea and Parkinson’s Disease, two diseases caused when specialized nerve cells in the brain begin to die off.

The first line of treatment for Parkinson's disease is drug therapy. Unfortunately, L-dopa, a precursor of dopamine which can be absorbed by the brain, helps only as long as there are some substantia nigra cells still alive to absorb the drug. Once that area of the brain is destroyed, L-dopa becomes ineffective, which until recently left the patient without any available treatment for this disorder. Now, in certain centres of excellence, pioneering fetal tissue transplants into the brain of Parkinson's patients show promise in slowing or even reversing symptoms of the disease. In this treatment, cells from the pre-brain structures of 6-8 week old fetuses are injected into the patient's striatum, where if all goes well they grow into a bundle of nerve cells that produce the needed dopamine. Patients with successful fetal tissue transplants have shown remarkable improvement in the severity of tremors and in their ability to move.

With such exciting results and millions of people in this country alone suffering from Parkinson's and other diseases that may be helped by fetal tissue transplants, patients and their advocates are urging further research into the use of stem cells. However, currently the only reliable source of fetal stem cells is selectively aborted human fetuses, collected from abortion clinics with the permission of the mother.

The present thesis examined the safety aspects of fetal neuronal tissue transplantation at a subcutaneous heterotopic site under local anaesthesia in different patients with severe idiopathic Parkinsonism, not responding to conventional drugs. All the cases passed through the voluntary consent protocol and were cleared by the Institutional ethical committee of the hospital.

Reference:

Curtis M. A., Faull R. L. M., Eriksson P. S. (2007) The effect of neurodegenerative diseases on the subventricular zone. Nature Reviews Neuroscience. 8, 712-723.