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Correspondence

All correspondence concerning the Journal should be addressed to:

Honorary Secretary

National Academy of Medical Sciences (India)

NAMS House, Ansari Nagar, Mahatma Gandhi Marg, New Delhi-110029

Tel.: 011-26589289 Email: nams_aca@yahoo.com

Website: www.nams-india.in

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In Memorial

During the year two most distinguished Fellows of the Academy, Dr. C.S. Bhaskaran and Dr. APJ Abdul Kalam died. Both are well known for their scientific research and love for teaching. Dr. APJ Abdul Kalam took it as a mission to teach students. He died while delivering a lecture at the Indian Institute of Management, Shilling. We pay our tributes to both of these scholars. May God grant them *Mukti* for what they did for the profession and for the students.

Editorial

The present issue of the Annals include some of the articles which reflect scientific excellence and development of new methodology in the prevention and new methods of treatment. The articles on 'Recent Concepts on Myocardial Regeneration' by Dr. Balram Airan and 'Biological Basis and Molecular Mechanism of Regeneration' by Dr. Sujata Mohanty emphasize role of Stem Cells in tissue regeneration. Gen. Amir Chand Oration delivered by Dr. Gayatri Rath on 'Potentiation of Curcumin and Wnt/β-catenin signaling in breast cancer' supports the use of Curcumin as natural dietary product in breast cancer treatment.

This volume is dedicated to the memories of **Late Dr. C.S. Bhaskaran** and **Dr. APJ Abdul Kalam** who have contributed immensely towards uplifting the Biomedical Sciences to the globally recognized new dimensions.

The contributors to this volume are outstanding and are dedicated to a life-long rigorous research keeping in view the national health needs which is also a significant objective of the National Academy of Medical Sciences (India).

Emeritus Editor

Prof. J.S. Bajaj

Dr. C.S. Bhaskaran

FAMS

Prof. C.S. Bhaskaran was a renowned Microbiologist and a Medical Educationist. Born on December 9, 1932, he graduated from Medicine from Madras University, and took M.D. (Bact & Path) from Andhra University. He had advanced training in Microbiology in London School of Hygiene & Tropical Medicine, London. He served as Professor of Microbiology for over 2 decades and worked as Additional Director and Director of Medical Education, Director, Institute of Preventive Medicine and Public Health Laboratory Services and Vice Chancellor of N.T.R. University of Health Sciences in Andhra Pradesh. He was an outstanding teacher, excellent research worker and an able administrator. He had trained several generations of postgraduate students and published research papers in journals of high impact.

He was the recipient of Fellowships of the Royal College of Pathologists, London; National Academy of Medical Sciences (India); Association of Basic Medical Sciences of India; Indian College of Pathologists; and A.P. Academy of Sciences. He was conferred Honorary Doctorate in Science (DSc. h.c), N.T.R. University of Health Sciences. He also received Dr. B.C. Roy National Award in the Category of Eminent Medical Teacher (MCI), Dr. H.I. Jhala Award, Dr. Siva Reddy Award, Dr. Shankar Benerjee Memorial Award.

He delivered Dr. Govinda Reddy Oration (NTR UHS), Dr. V.R. Khanolkar Oration (1999-2000) & Dr. B.K. Anand Oration (2009-10) of the NAMS and Dr. Lalitha Kameswaran Oration (Dr. MGR Tamil Nadu Medical University).

He was associated with several National and International Organizations such as MCI, ICMR, NBE, Planning Commission and Ministry of Health and Family Welfare, Govt. of India and WHO. As a recognition of his professional excellence and leadership, he was elected as the President of the Indian Association of Medical Microbiologists.

Dr. C.S. Bhaskaran had been associated with National Academy of Medical Sciences since his election as a Fellow in 1972 and was elected as Emeritus Professor of the Academy in recognition of proven track record of professional excellence and subject expertise of a high order. He had served the Academy with distinction and was Council Member (2006-09, 2009-12) and Vice-President of the Academy (2002-03, 2010-2012). He was elected and installed as President of the Academy in October 2012, and presided over Annual General Body meetings held in October 2013 at Jodhpur and October 2014 at Rishikesh.

With his qualities of equanimity, academic eminence, and professional excellence, Prof. C.S. Bhaskaran was a source of inspiration to the Academy, providing high quality of leadership. He was awarded Life Time Achievement Award in 2014 but he insisted that he will receive the award on the completion of his term in 2015.

His death in the early hours of July 6, 2015 left an irreplaceable void. We pay homage and pray to bestow peace on the departed soul and give strength to Mrs. Dr. Bhaskaran to bear this profound loss.

(Prof. J.S. Bajaj)

A Homage :

Dr. APJ Abdul Kalam

I had the unique privilege of meeting the two great scientists who pioneered the Space and Missile Technology in the country and brought India to the 5-6 Super Nations of the World. It was on 28 March, 1981 at the Investiture Ceremony of Padma Awards at the Rashtrapati Bhavan when Prof. Satish Dhawan was conferred Padma Vibhushan. ***The citation of Dr. Satish Dhawan made a special reference to the significant role he had played in building up the scientific and technological capability in the country in the field of space research and led a multi-disciplinary team of Indian scientists with eminent success.***

Dr. APJ Abdul Kalam was a recipient of Padma Bhushan. ***His citation highlighted a major contribution for the development of the first Indian Satellite Launch Vehicle SLV-3***.

I consider that it was my good fortune that I received Padma Shri at the same Investiture Ceremony and had the unique privilege of meeting these two great scientists of our country.

A highly gifted research and development engineer, Shri Abdul Kalam has made outstanding contributions to the National Space Programme and also to many institutions connected with aviation and advanced technologies.

Further development of Missile project was established with indigenous design capability for the guided missile systems, which were as good or better than missile systems in the world.

He was recipient of Bharat Ratna in 1997.

It was during the tenure of my Presidentship when he was elected Fellow of the National Academy of Medical Sciences in the field of Bio-Medical Engineering. I had the privilege to invite him to deliver a lecture in the Scientific Programme of 33rd Annual Conference of NAMS held on 19 March, 1994 at Tata Memorial Hospital, Bombay. The invitation was extended because of the fact that I was impressed by the development of low-weight (about 400 gm.) caliper instead of 4 Kg. which was being used for young polio affected children which he had developed as a spin-off of technology using the light material developed for the cones of missiles. Most remarkably the cost of lower weight caliper was about 1/4th of those which were being used earlier.

Immediately after following his lecture at Bombay on 19 March, 1994, I requested him to submit his bio-data for election as Fellow of the National Academy of Medical Sciences. He submitted his nomination on 31 March, 1994 and was elected a Fellow of the Academy in the discipline of Bio-Medical Engineering.

It was my privilege that we met several times in the Planning Commission in the year 1993-94 to discuss the medical application of missile technologies.

Even after becoming the President of India in 2002 and subsequently his major interest remained in the teaching, research and welfare of the children.

His death on 27 July, 2015 was at the time when he was delivering a lecture to the young students at the Indian Institute of Management, Shillong, continuing his mission of inspiring young minds.

(Prof. J.S. Bajaj)

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Text of the address by the President Dr.C.S. Bhaskaran, delivered at the Annual Convocation on 26th October 2013 at Jodhpur

Our esteemed Chief Guest of today's function Prof. R. Chidambaram, Principal Scientific Advisor and former Chairman, Atomic Energy Commission, our respected guest of Honor Prof. J.S. Bajaj, Emeritus President and Chairman, Academic Council, National Academy of Medical Sciences (India), an eminent cardiologist and the Past President Dr. S. Padmavati, NAMS Life Time Achievement Awardee, distinguished Members of the Council, Dr. Sanjeev Misra, Director, AIIMS – Jodhpur & the Chairman Organizing Committee, Dr. Sanjay Wadhwa, Hony. Secretary, NAMS and Dr. Kuldeep Singh, Secretary, Organizing Committee, distinguished Fellows & Members, newly elected Fellows and Members, friends, delegates, guests, ladies and gentlemen, I, as the 23rd President of the NAMS extend to you my warm greetings and welcome you all to the 53rd Annual Conference of the National Academy of Medical Sciences (India) being held at All India Institute of Medical Sciences, Jodhpur. We are indeed grateful to you Sir, for having spared your precious time for accepting our invitation to be the Chief Guest of today's function and grace the occasion, in spite of your busy schedule. We thank you for the same and for the support and goodwill.

We are very happy that this conference is being held in this historic city of Jodhpur, the former capital of Marwar state and presently the second largest city of Rajasthan State. It has become one of the major educational centres of learning and higher studies and research in India. The venue of our conference, the AIIMS - Jodhpur is one of the six newly started All India Institutes of Medical Sciences. This campus under the Directorship of Prof. Sanjeev Misra has provided us an excellent ambience and amenities for this Annual Conference. The Scientific Program of the conference is packed with two symposia, series of orations, award paper presentations coupled with free and poster presentations.

The National Academy of Medical Sciences (India) is established to promote knowledge in the field of medicine and allied disciplines, enhance the quality of professional education, identify the research potential, maintain high standards of professional and ethical practices, encourage medical and biomedical scientists in our country by recognizing their work and stimulate them to pursue research of the highest order, extend its application to the national health priorities, and to actively participate in the planning and formulation of national health programs. Our Academy is recognized by the Government of India as the nodal agency

for Continuing Medical Education for enhancing the knowledge and skills of the medical and paramedical professionals in the country.

It was in the year 1959 the Government of Andhra Pradesh initiated the proposal to establish an Academy of Medical Sciences. The Government of India recognizing the need has established the National Academy of Medical Sciences on par with other National Institutes of India. It was registered in 1961 as "Indian Academy of Medical Sciences" , later changed to "National Academy of Medical Sciences (India)" and inaugurated by the then Prime Minister Late Pandit Jawaharlal Nehruji. The first convocation was held on 8th December 1963 which was addressed by Dr.S. Radhakrishnan, the then President of India. In his convocation address he stated, *"I hope this Medical Academy which represents different sciences with men of achievements in their respective spheres, which offers incentives to men of promise that they can also get their distinctions, will be regarded as something to which all our youngsters will look forward to"*. He further said, *"A Fellowship of the Academy must be a matter of honor and not a matter of maneuvering or intrigue but a manner of straight forward work which is acknowledged as first class in nature. That should be the quality which we should encourage"*. This continues to be the principal directive of the Academy till to day and I hope it will continue for ever.

The Academy has been scrupulously fulfilling its objectives over the past five decades by following the traditions set by our eminent predecessors. In pursuance, every year we recognize outstanding contributions made by our biomedical scientists belonging to various disciplines in medicine and allied sciences and to confer Fellowships and Memberships to nurture and encourage talent, to award Academy Orations for the outstanding contributions in the field of medical education, research and health services and related sciences and Academy Awards to promote scientific advancement in the field of biomedical research and other commendations. These conferments and awards of the Academy has become a brand name for a highly cherished distinction conferred after a rigorous peer review process and it has been our endeavour to keep the excellence as the primary goal. At present we have 3 Hony. Fellows, 822 Fellows, 1685 elected members (MAMS) and 3707 registered members (MNAMS) on the live register of the Academy. This year 30 Fellows and 58 Members were elected.

The Academy has established a Life Time Achievement Award to honor Fellows who have made outstanding contributions to the growth and development of biomedical sciences over the past several decades and we are indeed very happy that Dr. Sivaramakrishna Padmavati, an eminent cardiologist, a very senior Fellow and the Past President of the Academy is conferred with this prestigious award for the year 2011 at this convocation and the Academy is greatly

honored by her gesture in accepting the award and we are grateful to her for the same.

During this Annual Conference we will have the privilege to listen to 10 orations by eminent personalities and 7 paper presentations by the NAMS Awardees and the Golden Jubilee Commemoration Award Lecture by the youngest Fellow of the year. Of these Dr. Janaki Memorial Oration and Dr. S.S. Sidhu Award and Dr. Vinod Kumar Bhargava Award are instituted this year. In addition two other important well structured programs in the fast developing areas of specialization one as CME – Regional Symposium on "Sleep Medicine" and the other as NAMS Scientific Symposium on "Regenerative Medicine" were held yesterday and this morning respectively.

I am happy to inform you that the National Development Council (NDC) had approved the 12th Plan Proposals of the NAMS with a recording that agencies such as NAMS can play useful role in developing programs for continuing medical education in the country. We greatly appreciate the efforts of Prof. Bajaj in ensuring that the role of NAMS is recognized by the NDC. Further steps are being taken to implement the proposals as indicted in the 12th Plan document.

CME Programs:

In order to fulfill the mandate, the Academy is giving top priority to Continuing Medical Education Programs (CMEs) and organizes them in the form of seminars, symposia and workshops in different specialties in different parts of the country and monitors the programs. All the CME topics are so chosen keeping in view of the national health relevance. We have two types of CME programs, one national/regional/ intramural programs planned and monitored by the NAMS Academic Committee and the other is extramural programs organized by the institutions / medical colleges, screened and reviewed by the NAMS CME Program Committee.

During the year 2012 -13, two intramural CME Programs -viz NAMS-PGI National Symposium on "Non-Alcoholic Fatty Lever Disease in Children and Adolescents" and NAMS-NFI National Symposium on "Micronutrient Deficiencies" were organized at PGI, Chandigarh and Nutrition Foundation of India, New Delhi respectively and 18 extramural CME programs were organized by various Medical Institutions in the country.

From this year 2013-14, it is proposed to organize at least 5 National/Regional Symposia /Intramural CMEs and 20 extramural programs every year on identified priority areas in health. So far, 3 Intramural programs viz CMEs on "Spina Bifida", "Ethics in Clinical Research" and a Symposium on "Sleep Medicine" were held at the All India Institutes of Medical Sciences, Rishikesh, Bhubaneswar and, Jodhpur respectively and 5 proposals are under process. Similarly, 5 extramural CMEs were held

so far this year and 3 are under process. Efforts are being made to get the NAMS CME Programs recognized by State Medical Councils and MCI to award credit hours to the participants attending NAMS CME programs.

We are also taking necessary steps to enhance the Learning Resource Material (LRM) availability through NAMS WEB SITE / Monographs etc., to establish a LRM Library and to streamline the publication of the Annals of the Academy.

Teleconnectivity:

NAMS has made a major effort to improve the outreach of CME program by establishing tele-linkages. The NAMS-PGI centre for Tele-education in the health sciences is the first centre to get connected to the medical colleges in Punjab, Haryana and also to some district hospitals in Punjab and Himachal Pradesh. Encouraged by this successful outcome, this year the NAMS-PGI Symposium was tele-linked with Shimla and Tanda with great success. We are making further efforts to optimally utilize the IT enabled services to increase the out reach of CME programme to more and more medical colleges through the National Knowledge Network.

NAMS-AIIMS Collegium:

The Academy in collaboration with the newly established All India Institutes of Medical Sciences in Jodhpur, Bhopal, Raipur, Patna, Bhubaneswar and Rishikesh has formed a NAMS-AIIMS collegium to facilitate academic cooperation between the NAMS and AIIMS and to strengthen the academic activities. For the present, it is proposed that each AIIMS will be conducting one symposium/CME during the year on selected topics on the basis of the priority areas as identified in the 12th Five-Year Plan.

Collaboration with Universities:

The NAMS in the year 2007 has signed a MOU with Guru Nanak Dev University, Amritsar. On similar lines, a MOU had been signed by the Academy with the Central University of Punjab, Bhatinda this year. The efforts are being made to enter into an agreement with other universities for training of middle level scientists in various advanced techniques. It is also contemplated to explore the possibility of developing academic collaboration with Universities of Health Sciences in India in order to evolve holistic and comprehensive strategy for improving the quality of medical education in the country.

During this year, the important developmental activities and achievements with particular reference to a) conceptualization of both the symposia held at this conference b) approval of the 12th Plan NAMS document, c) formation of NAMS-AIIMS Collegium and d) signing up of MOU with Central University of Punjab, Bathinda are entirely due to untiring efforts of Prof. Bajaj, Chairman, NAMS Academic

Council and but for him these would not have been accomplished. We are very grateful to him and in recognition of yeoman services rendered by him to the Academy so far and also appreciating his continuing efforts to strengthen the Academy, the NAMS Council at its last meeting resolved to confer the designation of "Patron" on Prof. J.S Bajaj, as a befitting gesture.

The Academy has also formulated schemes to mentor the post graduates and younger members of the faculty, junior and middle level specialists in different disciplines in various academic institutions by utilizing the expertise of the Emeritus Professors and senior Fellows of the Academy, to award travel fellowship to promote skill up-gradation in emerging technologies and acquire new skills at the chosen centers of excellence, to give Financial Grant for Travel to NAMS awardees to encourage participation of young professionals to present Scientific and Research Papers. Several professionals have already been benefited from these programs. The Academy also takes active part in formulating and developing the policy strategies and programs under Health and Family Welfare by participating in the Expert Group meetings and Consultative deliberations of the Government of India.

Presently, most of the medical colleges in our country with few exceptions are finding it difficult to impart right quality and quantity of medical education, the reasons for this being many and varied, which we need not go into at this juncture. However the need of the hour is get ourselves updated with the latest trends and keep abreast with the modern innovative technological practices and diagnostic methods. Though our Academy is over 50 years old, it is the ideally suited organization now geared up to meet these challenges to fullfill the demands and aspiration of the public at large and the medical profession in particular.

We have just now presented the scrolls to the newly elected Fellows and Members and felicitated Orators and Awardees including the Life Time Achievement Awardee. I on behalf of the Academy and on my own behalf heartily congratulate them for the distinctions conferred on them by the Academy on this memorable occasion.

We are very grateful to Dr. Sanjeev Misra, Director of the Institute and Dr. Kuldeep Singh, Additional Professor & Head Department of Pediatrics, AIIMS, Jodhpur and their team for having worked tirelessly to make this conference a truly memorable one.

Before I close, I would like to acknowledge the guidance, help and co-operation from our past Presidents Prof. J.S.Bajaj, Dr. Snehalata Deshmukh, Dr. P.K. Dave, Dr. Prema Ramachandran, Vice-President Dr. Manorama Berry, Treasurer Dr. Kusum Verma, other Members of the Council, Dr. Sanjay Wadhwa. Hony. Secretary - NAMS, and

all the staff of the Academy.

My special thanks are due to Prof. J.S.Bajaj who gave me the inspiration and direction as a friend, philosopher and guide which enabled me to discharge my functions during this year. Finally I, on behalf of the Academy and on my own behalf, sincerely express our grateful thanks to our honoured Chief Guest Prof. R. Chidambaram for having spared his precious time and for sharing his wisdom and thoughts with us today, which I am confident will go a long way to strengthen our Academy.

Thank you.

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Recent Concepts in Myocardial Regeneration

Sujata Mohanty[1], Sandeep Seth[2], Balram Bhargava[2], Balram Airan[3]
Stem Cell Facility[1], Department of Cardiology[2],
Department of Cardiovascular and Thoracic Surgery[3],
All India Institute of Medical Sciences (AIIMS), New Delhi, India

ABSTRACT

Functional restoration of the damaged heart presents a challenge as the available treatment options do not help in reduction of scar size after myocardial infarction or significant improvement of an impaired cardiac pumping ability in Heart Failure (HF). Nowadays, Stem Cell technology is rapidly gaining popularity as a way to improve the prognosis of patients with coronary artery disease and HF. Ideally, transplanted cells would mimic the lost myocytes morphologically and functionally. Therefore, we at AIIMS undertook following studies to evaluate the safety and efficacy of Stem Cell (SC) injection in Acute Myocardial Infarction (AMI) and Dilated Cardiomyopathy (DCM). A) To Study the Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection. B) Intracoronary stem cell implantation in patients with dilated cardiomyopathy. (B.1) pilot study- 6 months (B.2) final long-term (3-year) follow-up. C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with AMI - MI3 trial.

Keywords: Acute Myocardial Infarction, Dilated Cardiomyopathy, Heart Failure, Stem Cells.

Correspondence : Professor Balram Airan, Chief, CardioThoracic Centre, All India Institute of Medical Sciences, New Delhi, India 110029. Email: iactscon_2004@yahoo.co.in, Tel: 011-26593373.

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the world's leading causes of mortality. Cardiomyocytes are considered to be terminally differentiated and cardiac injury causes permanent myocardial loss resulting in cardiac dysfunction. There is loss of myocardial tissue during Myocardial Infarction (MI) which results in scar formation, progressive remodelling of the left ventricle (LV), and development of ischemic cardiomyopathy (ICM).

Myocardial infarction (MI) secondary to coronary artery disease is a leading cause of morbidity and death throughout the world. Although reperfusion therapies have provided dramatic advances in the treatment of acute MI, a substantial fraction of patients is not able to undergo successful reperfusion promptly. In patients having large MIs, loss of more than a billion cardiomyocytes can occur, overwhelming the hearts intrinsic reparative capacity. Without further intervention, the damaged myocardium is replaced by fibrous non-contractile tissue (scar), and the resulting left ventricular (LV) dysfunction can initiate a spiral of adverse remodeling progressing to end-stage heart failure.

The current conventional therapeutic strategies ameliorate the symptoms of heart failure but at the same time they fail to reconstitute the dead myocardium with functional new cardiomyocytes and vessels, ultimately failing to show any major improvement. Heart transplantation is an effective means of treating patients with heart failure. But the vast majority of patients are restricted by the age, the donor, surgical complications, medical costs, and so forth. In the search to improve the outcomes in patients with CVDs, a new approach has gained momentum during last few years which is repair of CVDs with Stem Cells. Cellular cardiomyoplasty is a new potential therapeutic approach that uses exogenous cells to repair regions of damaged myocardium. There are number of sources of Stem cells which have been investigated for cardiac therapy **(Table 1).**

The first cell source studied in detail in both animal models and humans was autologous skeletal myoblasts. Although myoblasts formed stable grafts in the heart, they failed to differentiate into cardiomyocytes and were unable to improve myocardial function. Autologous bone marrow mononuclear cells, which poses a broader differentiation potential than myoblasts, have also been tested in animal models and clinical trials. A range of rodent post-MI studies provided clear evidence of functional benefit resulting from transplantation of bone marrow–derived mononuclear cells to the infarcted myocardium; however, the mechanisms of benefit have been debated. Despite questions about underlying mechanisms, bone marrow mononuclear cells are being broadly tested in early stage clinical trials with results ranging from a small benefit on cardiac functional parameters to no significant effect (1,2). Patient-specific cardiac stem cells

Table 1. Cell Sources Investigated For Cardiac Therapy

Autologous	Allogeneic
Skeletal myoblasts	Fetal cardiomyocytes
Bone marrow-derived cells	Embryonic stem cells and derivatives
c-kit^{+}lin^{-}	
Bone marrow mononuclear cells	
Endothelial progenitor cells	Mesenchymal stem cells*+
CD34+	
CD133+	
Cardiac stem/progenitor cells	Parthenogenetic stem cells and derivatives
Side population	
C-kit+	
Cardiosphere derived	
Epicardial progenitors	
Spermatogonial stem cells and derivatives	
Induced pluripotent stem cells and derivatives*	

*can be both autologous and allogeneic.
+can be derived from multiple tissues, including bone marrow and adipose.

isolated from the adult heart hold promise (3,4). Experiments in animal models and more recently in early stage clinical trials have shown encouraging results testing autologous cardiac stem cells and cardiosphere-derived cells (5,6). However, scalability, senescence and dysfunction secondary to the underlying pathology are major potential limitations for cardiac stem cells (7,8). Alternatively, the use of mesenchymal stem cells (MSCs) has been investigated. Improved heart function following the transplantation of mesenchymal stem cells (MSCs) has been reported in animal models of acute MI as well as in clinical studies on patients with heart failure (9). Various favourable characteristics, such as multilineage differentiation potential, ability to evade the host immune system, immunomodulatory capacities and ease of proliferation *in vitro*, make MSCs particularly attractive for cell therapy (10). It has been well established that MSC infusion improves the function of infarcted myocardium (11). MSCs can be derived from a variety of tissues, including bone marrow and adipose. These cells can be extensively expanded in culture and exhibit apparent immune privilege.

MSCs transplanted post-MI animal hearts have shown benefits, which seem to be primarily paracrine in nature (12,13). MSCs have also been tested in early phase clinical trials, including MSCs treated with a cardiogenic cocktail, and show signs of functional benefits (14,15). **Table 2** highlights the different cell sources used for Cellular Cardiomyoplasty and their limitations.

Table 2. Cellular Cardiomyoplasty: Cell Sources and Limitations

Cell Source	Ethical Problems	Acquisition Concerns	Rejection	Oncogenicity
Fetal cardiomyocytes	Yes	Yes	Yes	No
Adult cardiomyocytes	No	Yes	No	Yes
Skeletal Myoblast	No	Yes	No	No
Embryonic Stem Cell	Yes	Yes	No	Yes
Marrow Stromal Cell	No	No	No	No

Clinical Trial Using MSCs:

A team from Rigshospitalet University Hospital Copenhagen (Copenhagen, Denmark) at the American College of Cardiology's 63rd Annual Scientific Session has suggested that heart failure patients may benefit from a new treatment in which stem cells derived from bone marrow are injected into the heart.

This study is the largest placebo-controlled, double-blind randomized trial to use mesenchymal stromal cells injected directly into the heart muscle to treat patients with chronic ischemic heart failure. A total of 59 patients with chronic ischemic heart disease and heart failure were included in the study. A small amount of bone marrow was extracted from each patient and the mesenchymal stromal cells were then isolated and induced to self-replicate. Patients were then given an injection containing either a saline placebo or their own cultured mesenchymal stromal cells directly into the heart muscle via a catheter inserted in the groin; a procedure requiring only local anesthesia. After 6 months, treated patients showed an 8.2-ml decrease in end systolic volume, the study's primary end point and a key measure of the heart's pumping ability. An increase in end systolic volume of 6 ml was observed in patients in the placebo group.

These results support previous findings from smaller studies that demonstrated reduced scar tissue in the heart in patients treated with stem cells. Researchers will now continue to monitor these patients in order to evaluate the long-term outcomes. A larger, Phase III clinical trial is now required in order to progress towards the acceptance of this treatment for widespread use in patients with ischemic heart failure (*Regen. Med.* (2014) 9(3), 255–257).

Embryonic Stem Cells & CVDs :

Embryonic stem cells (ESCs) provide another allogeneic cell source investigated for post-MI therapy and tested in animal models. ESCs have undoubted potency to generate all cell types present in the heart. ESCs and their derivatives have shown functional benefit in various animal MI models. Nevertheless, concerns about immune rejection, safety and the embryonic source of these cells have delayed clinical applications. In addition, other pluripotent stem cell sources, including spermatogonial stem cells and parthenogenetic stem cells, have been

suggested as potential cell sources for cardiac repair, but little data exist at this time on these cell sources and particularly with regard to human cells.

The recent discovery of induced pluripotent stem cells (iPSCs) and other advances in reprogramming technologies, such as induced cardiomyocytes, have provided more potential avenues for cardiac repair. Because iPSCs are produced by reprogramming somatic cells, such as dermal fibroblasts, they can provide autologous cells for patients, reducing the risk of immune rejection. Another promising feature of iPSCs is that they can be extensively expanded for the production of large quantities of potentially any cell type desired to repair the myocardium. Initial studies have begun to examine the use of iPSCs and their derivatives for cellular therapy to treat a variety of diseases using animal models (16).

A number of pre clinical studies have been done in MI model using iPSCs and their derivatives (**Table 3**).

Stem cells are the origin cells of various mature cells. They have the potential of self-renewal and differentiation. Either immediately after isolation or after expansion *in vitro*, stem cells are transplanted into a specific region of the heart, and ultimately replace and repair the myocardial necrosis or pathological cells; then the aim of curing heart failure can be achieved and it has brought a bright prospect for the treatment of heart failure. Many clinical trials using the stem cell transplantation for acute and chronic heart failure have been carried out. The interest is based on the assumption that left ventricular dysfunction is largely due to the loss of a critical number of cardiomyocytes and

Table 3: Preclinical Studies of Post –MI Cell Therapy Using iPSCs and Derivatives

References	Cell Source For Reprogramming	Reprogramming Method	Cell Types Transplanted	Delivery Method	Animal Model	Duration Of Study	Summary Of Results
Nelson et al	MEFs	Lentiviral-human (KOSM)	iPSCs	Intramyocardial injection	Mouse	4wk	↑Ventricular function↓ pathological remodeling. engraftment and differentiation into CMs SMs, ECs. No teratomas in immunocompetent mice. Teratomas in immunodeficient mice
Singla et al	Mouse H9c2 cardiomyoblasts	Plasmid-mouse (KOSM)	iPSCs	Intramyocardial injection	Mouse	2wk	↑Ventricular function↓ apoptosis, No teratomas in immunocompetent mice
Templin et al	Human cord blood	Lentiviral-human (NOLS)	iPSCs	Intramyocardial injection	Pig	12-15 wk	iPSCs coinjected with human MSC survived and differentiated into endothelial cells. iPSCs injected alone failed to survive. Pigs were immunosuppressed

that it may be partly reversed by implanting new contractile cells into the post infarction scars or regions of wall thinning.

Recently, the results of a few small trials were reported that suggest a beneficial role of stem cell therapy in nonischemic dilated cardiomyopathy (17-19). Kalil et al. (17) showed intramyocardial transplantation of bone marrow stem cells in dilated cardiomyopathy patients with improvement in functional class without improvement in left ventricular function. Wang et al. (18) also found similar results after intracoronary infusion of autologous mesenchymal stem cells. Kaparthi et al. (19) showed improvement in functional status and left ventricular function after intracoronary autologous bone marrow mononuclear cells infusion. Resident CMPCs by contrast have only relatively recently been identified, but are already generating excitement because they appear to differentiate into bona fide cardiomyocytes *in vitro* with high efficiency. This is exceptional for any adult stem cell source studied to date.

While experimental studies and early phase clinical trials tend to support the concept that cell therapy may enhance cardiac repair,several key issues still need to be addressed before introduction into routine clinical practice. These include (1) the optimal type of donor cells in relation to the clinical profile, (2) the mechanism by which cell engraftment improves cardiac function, (3) optimization of cell survival, (4) development of less invasive cell delivery techniques, and (5) the relevance to non-ischaemic heart failure.

As, there was no clinical data on the role of stem cell therapy in nonischemic dilated cardiomyopathy; therefore we undertook a pilot study of intracoronary stem cell implantation in patients with dilated cardiomyopathy. From a cohort of 44 patients, 24 were randomly allocated to the stem cell therapy arm and 20 to the control arm. There was a significant improvement ($p < 0.001$) in NYHA (New York Heart Association) functional class in the treatment arm, with 16 patients (62%) improving by at least 1 functional class, as compared to only 2 patients (10%) improving in the control arm.

In continuation with the previous study, next study was again in DCM patients (n=85) with a follow up of 3 years. The EF improved in the treatment arm by 5.9% with a reduction in end-systolic volumes and no change in end-diastolic volumes. There was no significant improvement in the EF in the control patients.

MATERIALS & METHODS

A) Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection:

Patients with Coronary artery disease (CAD) undergoing CABG and with reduced LV function with an area of nonviable myocardium were included. 86% had a documented previous MI, 43%

had hypertension, 36% were chronic smokers and 14% had diabetes.

Materials & Methods:

35-40 ml of bone marrow was aspirated from patients giving mean cell count of 15±22 million/ml. Number of injections per patient ranged from 25 to 48 with amount of 0.25cc/sq cm. All patients underwent serial echocardiograms and stress thallium 20-segment analysis.
Mean follow up was14.5±3 months.

B) Role of intracoronary implantation of stem cells in dilated cardiomyopathy (DCM):

B.1) Short term study: In this study 44 patients were included, who had dilated cardiomyopathy with an ejection fraction (EF) of ≤35%, where New York Heart Association (NYHA) functional class II or more symptomatic for more than 6 months, had normal coronary arteries, and had no other comorbidities such as chronic renal or liver failure or any malignancy. Out of which, 24 were randomly allocated to the stem cell therapy arm and 20 to the control arm.
Mean follow-up period was 6 months.

B.2) Long Term Study: This includes the final long-term (3-year follow-up) results of the trial. The study included patients between 15 and 70 years of age with idiopathic dilated cardiomyopathy with normal coronary arteries, an ejection fraction (EF) of <40%, and no other severe comorbidities (e.g., chronic renal failure).

This was an open-label, randomized trial in which 85 patients were enrolled. The end points of the study were 1) change in New York Heart Association (NYHA) functional class, 2) change in quality of life as per the Kansas City Cardiomyopathy Questionnaire (KCCQ), 3) change in left ventricular function (Vivid 7TM, Wipro GE Healthcare, offline analysis using Simpson's method), and 4) mortality. An endomyocardial biopsy was performed in 8 patients.

The mean follow-up period was 28 ± 9 months.

Materials & Methods:

50-60 ml bone marrow was aspirated from the iliac crest of the patients. Mononuclear cells were separated from the bone marrow using Ficoll density gradient separation. The mononuclear cells constituted 89 ±2% of the cells, were 28 ±16 million /ml, and CD 34+ cells and were 1.6 million/ml. The viability of these cells was 99 ±1%. The patients then underwent right heart catheterization and endomyocardial biopsy from the right side of the interventricular septum. The coronary sinus was then engaged using a Swan-Ganz catheter (Arrow International, Reading, Pennsylvania) that was passed up the coronary sinus, and the balloon was inflated. This was done so that the coronary circulation was slowed and the stem cells would get more time to transmigrate into the myocardium. Once the coronary sinus catheter was inflated, the stem cells were slowly injected into

the coronary arteries by hooking the arteries with a Judkins catheter. Two-thirds of the mononuclear cell concentrate was injected into the left coronary artery and one-third was injected into the right coronary artery. The coronary sinus balloon was kept inflated for 3 min during the intracoronary injection.

The patients were kept under monitoring for 24 h with electrocardiographic monitoring and serial cardiac enzymes. Follow-up was done at 1 week, 1 month, and then every 3 months for 1 year. At 3 months, Holter monitoring, an echocardiogram, and an endomyocardial biopsy were repeated. An echocardiogram was also repeated at 1 year. Left ventricular function assessment was performed offline by the modified Simpson method by 2 observers blinded to the underlying treatment. All patients were on the maximum tolerated doses of angiotensin-converting enzyme inhibitors and beta-blockers. Diuretic doses (including frusemide and torsemide, and spironolactone) were adjusted to ensure the absence of pedal edema. The end points of the study were: 1) change in NYHA functional class, 2) a change in left ventricular function, 3) mortality, and 4) endomyocardial biopsy and histopathologic evaluation.

Continuous variables were compared by a Wilcoxon 2-sample test (for within-group differences) and the Mann-Whitney U test (between-group differences). Differences in mortality and change in functional class between the 2 groups were compared by the Fisher exact test. A value of $p < 0.05$ was considered statistically significant. All analyses were performed with SPSS for Windows.

C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with Acute Myocardial Infarction - MI3 trial (Mononuclear infusion in Myocardial infarction, Multicentric-Trial, India):

In this study, 250 patients with AMI were included. They were given Intracoronary infusion of either autologous bone marrow derived mononuclear cells (MNC) or standard of care medical therapy.

Materials & Methods:

The study was a randomized multicentric phase III trial to evaluate the efficacy of stem cell in improvement of LV function in patients with AMI. Patients of AMI following left anterior descending (LAD)artery occlusion from five premier centres namely, Army Hospital (Research and Referral), New Delhi; Military Hospital, Cardio Thoracic Centre (MHCTC), Pune; Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGI), Lucknow; Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh; Christian Medical College (CMC), Vellore and All India Institute of Medical Sciences (AIIMS), New Delhi were included in the study w.e.f 07 July 2007 to 08 July 2010.

Patients aged 20-65 years

presenting with first acute ST elevation anterior wall myocardial infarction (AMI) who underwent coronary angiography (CAG) between 1 - 3 weeks were included in the study if they fulfilled the following (a) Killip Class I - III at admission; (b) Proximal and/or mid LAD artery involvement on CAG and (c) LVEF of 20-50% by multigated acquisition scan (MUGA).

Patients with multi-vessel coronary artery disease (CAD), pulmonary edema, Killip class IV, advanced renal or hepatic dysfunction, associated mechanical complications like ventricular septal rupture, previous history of angioplasty or significant circumflex and right coronary artery (RCA) involvement, LVEF < 20% by echocardiography (ECHO), percutaneous coronary intervention (PCI) done within 2 hrs of AMI, and pregnant women were excluded from the study.

RESULTS

A) Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection:

All the patients underwent serial echocardiograms ,ECG and stress thallium 20 segment analysis. There was a significant improvement in New York Heart Association class from a baseline of 2.9±0.7 to 1.25±0.6 (P<.001). Echocardiographic LV ejection fraction analysis revealed evidence of improvement to 41% ± 9%. A mean 3.7±2.6 segments showed improvement, 10.0±1.6 segments showed no change and 2.3±2.6 segments showed worsening. Overall, 56.1% of infarcted areas injected with stem cells improved (**Fig. 1**).

On stress thallium 20 segment analysis at last follow-up, the number of scarred segments reduced from 5.4±2.7 to 4.6±2.6, and the number of normal segments increased from 6.2±3.9 to 8.2±4.1 (**Fig. 2**).

On stress thallium evaluation of injected infarcted segments (n=7±2), 1.8±1.9 segments showed improvement, the other remained same and none worsened.

Bone-marrow derived stem cell transplantation during Coronary artery bypass grafting (CABG) is feasible and safe and the BM obtained from sternum at the time of CABG provides an adequate number of stem cells.

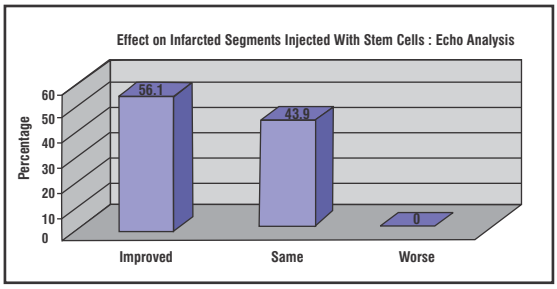


Fig. 1: Effect on infarcted segments injected with stem cells: echocardiography analysis

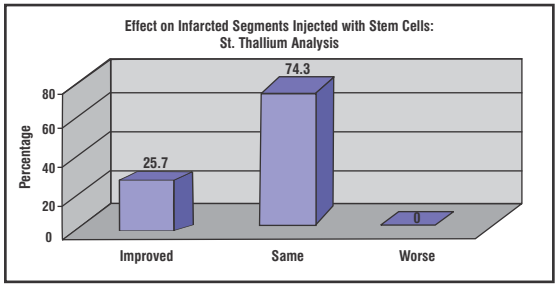


Fig. 2: Effect on infarcted segments injected with stem cells: stress thallium analysis

B) Role of intracoronary implantation of stem cells in dilated cardiomyopathy (DCM):

B.1) Short term Follow-up (6 months): 24 patients underwent intracoronary stem cell injection with coronary sinus blockage. Four patients died during the 6-month follow-up. Overall EF showed a small but significant improvement of 5.4%. There was a decrease in end-systolic volumes, but no change in end-diastolic volumes. Endomyocardial biopsy done at 3 months showed no significant change in the number of myocytes or capillaries, but the ratio of capillaries to myocytes showed an insignificant increase. There were soft data to suggest cell proliferation (binucleate cells and Ki 67 positivity).

B.2) Long term Follow-up (up to 3 years): Two patients in the treatment arm were lost to follow-up, and another 2 patients underwent biventricular pacing. Among the remaining 41 patients, 10 (24.4%) patients died within 3 years. There were 12 NYHA functional class IV patients, and of them, 6 died during the follow-up period and 5 patients showed improvement (1 patient to class I, 1 patient to class III, and the remaining 3 patients to class II). Mortality was not significantly different between the treatment and control arms. The EF improved in the treatment arm by 5.9% with a reduction in end-systolic volumes and no change in end-diastolic volumes. Both NYHA functional class III and IV groups in the treatment arm showed improvement, although the effect on the NYHA functional class III patients (EF: 23.6±10.6.% to 30.1±11%) was greater than that on the NYHA functional class IV patients (EF: 20.1 ±9% to 24 ±13.8%). There was no significant improvement in the EF in the control patients **(Table 4)**. There was a significant improvement in quality of life as assessed by KCCQ and functional status on long-term follow-up in the treatment group **(Table 4)**.

In summary, the clinical follow-up results of a first-in-man pilot study of stem cell therapy in patients with dilated cardiomyopathy at the completion of 3 years of follow-up demonstrate that the benefit is sustained and without any long-term side effects. Although the effect was less in patients with more severely damaged myocardium. This study establishes the long-term safety and long-term efficacy of this therapy in dilated cardiomyopathy.

The data of the analysis at 6 months are presented. Four patients died between 1 and 3 months in the treatment arm. Three died of progressive heart

Table 4: Evaluation at Follow up of Cell Therapy

	Treatment Arm (n = 41)		Control Arm (n = 40)	
	Baseline	3 Yrs	Baseline	3 Yrs
Clinical data				
Age, yrs	45 ±15		49 ± 9	
Sex, male	33		35	
NYHA functional class for dyspnea				
I	0	4 (9)	0	9
II	0	22 (54)	14 (35)	10 (25)
III	29 (71)	6 (15)	12 (30)	18 (45)
IV	12 (29)	9 (22)	14 (35)	12 (30)
Mortality		12 (24.4)		14 (30)
KCCQ				
Functional status score	51.19 ± 19.90	67.02 ± 21.8*	51.52 ± 18.12	52.74 ± 18.8†
Clinical summary score	59.81 ± 20.27	75.22 ± 18.31*	59.95 ± 18.44	61.17 ± 19†
Echocardiography data				
End-systolic volume, ml	137.3 ± 62.6	120 ± 52*	145.7 ± 74.7	147.8 ± 79.9†
End-diastolic volume, ml	176.7 ± 76.40	166.5 ± 65.5	184.9 ± 94.6	187.7 ± 98.8
Ejection fraction, %	22.5 ± 8.3	28.4 ± 11.8*	20.8 ± 9.3	21.2 ± 9.2†
Treatment at 3 yrs				
ACE inhibitor or ARB (ramipril equivalent), % taking drug		41 (100)		40 (100)
Dose, mg		7.5 ±1.2		7.4 ±1.7
Beta-blocker (carvedilol equivalent), taking drug		29 (70)		29 (72)
Dose, mg		12.5 ± 5		12.1 ± 3

Values are mean ± SD or n (%) of patients. Continuous variables were compared using a Wilcoxon 2-sample test (for within-group differences) and the Mann-Whitney U test (between-group differences). Differences in mortality were compared by the Fisher exact test. All analyses were performed with SPSS for Windows (version 10.0.1, 1999, SPSS Inc., Chicago, Illinois). *p < 0.05 between baseline and 3 years for treatment group. †p < 0.05 between treatment arm and control arm.
ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; KCCQ = Kansas City Cardiomyopathy Questionnaire; NYHA = New York Heart Association.

failure and 1 experienced sudden cardiac death. The mortality was not significantly different (p value not significant) between the treatment and control (2 patients died) arms. There was a significant improvement (p < 0.001) in NYHA functional class in the treatment arm, with 16 patients (62%) improving by at least 1 functional class, as compared to only 2 patients (10%) improving in the control arm. The EF improved by 5.4% (20 ±7.4% to 25 ±12%, p ± 0.05) (**Fig. 3**) with a reduction in end-systolic volumes (144 ±85 ml to 116 ±68 ml, p± 0.05) and no change in end-diastolic volumes.

None of the patients who were functional class IV and had recently required inotropic infusions showed any improvement. There was no significant improvement in the EF in the control arm (baseline EF:16 ±5.4% to final EF: 16 ±4.7%). Endomyocardial biopsy was performed at 3 months. Histopathology revealed no evidence of persisting stem cells, no evidence of any new immature myocytes, and also no evidence of any inflammation, infarction or neovascularization.

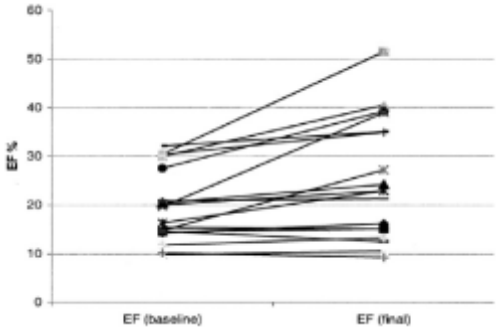


Fig. 3: Change in ejection fraction (EF) after 6 months of follow-up

Two patients had scattered binucleate cells and evidence of cell proliferation (Ki 67). (**Fig. 4**) Morphometric analysis showed no change in the absolute number of myocytes (457 ±210 cells/mm2 [before] and 340 ±150 cells/mm2 [after]). There was also no change in the number of capillary endothelial cells (stained by CD34 class II, 696 ±206 cells/mm2 [before] and 569 ±150 cells/mm2 [after]). The ratio of capillaries to myocytes showed an increase, but it was not significant (1.7 ± 0.7 to 1.9 ±0.8). There was no significant difference between the mortality in the treatment (16.7%) and control (10%) arms. In the control group, 2 patients died. There was no significant change in functional class or left ventricular function in this group. In summary, 24 patients underwent intracoronary stem cell injection with coronary sinus blockage. Four patients died during the 6-month follow-up. Overall EF showed a small but significant improvement of 5.4% (**Fig. 3**). There was a decrease in end-systolic volumes, but no change in end-diastolic volumes.

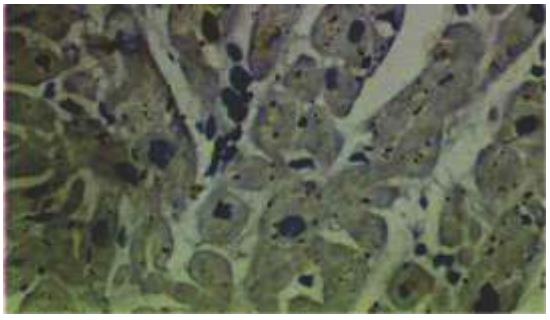
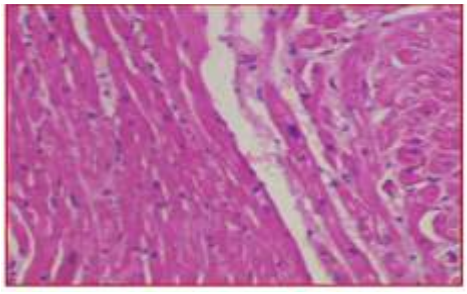
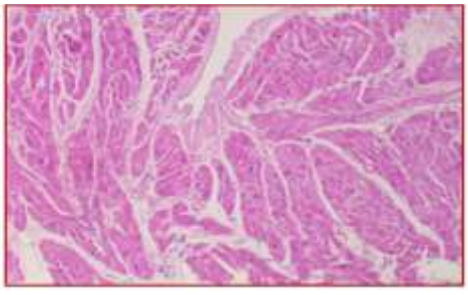


Fig. 4: Presence of Ki67 (Proliferation seen in one patient)

Endomyocardial biopsy done at 3 months showed no significant change in the number of myocytes or capillaries, but the ratio of capillaries to myocytes showed an insignificant increase. There were soft data to suggest cell proliferation (binucleate cells and Ki 67 positivity) (Fig. 4). Also, Hematoxylin Eosin Staining showed that there was no

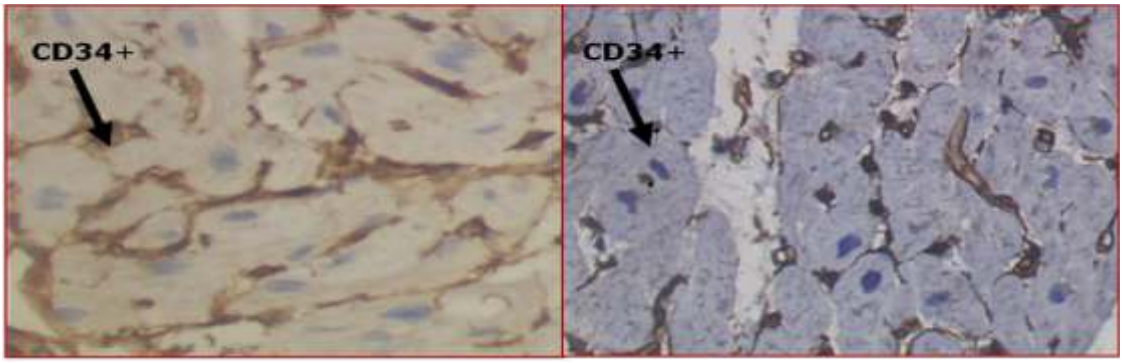


- Pre
- Hypertrophy of fibers with nuclear enlargement
- Mild interstitial fibrosis



- Post
- No abnormal vasculature
- No inflammation
- No primitive cells

Fig. 5: Hematoxylin Eosin Staining of the Cardiac biopsy tissue of the patient



- MYOCYTE : CD34+ Ratio
  - Pre stem cell 1 : 0.8
  - Post stem cell 3 wks 1 : 1.5
    6 mths 1 : 1.4

Fig. 6: Presence of Cd34+ Stem cells after Stem Cell Transplantation

inflammation and no abnormal vasculature formation occurred supporting the safety of stem cell injections (**Fig. 5**). The ratio of myocyte: CD34 cells was slightly increased at 3 months, however this was insignificant (**Fig. 6**).

C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with Acute Myocardial Infarction - MI3 trial (Mononuclear infusion in Myocardial infarction, Multicentric-Trial, India):

Between 07 July 2007 and 08 July 2010, 621 patients were screened to assess their eligibility for participating in the trial. Two hundred and fifty patients were randomly assigned, in 1:1 ratio, either to a non Stem Cell Treatment (SCT) arm (n: 125) that received standard of care medical therapy or to a SCT arm (n: 125) that received intracoronary infusion of MNCs in addition to standard of care medical therapy. In the SCT arm of the 125 patients, a total of 114 patients received the stem cells. While in the non SCT arm, all 125 patients received standard of care medical therapy as per current guidelines after PCI. The final cohort followed up for six months included, 109 patients in SCT arm and 117 in non SCT arm (**Fig. 7**).

RESULTS:

Since only 71 patients received the predefined cell dose, a stratified analysis of this group of patients was done with a nested cohort matched for age and sex. The baseline LVEF was similar in both groups (34.22 ±7.03% in SCT vs. 35.75 ± 4.1% in non SCT group). The difference in LVEF observed at the end of six months was approximately 3 per cent (7.03 vs. 4.1%) with a possible benefit in the SCT group (**Fig. 8A**); however, this was not significant. Stratified analysis comparing 38 trial deviates with a nested cohort from

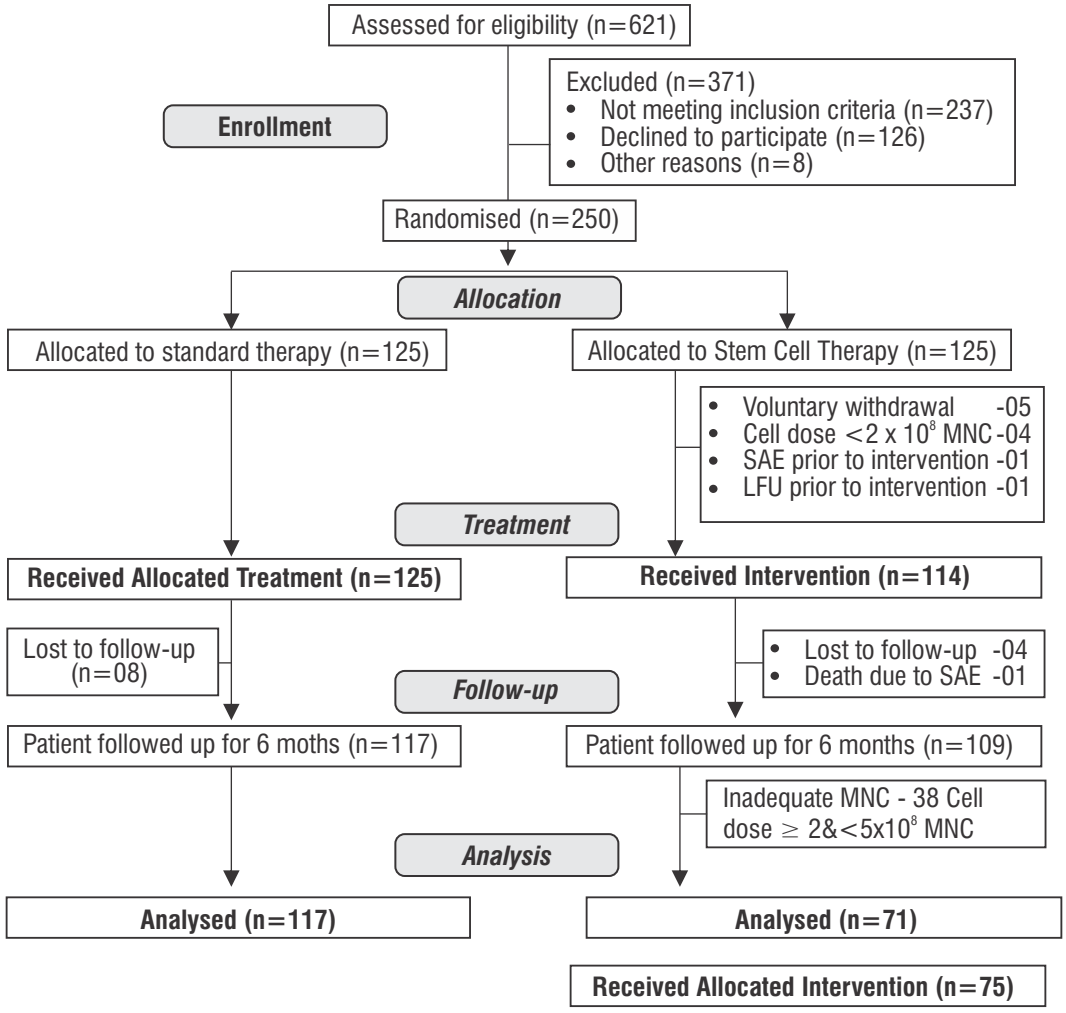


Figure 7.Consort diagram depicting patient enrolment and follow up

the non SCT group showed no significant improvement between the two groups (**Fig. 8B**). It was observed that the baseline LVEF did not differ significantly between the two groups. At six months, LVEF showed an increase in both groups. The mean change in LVEF from baseline to six months being 5.17 ± 8.90 per cent in non SCT group and 4.82 ± 10.32 per cent in SCT group. The median change in LVEF from baseline to six months was 4% in non SCT and 3.5 % in SCT group. However, the difference was not significant. It was found that the cell dose showed a positive impact when infused in the intended dose of ≥ 5 X 10^8 (n=71) when compared with a subset of trial deviates (n=38) who did not receive the predefined cell dose, namely ≥ 2 or <5 x 10^8 cells (**Fig. 8C**). Also , there was no difference noted in the group infused stem cells prior to or beyond 10 days (upto 21

days) of onset of AMI. There was no impact of age and baseline LVEF noted on the primary outcome (**Fig. 8D**).

Adverse effects (AEs) and serious adverse events (SAE) recorded during six months follow up were equally distributed in both the groups with no significant difference.

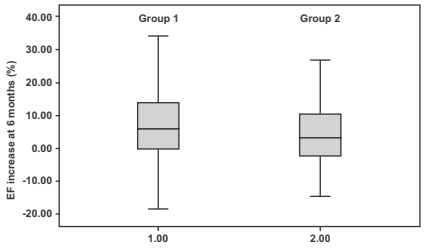


Fig. 8A. Stratified analysis of SCT group with nested cohort: Effect on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, SCT (n=71) and Group 2 Nested cohort from non SCT group (n=71). Actual increase in EF at 6 months between SCT group (7.03 ± 10.33 %, Median 6, IQR 0-14) and nested cohort from non SCT arm (4.1 ± 9.1%, Median 3.01, -2.15-10.45) was not significantly different.

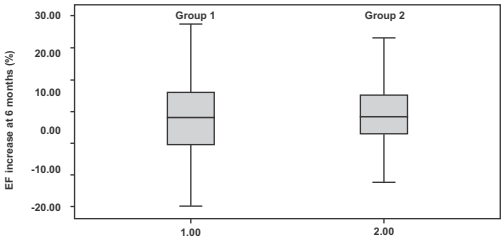


Fig. 8B. Stratified analysis of Trial deviates with nested cohort: Effect on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1,Trial deviates (n=38) and Group 2, Nested cohort from non SCT arm (n=38). Actual increase in EF at 6 months between trial deviates group (2.75 ± 9.6%, Median 3.25, IQR -3.91-9.49) and nested cohort from non SCT arm (4.37 ± 8.87% Median 3.5, -0.75-8.86) was not significantly different.

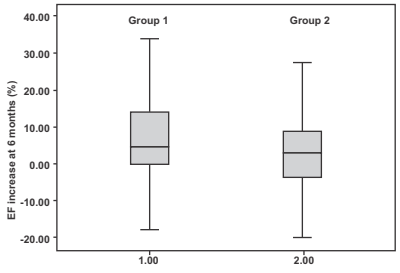


Fig. 8C. Impact of cell dose administered on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, SCT (n=71) and Group 2, Trial deviates (n=38). Actual increase in EF at 6 months between SCT group (7.03 ± 10.33%, Median 6, IQR 0-14) and Trial deviates (2.75 ± 9.6%, Median 3.25, IQR -3.91-9.49) was significant (P<0.05).

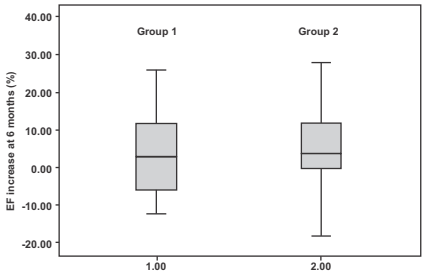


Fig. 8D. Impact of timing of infusion in SCT arm on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, early (infusion given in < 10 days, n=21) and Group 2, late (infusion given between day 10 to day 21, n=50). : Actual increase in EF at 6 months between the early group (6 ± 10.45 %, Median 7.5, IQR 0.49-14) and late group (7.47 ± 10.97, Median 4, IQR -2 - 14) was not significant.

DISCUSSION

Cell-based therapy for ischemic cardiomyopathy and heart failure has emerged as a highly promising therapeutic approach that will expand the benefits obtained by current pharmacologic and revascularization approaches by directly reversing scar formation and promoting myocardial regeneration. The next stage of development for the clinical use of cell therapy should focus on investigating novel formulations, particularly the best cell type(s) and/or cell combinations to use and understand the mechanisms by which various stem cells interact with host cells and/or each other and elicit their regenerative effect. In summary, the clinical follow-up results of a first-in-man pilot study of stem cell therapy in patients with dilated cardiomyopathy at the completion of 3 years of follow-up demonstrate that the benefit is sustained and without any long-term side effects.

This study establishes the long-term safety and long-term efficacy of this therapy in dilated cardiomyopathy. This is the first study of stem cell therapy in dilated nonischemic cardiomyopathy. Over a 6-month period, there was a small albeit significant improvement in ventricular function. Previous clinical studies have also shown a small degree of change in ventricular function of a similar magnitude (20, 21). Laboratory experiments in nonischemic dilated cardiomyopathy (22) have previously suggested that benefit from stem cell therapy in this group comes mainly from a decrease in fibrosis and an increase in vascularity, but no evidence has been found supporting transdifferentiation of stem cells to myocytes. Our data also suggest that the benefit of stem cell therapy could be a paracrine effect with changes in vascularity, perhaps stimulation of cell proliferation, or by some still-unexplored mechanism. We did not find any evidence of transdifferentiation. In this study, we wish to highlight a number of issues. It is the first study to show the benefit of stem cells in nonischemic dilated cardiomyopathy, and the first study that uses coronary sinus occlusion to increase cell contact time. It is also the first study in which we have endomyocardial biopsies performed after stem cell therapy.

It provides a stimulus for exploring the benefits of stem cell therapy in nonischemic dilated cardiomyopathy. A double-blind study is being planned to further explore the benefit seen in this preliminary study. The small magnitude of benefit could perhaps be because all patients in this study were in very late stages of their cardiomyopathy, and we probably need to consider stem cell therapy at a much earlier stage. Endomyocardial biopsy performed for the first time in stem cell therapy, shows no evidence of transdifferentiation of stem cells to myocytes but provides soft data pointing to a possible paracrine effect.

Similarly, the results of MI3 trial demonstrates that autologous MNCs can be safely administered in patients with AMI and the dose of stem cells has important role in determining its efficiency in regeneration (23).

Although there has been significant progress in the clinical translation of cell therapy over the past decade, uncertainties remain regarding the most efficacious cell type, source and quantity, as well as route and timing of delivery (24). Collectively, these issues highlight the need for further investigation of the mechanisms underlying stem cell survival, plasticity, and function.

Future Prospects :

Stem cell treatment is gaining momentum in treatment of CVDs but still a lot needs to be explored so that its use can be translated into better clinical outcomes. Therefore, pharmacologic and genetic strategies are being developed in an effort to improve stem cell survival, homing, and engraftment (25, 26, 27-32). Different strategies are being explored to utilize the combination of different stem cells or of cell and gene therapy (25, 26, 27-32). In addition, the discovery of microRNAs as regulators of cardiovascular biology and stem cell differentiation have made them attractive targets to optimize cell-based therapies. One of the major challenges of cell-based therapy is the survival of cells after delivery into the recipient tissue microenvironment. Ischemia creates a hostile microenvironment due to locally expressed pro-inflammatory and pro-apoptotic cytokines inducing cell death. Various approaches to inhibit local inflammation and promote cell survival and tissue regeneration are being investigated, including preconditioning, by *in vitro* incubation of stem cells with pro- survival factors, or transfection of stem cells with pro-survival or antiapoptotic genes prior to cell delivery (25, 26, 27-32).

New methodologies are being developed for direct differentiation of fibroblasts into beating cardiomyocytes. Three transcription factors (TFs), GATA4, Mef2c, and Tbx5, are essential for cardiac myocyte differentiation for direct reprogramming of heart fibroblasts to cardiomyocyte formation *in vitro* (33). Recently, it was shown that these TFs induce cardiomyocyte formation not only *in vitro* but also *in vivo* (34).

Use of Stem Cells has shown beneficial effects in hearts with post myocardial infarction LV remodelling (MI) and has emerged as a highly promising therapeutic approach. The next stage of development for the clinical use of cell therapy should focus on applying a prefabricated bioartificial cardiac tissue (35-36), a "cardiac muscle patch", over the surface of a myocardial infarct and investigating novel formulations, particularly the best cell type(s) and/or cell combinations to use and elucidation of the mechanisms by which various stem cells interact with host cells and/or each other and elicit their regenerative effects.

Acknowledgement:

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Potentiation of Curcumin and Wnt/β-catenin signaling in breast cancer

Gayatri Rath[1]*, Poonam Jawanjal*[1]*, Chandraprakash Prasad*[2]
Department of Anatomy, Vardhman Mahavir Medical College and Safdarjung hospital, New Delhi[1], Cell and Experimental Pathology, Lund University, Clinical Research Centre, Ent 72, Bldg 91, Fl 11 Malmö University Hospital, UMAS, SE205 02 Malmö, Sweden[2]

SUMMARY

Breast cancer is the commonest malignancy among females worldwide including India. In recent years, the use of natural dietary product in breast cancer treatment has attracted great interest. Curcumin is the active component of turmeric which possesses anti-oxidant, anti-inflammatory, anti-ageing and anti-carcinogenic properties against wide range of diseases. Both *in vitro* and *in vivo*, studies have revealed that curcumin targets various genes involved in vital cellular processes. The curcumin mediated inhibition of human breast cell growth is arbitrated by certain signaling cascades including modulation of Wnt/β-catenin pathway. This signaling is aberrantly activated in breast cancer and found to be associated with its aggressive phenotype. Curcumin inhibits the expression of various component of this signaling in order to suppress the breast tumor growth. Current review summarizes the studies revealing the preventive and therapeutic potential of curcumin with an emphasis on its multi-targeted biological and molecular effects towards Wnt/β-catenin signaling in a breast carcinoma model.

Keywords: Breast cancer, curcumin, Wnt/β-catenin pathway, therapeutic modulation.

Correspondence: Dr. Gayatri Rath, Director Professor, Department of Anatomy, Vardhman Mahavir Medical College & Safdarjung Hospital, Room No. 111, New Delhi–110029, India; Phone +91–11–26716440, Fax +91–11–26589821, e-mail:gayatrirathvmmc@gmail.com

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INTRODUCTION

The malignancy of breast is often associated with high female mortality worldwide (1) and is second most common cancer among Indian females (2). In India breast cancer is highly frequent in urban Indians as compared with rural ones. This significant variation in incidence of breast cancer may be associated with the lifestyle related and reproductive/ hormonal related risk factors. The most important reproductive factors that enhance the risk of breast cancer include a long menstrual history, nulliparity, recent use of postmenopausal hormone therapy or oral contraceptives and late conception. Alcohol consumption also increases the risk of breast cancer (3). Among all these predisposing risk factors family history as well as mutations in BRCA-1 and BRCA-2 genes may also play a significant role in development of breast cancer (4). Breast cancer showed considerable heterogenecity with regards to its biological, histological and clinical behaviour (5). The criterion of breast cancer sub typing is primarily based on the presence or absence of estrogen receptor (ER) or the progesterone receptor (PR), or the up regulation of the human epidermal growth factor receptor 2 (HER2) (6,7). The cases which show positivity for all these receptors are considered as triple positive and those that show negativity are considered as triple negative breast cancers. In India, mostly the breast cancers are ER/PR negative and are characterized by aggressive clinicopathological features such as higher tumor size, tumor grade and a higher rate of lymph node positivity (8). Hence, due to the involvement of these factors in the process of development of breast cancer it has been envisaged that alteration of cellular proteins may play a major role, probably through the activation of several signaling pathways. The over activation of cellular signaling, then results in tumor expansion, cell proliferation, invasion and metastasis (9).

The breast cancers are often diagnosed in advanced stages and the biology of these tumors is not well understood till date. Despite the availability of standard protocol for breast cancer treatment like, chemotherapy, surgery or targeted therapy, the patient's outcome and response to therapy remains inconstant. The use of chemotherapeutic agents such as cisplatin, paclitaxel, carboplatin, bevacizumab, doxorubicin, cyclophosphamide, docetaxel, and epirubicin (10, 11) is always associated with their cytotoxic effects which again affect the health of patients. Hence, there is an urgent need to identify certain components that can be used in breast cancer treatment with lesser side effects. In this respect, identification of certain natural products with their targeted anti-cancerous properties can serve as a better therapeutic avenue for the treatment of breast cancer.

Curcumin :

Curcumin [1,7-bis(4-hydroxy 3-methoxy phenyl)-1,6- heptadiene-3,5-dione, Fig. (1)] is a bioactive component

Fig-1: Solid turmeric containing yellow coloured curcuminoids. The chemical structure of curcumin (Diferuloylmethane), IUPAC name: (1E,6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3,5-dione; MF: C21H20O6; MW: 368.37.

of Indian spice i.e. turmeric. It is used as a colouring agent in many Indian recipes. It has also been the important component of Indian Ayurvedic medicine and is used traditionally to treat wounds and respiratory disorders (12, 13).Biologically it is yellow coloured curcuminoid, a polyphenol derivative of rhizome of Curcuma longa (ginger family, Zingeberaceae) that makes approx. 2-6% of turmeric (11). It was first isolated in 1815, obtained in crystalline form in 1870, and identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E)ordiferuloyl methane (9, 11). Chemically, curcumin depicts a bis-α, β-unsaturated β-diketone (commonly called diferuloyl methane) structure that exhibits ketoenol tautomerism, having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline media (14).

Properties of Curcumin :

It has been extensively reviewed that curcumin can fight against wide range of diseases, due to its anti-inflammatory, anti-oxidant, anti-ageing, antimicrobial and insecticidal properties (11). Therefore, number of studied have been carried out to establish its biological as well as clinical role in various chronic diseases, including diabetes, cardiac disorders, arthritis, obesity, depression, Alzimer's and autoimmune diseases (15-20).

Anti-tumorogenic property of Curcumin :

In addition to various biological properties, curcumin has also been started using therapeutically due to its unique anti-cancerous property. The anti-tumorigenic effect of curcumin has been analysed in number of human cancer from last 50 years, which showed that curcumin affects the cancer at its initiation as well as progression stage. The in-vitro studies which were carried out on various cancer cell lines proved that curcumin plays anti-tumoral, chemo-preventive as well as chemo-radio sensibilizing role in various primary as well as recurrent malignancies. Overall, curcumin has a potential role not only to prevent but also to cure cancer (9,

21-23). The anti-cancerous potential of curcumin with respect to its tumor growth suppressive activity have been reviewed in almost all kind of cancer including, malignancies of digestive system (esophageal, gastric, intestinal, colorectal, hepatic, pancreatic), urogenital system (kidney, bladder, prostate), reproductive system (cervix, ovary), haematological system (leukaemia, lymphoma, multiple myeloma). In addition, curcumin is also found to be effective in pulmonary, thymic, brain and bone cancers (24).

It has been observed that curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancer through interacting with multiple proteins (25). The underlying molecular mechanism of effect of curcumin may be due to its ability to target the various cellular signaling and their components. The precise mechanisms by which curcumin selectively kills cancer cells is not fully disclosed; however it is proposed that the anti-cancer potential of curcumin may depend on its ability to suppress the proliferation of cancer stem/progenitor cells and their progenies, through multiple molecular mechanisms (13). Curcumin modulates various oncogenic signaling pathways i.e. inflammatory pathways (TNFα, IL-1, IL-6), protein kinases pathway (mTOR, JNK, MAPK, AKT/PKB, Cox-2), cell proliferation and survival pathways (CDKs, Cyclin E, Cyclin D, c-Myc, c-Jun, Bcl-2, Bcl-xl, cFILP, XIAP, c-IAP1), mitochondrial and caspase activation pathway (Caspase-8, 9, 3) (26). Apart from this, certain receptors like EGFR/erbB1, erbB2/HER2, IGF-1R, PPAR-γ, SHH/GLIs are also the potential target for curcumin activity (9, 27-29). It also modulates the metabolizing biotrans forming enzymes (Phase-I and Phase-II) which are responsible for activation of pro-carcinogens during carcinogenic process. Studies have shown that curcumin inactivates cytochrome P450 (CYP) activity and up-regulates GSTs (30-33).

Curcumin in Breast Cancer:

In preclinical studies, the effect of curcumin have been extensively analysed in wide range of malignant, metastatic, ER/PR/HER2$^{-/+}$ breast cancer cell lines and it has been observed that curcumin effectively counteract their growth, thereby suggesting a multifaceted role of curcumin in breast malignancy. Verma et al, 2003, studied the effect of curcumin on MCF-7 (ER+/PR+) breast cancer cell lines and suggested that curcumin exerts the anti-proliferative impact on breast cancer cells through inhibition of PS2 and transforming growth factor-β (TGF-β) gene expression. Furthermore they also reported that curcumin effectively breaks the copper-protein moieties of cells thereby reducing the growth of MCF-7 (ER+), T47D and MDA-MB-231 (ER-) cells (34). Curcumin exerts anti proliferative action mainly by interfering at the various steps of cell cycle. On experiments on MCF-7 and MDA-MB-231 breast cancer cell lines, Prasad et al, 2009 showed that, curcumin inhibits proliferation of breast cancer cells by inducing G2/M cell cycle arrest, leading to apoptotic cell death through p53

dependent manner (35). In addition, Chiu et al, 2009; showed that curcumin inhibits cell proliferation as well as migration by trans-activation of Bax/Bcl-2 proteins ratio and inactivation of NF-Kβp65 in breast cancer MDA-MB-231 cells (36). Altenburg et al, 2011 studied the effect of curcumin on SK-BR-3 (ER-/PR-/HER2+) breast cancer cell lines and observed that the breast cells supplemented with DHA enhance their curcumin uptake and thereby increased the cellular content of peroxisome proliferator-activated receptor gamma (PPAR-γ) and phospho-p53 (37).

Apart from these studies, there are reports which further suggest that curcumin also inhibits the breast tumor angiogenesis by targeting the pro-angiogenic proteins. It has been observed by Chakraborty et al, 2008, that curcumin suppresses tumor angiogenesis in breast cells by preventing osteopoitin mediated VEGF expression (38). Similar to these findings, Carroll et al, 2008 also reported that curcumin helps in the reduction of angiogenesis in T47-D (ER+) breast cancer cell line by reducing medroxy progesterone acetate (MPA) induced expression of VEGF. Furthermore they suggested that, the level of VEGF was not affected in progesterone or progestin exposed cells by curcumin through MPA (39). In another study, curcumin showed the inhibitory effect towards α6β4 integrin signaling in breast cancer cells by sub-cellular localization of α6β4, resulting in the loss of association between epidermal growth factor receptor (EGFR) and Akt receptors (40).

Evasion of apoptosis by tumor cells is the hallmark characteristic of cancer. To bring the apoptosis of neoplastic cell has always been a pre-requisite for the suppression of tumor growth. Number of experiments demonstrated that curcumin induces apoptosis and inhibits cell growth through modulating apoptotic proteins of intrinsic as well as extrinsic apoptotic pathways in cancer cells. Curcumin triggers the apoptosis of breast cancer cells by down regulating IGF-1 system molecule (important for breast cancer development) i.e. IGF-1 (41). In addition, curcumin also suppresses the microtubule instability and stimulates the activation of mitotic checkpoints for the induction of apoptosis in MCF-7 (ER+/PR+) breast cancer cells (42). In triple negative breast cancer cells it induces DNA damage and brings the cytoplasmic preservation of breast cancer type 1 susceptibility protein (BRCA1) (43). Curcumin also induces the expression of miR-15a and miR-16 by epigenetic regulation thereby reducing the Bcl-2 protein expression in breast cancer cells (44). In another study, it was observed that curcumin low doses induces p27 expression and inhibits Skp2, Her2, Cyclin E and CDKs activity in MDA-MB-231 (ER-/PR-/low HER2) cells, suggesting its probable involvement in the growth inhibition. It was also reported that curcumin in high doses was able to induce apoptotic death in MDA-MB-231 (ER-/PR-/low HER2) cells, due to the cleavage of PARP and activation of caspase 3 (45).

Cancer cells have the capability to

evade the host immune system by secreting multivesicular bodies, called exosomes (9). Curcumin has been shown to reverse the inhibitory action of exosomes towards Jak3- mediated activation of Stat5 for natural killer (NK) cells functions in breast cancers (46). Purified exosomes from TS/A breast cancer cells inhibits IL-2-induced natural killer (NK) cell cytotoxicity. The dietary polyphenol curcumin partially reverse the tumor exosome-mediated inhibition of NK cell activation, which is mediated through the impairment of the ubiquitin-proteasome system (47). Apart from that, in line with various *in vitro* studies, some *in vivo* studies were also carried out to delineate the role of curcumin in breast cancer. The overall activity of curcumin with respect to its targets in breast cancer is summarized in **Table-1**.

Table 1: *In vitro* studies depicting anti-cancerous role of curcumin in breast cancer

Breast cancer cell lines	Effect of curcumin on cancer cells	Proteins targeted	References
MCF-7 (ER/PR+), T47D and MDA-MB-231(ER-)	Anti-proliferative, inhibition of cell growth	↓PS2,↓ TGF-β	Verma *et al*, 2003
MCF-7 and MDA-MB-231	Inhibits cell proliferation and induced apoptosis	↑ Maspin, ↓ Bcl-2,↑ p53	Prasad *et al*, 2009
BT-20, T-47D, SKBR3 and MCF- 7	Arrest cell cycle at G2/S phase and inhibits cell proliferation	↓ODC	Mehta *et al,* 1997
MDA-MB-231	Inhibition of cell proliferation and migration	↑Bax, Bcl-2 ratio; ↓ NF-Kβp65	Chiu *et al*, 2009
SK-BR-3, MDA-MB-231, MDAMB- 361, MCF7 and MCF10AT	Inhibition of cell proliferation, cell cycle progression and metastasis, induction of cell cycle arrest	↑ PPARg, ↑phospho-p53	Altenburg *et al*, 2011
Breast cancer tissue cell culture	Abrogation of osteopontin-induced VEGF expression	↓VEGF	Chakraborty *et al*, 2008
T47-D	reduces medroxyprogesterone acetate (MPA) induced expression of VEGF	↓ VEGF	Carroll *et al*, 2008
MDA-MB-231	inhibitory effect toward α6β4 signaling	↓ α6β4, ↓EGFR/AKT	Soung *et al*, 2011
MCF-7	Induces apoptosis	↓IGF-1,↓ IGFBP-3, IGF-1R tyrosine ↓Kinase	Xia *et al*, 2007
MCF-7	Induces apoptosis	↓microtubule instability	Banergee et *al*,2010
MCF-7 (ER+/PR+)	Induces apoptosis	↑ BRCA-1	Rowe *et al*, 2009
MCF-7	Epigenetics changes and apoptosis	↑ miR-15a & miR-16; Bcl-2	Yang *et al,* 2010
MDA-MB-231/Her-2	Repressed cell proliferation, induced G1 arrest and triggered apoptosis	↑p27,↓Skp2, ↓Her2, ↓ Cyclin E, ↓CDKs,↓ cleavage of PARP, ↑caspase 3	Sun *et al.*, 2012

Abbreviations: PS2, an estrogen-induced RNA messenger; TGF-β, transforming growth factor-β, Bcl-2, B cell lymphoma-2; ODC, Ornithine decarboxylase; Bax, BCL2-associated X protein; NF-Kβp65, Nuclear factor kappa-light-chain-enhancer of activated B cells; PPARg, Proliferator-activated receptor gamma; VEGF, Vascular endothelial growth factor; EGFR, Epidermal growth factor receptor; IGF-1R, IGF-1 receptor; IGF-1, Insulin like growth factor-1; BRCA1, Breast cancer type 1 susceptibility protein; miR, micro RNA; Skp2, Sphase kinase protein 2; HER-2, Human epidermal growth factor receptor 2; CDKs, Cyclindependent kinases;BRACA-2, Breast cancer type 2; ER, Estrogen receptor; PR: Progesterone receptor; TNF, Tumor necrosis factor; IL-1, Interleukin-1; IL-6, Interleukin-6; JNK, c-Jun NH2-terminal kinase; Cox-2, Cyclooxygenase-2; SHH, Sonic hedgehog; DHA, Docosahexaenoic acid, NK, Natural Killer cells; Fzd, Frizzled; LRP, low density lipoprotein receptor-related proteins; APC, Adenomatosis polyposis coli;GSK3β, Glycogen Synthase kinase-3β; CK-I, casin kinase-1;Dvl, Deshevelled; TCF/LEF, T-cell factor/lymphoid enhancer factor; PI3K, Phosphatidylinositol-3-kinase; EMT, Epithelual Mesenchymel Trasition; PAC, 5-bis(4 hydroxy-3-methoxy benzylidene-)-N-methyl-4-piperidine; EAC, 1,7-Bis (4-hydroxy-3-ethoxyphenyl)-1,6-heptadien-3,5-dione; PLGA, polylactide-co-glycolide analog; PEG, Poly ethylene glycol

Curcumin and other signalling pathways in Breast Cancer :

Curcumin exerts its anti-tumorigenic activity towards wide range of proteins that are the important components of various cellular signaling pathways. As, cancer is a multifactorial disease, it is always impossible to target individual protein to suppress the tumor growth. Hence, targeting whole signaling pathway by inhibiting its all regulatory components may prove as a promising strategy for improvement of therapeutic value. Signaling pathways like canonical Wnt/β-catenin, Notch and Hedgehog are aberrantly activated in breast cancer and play a significant role in the disease development and progression. The effect of curcumin towards these signaling pathways has been analysed in multiple human cancers and observed that it modulates these signaling pathways by inhibiting them. Wang et al, 2006, reported that curcumin induces reduction of Notch-1 expression by activating hairy and enhancer of split (Hes)-1 and BclxL protein levels (48). Similarly, it has found that curcumin and its analog inhibit Wnt/β-catenin signaling in wide range of cancers. In breast cancer, where this signaling is aberrantly deregulated, the therapeutic intervention of this signaling could be the prime target of curcumin activity.

Wnt/β-catenin pathway :

The Wnts comprise of secreted cysteine-rich 39-46 kDa glycoproteins. Till date 19 members of Wnt family has been discovered in mammals (49). The Wnt gene family, the name is derived from the drosophila segment polarity gene-Wingless and the murine mammary virus gene Int-1 (50). They regulate key developmental processes including cell-fate determination, cellular differentiation, proliferation, motility and the establishment of primary axis of the body during vertebrate embryogenesis (51-53). In the adult, Wnt regulates

hematopoiesis, osteiogenesis, angiogenesis and adipogenesis (54, 55). Wnt proteins are differentiated into two group-canonical and non-canonical on the basis their transformation capabilities in cell lines or *in vivo*.

The canonical signaling gets activated by binding of specific Wnt proteins (e.g. Wnt-1; Wnt-3a etc) to the seven transmembrane domain Frizzled (Fzd) receptors on the cell membrane. Fzd proteins unite with their co-receptors, the low density lipoprotein receptor-related proteins (LRP5 or LRP6), to activate downstream Wnt signaling (56). In absence of activated Wnts, β-catenin (hallmark protein of the pathway) is phosphorylated by the destruction complex, i.e. protein complex containing adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3β (GSK3β) and casein kinase I (CKI). Phosphorylated β-catenin is ubiquitinised with the help of β-TrCP and projects for proteolysis mediated degradation (57, 58). Binding of Wnts to receptors (Fzd and LRP5/LRP6) leads to the translocation of axin to the cell surface which binds with LRP5/LRP6, followed by phosphorylation of the cytoplasmic protein i.e. Dishevelled (Dvl) (59, 60,). These events ultimately consequence into the degradation or inhibition of the destruction complex, which in turn leads to accumulation of β-catenin in the cytoplasm and finally translocation into the nucleus, where it forms a complex with the T-cell factor/lymphoid enhancing factor (TCF/LEF) family of transcription factors, and activates transcription of Wnt

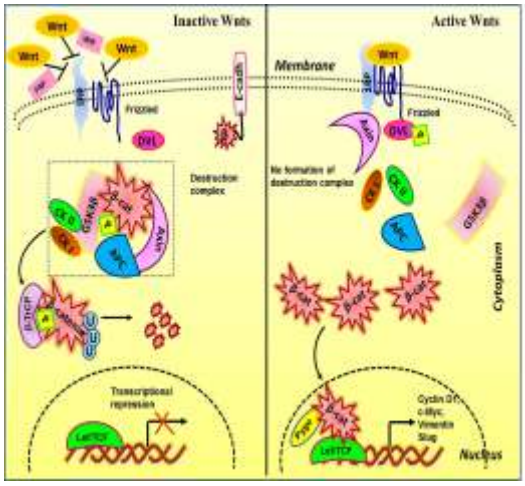

Fig- 2: In the absence of active Wnts ligand (left panel),β-catenin is associated with E-cadherin at the cell membrane. It bind to the destruction complex comprising Axin, adenomatous polyposis coli (APC)(Scaffolding proteins), glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1). The bounded β-catenin is then phosphorylated by GSK3β, ubiquitinised by beta-transducing repeat-containing protein (β-TrCP) and then degraded by proteasomal activity.
The active Wnts (right panel) bind to their transmembrane receptors Frizzled and LRPs, the formation of destruction complex is inhibited. This results in the stabilization of β-catenin in cytoplasm and translocation into nucleus. In the nucleus, it gets associated with Lef/TCF transcription factors and activates transcription of various target genes.

target genes, including c-Myc and Cyclin D1 (**Fig. 2**).

Wnt/β-catenin pathway in Breast Cancer :

Several studies have indicated the role of Wnt signaling pathways in human breast cancer. Hyper activation of the canonical Wnt/β-catenin pathway, caused by mutations in β-catenin, APC and axin, is one of the most frequent abnormalities found in other human cancers. In breast cancer, however, evidence of such mutation is sparse but β-catenin mutations have been detected in 45% cases of breast fibromatosis (61). In contrast, there is strong confirmation for elevated levels of nuclear and/or cytoplasmic β-catenin detectable by immunohistochemistry in over 50% of breast carcinomas (62-64) which correlates with the expression of its target gene Cyclin D1 and poor prognosis of breast cancer patients (64, 65).

Studies have described overexpression of Wnt proteins (Wnt2, Wnt7b, Wnt10b, Wnt13/2b and Wnt14) in breast cancer compared to normal tissues. (61, 66-70). Wnt/β-catenin pathway also showed a cross talk with phosphatidyl inositol 3-kinase/Akt and mitogen-activated protein kinase signaling pathways and two diverse receptors i.e. ER-α/EGFR (71). In addition, the various components of this pathway have also been modulated in breast cancer. The epigenetic loss of sFRP1 (negative regulator of pathway) gene has been observed in primary breast cancers and is associated with poor prognosis (72-74). Whereas, it has also been found that overexpression of DVL-1 (transducer of pathway) gene plays an important role in breast tumorigenesis through derangement of the Wnt signaling pathway (75, 65). Prasad et al 2008 reported that Wnt/β-catenin pathway components such as DVL, β-catenin and Cyclin D1 are up-regulated in invasive ductal breast carcinoma. Furthermore they also showed that the aberrant expression of these proteins were associated with advanced tumor stage of patients, hence proposed the clinical utility of these proteins in breast cancer (65). In another study, Prasad et al, 2008; showed that promoter methylation leads to loss of CDH-1 (E-cadherin) and APC genes required for the activation of Wnt/β-catenin signaling in breast cancer (76). In line with previous studies, Prasad et al, 2009 again provided the clinical evidences of modulation of expression of components of Wnt signaling like, E-cadherin, slug and GSK3β and their association with Epithelial Mesenchymel Transmission (EMT) in breast cancer (77). An APC truncation leading to β-catenin up regulation has also been shown both at cytoplasmic levels and in total cellular extracts in breast cancer cell line DU4475 (78). Transcriptional repression of APC by promoter hyper-methylation in breast cancer has been studied and found to correlate to poor prognosis in breast cancer (79-81). Moreover up-regulation of downstream targets of Wnt/β-catenin signaling viz. Cyclin D1 and c-Myc was also reported in breast cancer which was then found to be associated with progression of disease.

Activity of curcumin on modulation of Wnt/ β-catenin pathway in breast cancer :

Altered functions or levels of components of the Wnt/β-catenin pathway are associated with cancer, and other diseases (82). Therefore, Wnt/β-catenin signaling cascade increasingly attracts considerable attention of cancer researchers and pharmacologists. Dysfunctional Wnt/β-catenin signaling creates continuous transcription of many target genes supporting cell proliferation. The uncontrolled activation of transcriptional factor, β-catenin is mainly responsible for up-regulation of various proliferative genes resulting progression of disease. Hence therapeutic inhibition of this protein is the need of hour for the management of breast cancer. There are few approaches like antisense monoclonal antibodies, anti-sense oligos, RNA interference protein knockdown and chemo-sensitizers have also been developed. The main target of all these strategies is to inhibit the β-catenin activity and enhance its proteasomal degradation process, thus preventing the expression of Wnt target genes. Several reports suggest that curcumin and its analog (CHC007) are good inhibitors of β-catenin/Tcf signaling in gastric, colon, and intestinal cancer cells (9, 11, 24). Jaiswal et al 2002; suggested that curcumin suppresses the β-catenin protein activity through caspase-3-mediated blocking in intestinal cancer HCT116 cells (83). It has also been reported that curcumin controls β-catenin/TCF transcription activity by bringing down the nuclear volume of β-catenin as well as TCF-4 proteins in gastro-intestinal cancer cells (84). Moreover, the activity of Frizzled-1 receptor was also affected by curcumin (85). Curcumin also down regulates p300 in colon cancer for the attenuation of response of β-catenin to Wnt3a (86).

In human breast cancer cells, curcumin modulates β-catenin pathway by inhibiting cell proliferation and induction of apoptosis (87). Prasad et al, 2009 analysed the effect of curcumin on proliferation and modulation of components of Wnt/β-catenin signaling in breast cancer cells (MCF-7 and MDA-MB-231). They examined the effect of curcumin on breast cancer cells at different doses and its activity was detected by investigating Wnt/β-catenin signaling proteins by immune fluorescence, flow cytometry and western blotting. In these experiments, they observed that curcumin exerts a cytotoxic effect on MCF-7 cells with 50% inhibitory concentration (IC50) of 35 uM, while IC50 for MDA-MB-231 was 30 uM. Upon curcumin treatment, there was increased percentage of cells in G2/M as well as G0/G1 phases, for both the breast cancer cell lines. Furthermore, curcumin treatment decreased the levels (2 folds) of Wnt/β-catenin pathway proteins i. e. Dvl, β-catenin, Cyclin D1, and Slug. Increase in the expression of active GSK3β and E-cadherin in curcumin treated cells were also observed. From these findings, authors suggested that curcumin inhibits cell proliferation and induces cell death in these breast cancer cell lines. Furthermore, curcumin treatment reduced

the cellular level of Wnt signaling proteins in breast cancer cells thereby inducing β-catenin phosphorylation and its subsequent degradation. Overall they concluded that curcumin inhibits Wnt/β-catenin signaling by potentially inhibiting activity of its key components. One of the key mechanism that comes out from this study was curcumin mediated inhibition of Wnt/β-catenin induced EMT (88). The targeted inhibitory activity of curcumin on Wnt/β-catenin pathway proteins is illustrated in **Fig.3**

Bioavailability, pharmacokinetics and doses of curcumin :

The therapeutic ability of curcumin is usually compromised due to its low bioavailability. In various studies

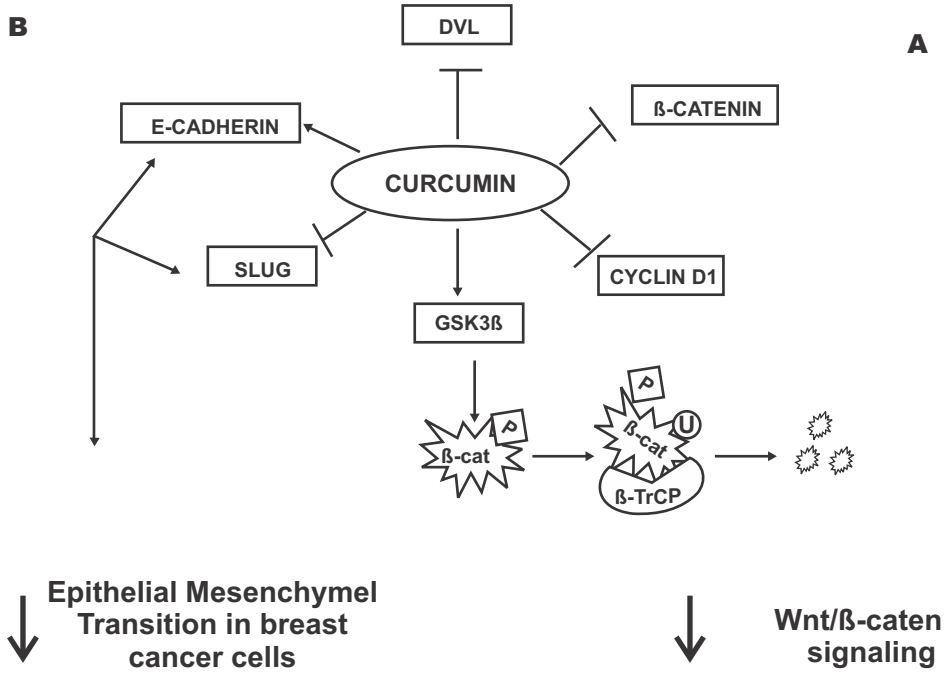

Fig-3: Schematic representation of the role of curcumin in modulation of Wnt/β-catenin signaling proteins in breast cancer cells.
Dual role of curcumin:
A. Inhibition of growth of breast tumor via modulation of Wnt/β-catenin signaling proteins. Curcumin down-regulates the expression of dishevelled (Dvl), β-catenin and Cyclin D1, but significantly retains the cellular expression of active GSK3β, leading to the phosphorylation, ubiquitination and proteasomal degradation of β-catenin.
B. Suppression of Epithelial Mesenchymel Transition through the activation of E-cadherin and inactivation of slug in breast cancer.

performed, it displayed great metabolic variability, low plasma level and poor tissue distribution. It gets poorly absorbed in the gastrointestinal tract as well as extensive metabolism through oxidation, reduction, glucuronidation and sulfation, yielding less active metabolites, and rapid elimination from the body (11, 89, 90). Some clinical studies that have been carried out on fasting volunteers and patients with precancerous lesions also revealed that the serum level of curcumin was undetectable even after administrating high dose of it (up to 2000-8,000mg) daily, which also exhibited curcumin's poor bioavailability (91 ,92). Sharma et al, 2005, suggested that the truncated systematic bioavailability of curcumin probably have been due to its hydrophobic nature, low absorption and metabolic biotransformation in intestine and liver (93). Therefore, to overcome the pharmacokinetics limitation and to improve the therapeutic value of this dietary component, various approaches like liposomal curcumin, curcumin phospholipid complex, structural analogs of curcumin, curcumin nanoparticles and curcumin conjugations have been developed and tested (94-98) in order to improve curcumin drug delivery. Among all these strategies, the efficacy of curcumin analogs and curcumin nanoparticles has been extensively tested in breast cancer. Importantly, two novel non-toxic curcumin analogues have been investigated to possess anti-breast cancer properties, namely 5-bis(4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidine(PAC) and 1,7-bis-(4-hydroxy-3-ethoxyphenyl)-1,6-heptadien-3,5-diene (EAC).PAC showed higher stability in blood, higher water solubility, greater bioavailability and bio-distribution than curcumin(9, 11). PAC displayed five times higher efficiency than curcumin and also EAC at inducing apoptosis on ER-negative-MDA-MB-231 cells via an internal mitochondrial route (9, 11). *In vivo* experimental results on MDA-MB-231 cell xenografts have proven that PAC exhibited the anti tumoral effects by inhibiting NF-kB and its downstream effectors (cyclin D1 and Bcl-2), p21WAP1, Survivin and activating the caspase cascade (99).

Curcumin encapsulated with nanoparticulate formulation based on poly lactide-co-glycolide (PLGA), along with a stabilizer polyethylene glycol (PEG)-5000 showed more potency than crude curcumin, in inducing anti-proliferative and apoptotic effects against MDA-MB-231 cells (98). Transferrin-mediated solid lipid nanoparticles (Tf-CSLN) increased photostability and enhanced its anticancer activity against MCF-7 breast cancer cells (100). Tang et al. 2010, reported that an intracellular-labile amphiphilic surfactant (- like curcumin prodrug-curcumin) conjugated with two short oligo (ethylene glycol and Curc-OEG) chains via beta-thioester bonds acted as vehicles for doxorubicin and camptothecin treatment. On delivery to drug-resistant cells, it greatly enhanced the cytotoxicity of the loaded drug in nude mice inoculated with MDA-MB-468 cells (101).

Potential risk and side effects of

curcumin :

Phase I clinical trials discovered that curcumin could be safely administered at very high doses up to 6 g per day (102). However, it has also been observed that curcumin bears the blood-thinning effect that may decline blood flow, leading to ischemic stroke (103). In addition to its anti-oxidant effect, curcumin also demonstrate pro-oxidant effect (104). Curcumin inactivated the cancer cytotoxic agent by blocking JNK pathway in breast cancer (105). The studies performed on breast cancer cells clearly showed that curcumin inhibits mechlorethamine, doxorubicin and camptothecin and induces apoptosis of these cells (106).Therefore, more importance should be given towards the establishment of efficacy of curcumin in breast cancer treatment.

Conclusion :

From the last few years, cancer researchers thoroughly examined the anti-tumorigenic effects of curcumin. Studies suggest that this polyphenol affects various pathways and signaling proteins critical for tumorigenesis. Therefore, it has potency in cancer prevention and also cancer treatment. Among the number of pathways, Wnt/β-catenin signaling is aberrantly up-regulated in breast cancer and the modulation of its key components is found to be associated with aggressive phenotype of these tumors. Therefore, therapeutic intervention of this signaling is the topic of great interest in breast cancer treatment. The use of novel curcumin synthetic analogs and nanotechnology- based formulations represented a potential alternative strategy of great clinical interest for overcoming the high metabolic instability and poor bioavailability of curcumin. Overall, curcumin and its analog needs significant research to establish its therapeutic potential with less limitation and enhanced action in breast cancer.

Conflict of interest:

Authors do not show any conflict of interest.

Authors contributions:

Experimental analysis was carried out by Dr. Chandraprakash Prasad. Poonam Jawanjal helped in acquisition of data and references as well as writing the manuscript. Dr. Gayatri Rath reviewed the results of experiments and has compiled the manuscript.

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Biological Basis and Molecular Mechanism of Regeneration

Sujata Mohanty, Anupama Kakkar, Manisha Singh*
Stem Cell Facility, DBT- Centre of Excellence for Stem Cell Research,
All India Institute of Medical Sciences, New Delhi.

ABSTRACT

Stem cells are responsible for regenerating the lost and damaged cells and tissue. Because of this known fact, regenerative medicine has emerged as a promising field to explore. As a result of exhaustive series of research, in 2012, Shinya Yamanaka and John B Gurdon were awarded the Nobel Prize for their remarkable contribution in the field of Physiology and Medicine. Stem Cell Facility (SCF) at AIIMS was established in 2005 and was the first centre in North India to start stem cell trial. Department of Biotechnology has granted Centre of Excellence in stem cell research to SCF, AIIMS to carry out basic, pre- clinical and clinical research. The main purpose of exploring all these various aspects of research is to study the molecular biology involved in the process of stem cell differentiation, mode of action of stem cells and their pre-clinical and clinical implications in terms of homing of stem cells at the site of damage, differentiation into desired cells and interaction with the tissue *in vivo* and finally, in disease prognosis

Keywords: Regeneration, Stem Cells, Regenerative Medicine, Embryonic Stem Cells, Adult Stem Cells.

Correspondence: * Dr. Sujata Mohanty, Stem Cell Facility, DBT- Centre of Excellence for Stem Cell Research, 1st Floor ORBO Complex, All India Institute of Medical Sciences, New Delhi- 110029, E-mail: drmohantysujata@gmail.com, Phone : +91-9810291336, +91-11-26593085

INTRODUCTION

What is Regeneration?

Regeneration is the process of renewal, restoration, and growth that makes genomes, cells, organs, organisms and ecosystems resilient to natural fluctuations or events that cause disturbance or damage. The regeneration of lost body parts and injured organs has captured the human imagination since the time of the ancient Greeks. Every living organism from bacteria to human has the capability to regenerate (Fig. 1). Regeneration can either be complete (where new tissue is same as the lost tissue) or incomplete (where after the necrotic tissue comes the fibrosis) (1, 2).

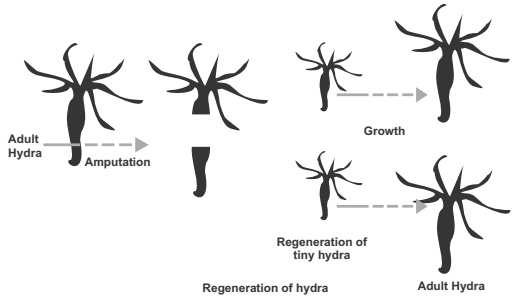

Fig 1: Process of regeneration in Hydra (http://www.tutorvista.com/content/biology/biology-iv/growth-regeneration ageing/regeneration.php)

Stem Cells and types:

In multicellular organisms, stem cells play a very important role in the tissue regeneration *in vivo*. These are defined as the biological cells, found in multi- cellular organisms, which are capable of both self renewal and differentiating into various diversified cell types. There are various types of stem cells present in the human body. Embryonic stem cells (ESC) are the pluripotent cells, obtained from the inner cell mass of blastocyst stage of embryo. Adult stem cells (ASC) are the multipotent cells, present at the local niche of every tissue. ASC can be obtained from tissues like bone marrow, umbilical cord, umbilical cord blood, adipose tissue, dental pulp, skin, hair follicle, parotid glands, etc. (Fig. 2) There are several pros and cons of these stem cell sources.

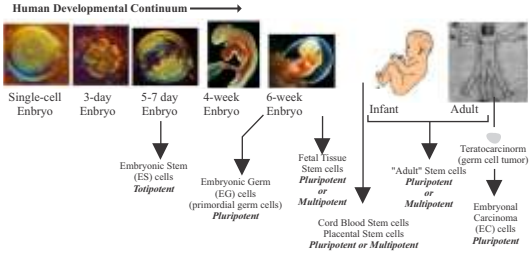

Fig 2: Different sources to obtain Stem Cells of varied potencies (cyhsanatomy1.wikispaces.com)

Ethical Issues and other issues:

Although ESCs are pluripotent and can be used to study the embryological development processes, yet they have limitations in not being immunologically naive and having ethical issues as well. Moreover, it is very cumbersome to isolate culture and maintain ESCs. These issues can be overcome with ASCs but they lack in their potency to differentiate into cells of any lineage. Moreover, ASCs have the trans-differentiation potential to differentiate cells of other lineages also. As they are

immunologically naive, these cells are good for translational purposes.

Mode of Action of Stem Cells:

On transplantation *in vivo*, stem cells interact with the native tissue via various modes of action like differentiation, fusion, paracrine factors (immunomodulation, releasing angiogenic factors, anti- apoptotic factors and anti- oxidative factors) and by releasing microvesicles and miRNA (3). Differentiation of stem cells require various factors like FGF2, FGF8, SHH, BDNF, TGFβ, etc. depending upon the cell lineage into which they are to be differentiated. This fact has been fully explored by various research groups of the world. These growth factors are being used *in vitro* to study the differentiation pattern of stem cells into cells of different types. *In vitro*, stem cells can be differentiated by the process of reprogramming also (4, 5). Cell fusion is a process that has an important biological role in the development, physiology and disease of multicellular organisms. For example, we have the zygote formation and organogenesis of various tissues, such as placenta, bone and skeletal muscle. Cell fusion is triggered by inflammation. It is important to note that lipid bilayer membranes do not spontaneously fuse, and that fusion between membranes involves a highly intricate choreography of lipids and proteins (6).

Apart from these, stem cells mend the injury by paracrine and endocrine effects, i.e., by immunomodulation by regulating macrophages, dendritic cells, B and T lymphocytes, NK cells, etc. (7), angiogenic factors like vascular endothelial growth factor (VEGF), stromal cell derived factor- 1 (SDF1), fibroblast growth factor- 2 (FGF2), etc. these factors function mainly by CXCR4 pathway (8). Anti- apoptotic factors involved in this procedure are brain derived growth factor (BDNF), hepatocyte growth factor (HGF), insulin growth factor (IGF), etc. involved in the interaction of stem cells in *in vivo* conditions (9). Besides making inflammatory response, stem cells interact by releasing anti- oxidative factors/ mediators like IGF, platelet derived growth factor (PDGF), superoxide dismutase (SOD), HGF and IL-6 (10). Despite the bioactive molecules secreted by MSC, Bruno et al have showed that inside the conditioned medium also there are some micro vesicles. These micro vesicles (MVs) are circular membranes fragments that shed from the cell surface membrane carrying protein and lipids from the membranes of the cells from which they originate. It has been shown that stem cells are able to shed off these MVs and they contain mRNA and micro RNA for the amelioration of injured site (11) (Fig. 3).

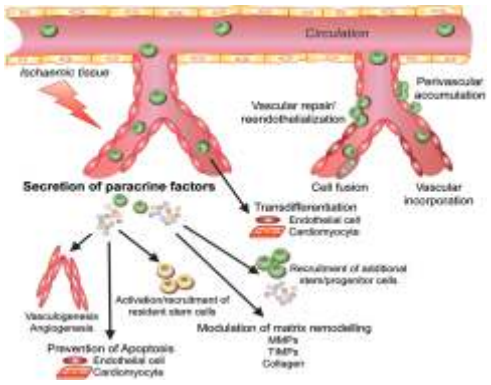

Fig. 3: Proposed mechanisms of ischaemic tissue repair via stem and progenitor cell- based therapies (Ref: European Heart Journal, 2011: doi:10.1093/eurheartj/ehr018)

Significance of mitochondria in Repair and Regeneration of damaged tissue:

Studies have shown the role of mitochondria in maintenance of pluripotency, differentiation and reprogramming of iPSCs. While glycolytic energy is observed at pluripotent state, mitochondrial oxidative phosphorylation (mtOXPHOS) is necessary for cell differentiation. It is hypothesized that reprogramming of somatic cells towards a pluripotent state, by somatic cell nuclear transfer (SCNT), transcription-induced pluripotency or creation of pluripotent cell hybrids, requires acquisition of mitochondrial properties characteristic of pluripotent blastomeres and ESCs. In a recent study, it was reported that cells and tissues created through nuclear transfer can be rejected by the body because of an immune response to the cell's mitochondria. In addition, differentiated stem cells and pluripotent stem cells differ in mtDNA copy number, ATP production, mtOXPHOS and total cell (12). Mitochondria are also known to mediate stress-induced apoptosis in embryonic stem cells (13). However, the mechanisms and exact associations of mitochondrial activities associated with stemness and cell differentiation remains elusive. In addition, there is evidence that suggest that MSCs can rejuvenate damages cells by mitochondrial transfer (Fig. 4). Miro1/Rho1 mitochondrial transport proteins positively regulate the transfer of mitochondria from MSCs to the damages cells with dysfunctional mitochondria (14, 15). It has been shown that Miro1 over expression can lead to enhanced therapeutic efficacy and reversed inflammation by releasing anti-inflammatory products (16). The presence of oxidative stress and imbalance in potassium efflux and membrane potential is associated with inflammatory responses (17, 18). The role of mitochondria in immunomodulatory function of MSCs remains widely unknown.

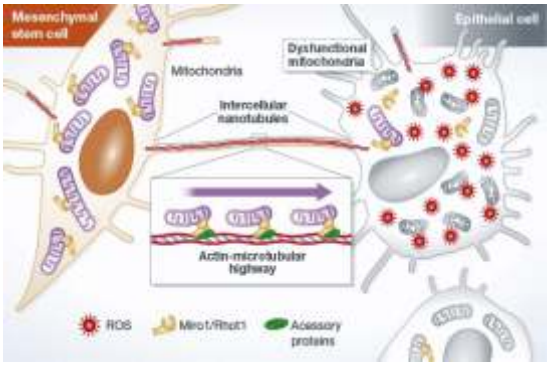

Fig. 4: Mesenchymal Stem Cells (MSC) act as mitochondrial donors during attenuation of lung inflammation and injury. Mitochondrial donation is an essential part of the MSC therapeutic effect in these models and is positively regulated by Miro1 / Rhot1 mitochondrial transport proteins. (The EMBO Journal. 2014;2;33(9):994-1010)

Role of Exosomes secreted by Mesenchymal Stem Cells in Tissue Repair & Regeneration:

Exosomes are small membrane vesicles (between 30 and 100 nm in diameter) of endocytotic origin that are secreted by most cells in culture. They seem to form by invagination and budding from the limiting membrane of late endosomes, resulting in vesicles limited by a lipid bilayer containing cytosol from the producing cells. Fusion of the exosomes with the plasma membrane of the recipient cell, allows transfer of the internal components to the target cell and thus, the transfer of information (Fig. 5). It has been long understood that MSCs can lead to repair of damaged tissue by differentiating to the cell type, for example to cardiomyocyte in case of myocardial infarction. However, several studies suggest that contrary to the previously known belief, new hypothesis secretion of complex paracrine factors for tissue repair and regeneration (19, 20). Several growth factors including vascular endothelial growth factors (VEGF) and basic fibroblast growth factor (bFGF) (21).

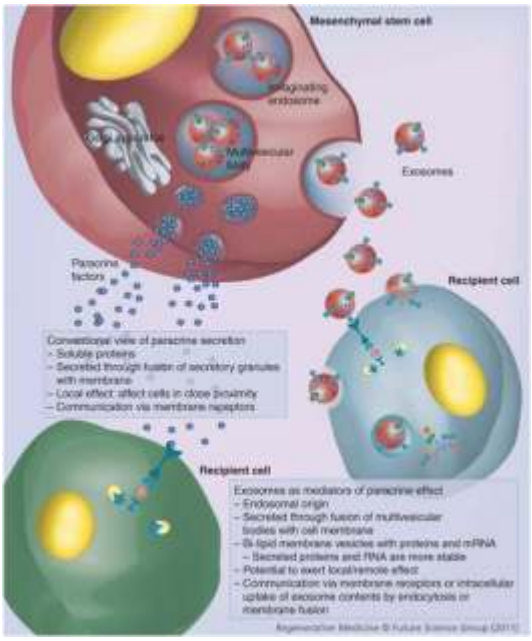

Fig. 5: Proposed mode of action of exosomes released by Mesenchymal Stem Cells (beyondthedish.wordpress.com)

Stem Cells in regenerative medicine:

Regenerative medicine is the "process of replacing or regenerating human cells, tissues or organs to restore or establish normal function". Stem cells play a very indispensable role here. Now a days, scientists all over the world are trying to prepare biocompatible scaffolds that support the growth and differentiation of stem cells and can also act as a mode of transport of these stem cells at the localised site. Various research groups all over the world have reported scaffolds that are differentially compatible to neurons, cardiomyocytes, osteocytes, chondrocytes, etc. Generation of disease specific Induced Pluripotent Stem Cells (iPSC) represents a clear breakthrough in regenerative medicine. iPSCs were first developed in mouse cells in 2006, followed by development of human iPSCs in 2007 by the group headed by Prof. Shinya Yamanaka at Kyoto University. Four important genes involved in the development of iPSCs are Oct3/4, c-myc, Sox2 and Klf-4 (22). Hence, it may be inferred from this point that regenerative medicine holds a potential scope in cell based therapy for the most dreadful

degenerative diseases.

At the Stem Cell Facility, AIIMS, we are carrying on research at all the three stages, i.e., basic, pre- clinical and clinical. Basic research is going on in the field of differentiation of mesenchymal stem cells from various sources like bone marrow, umbilical cord, umbilical cord blood, skin, hair follicle, etc. into neuronal cells, cardiomyocytes, chondrocytes, osteocytes and adipocytes, etc. The pre-clinical research includes the transplantation of cell- loaded biocompatible scaffolds into the rat model to study the bone formation (unpublished data). We have also studied the effect of transplantation of mononuclear cells in the brain infarction, nerve injury and spinal cord injury in rat model having damaged sciatic nerve. Under the clinical research, patient trials for myocardial infarction, vitiligo, stroke, corneal defects, etc. are going on. Limbal stem cells have been established to be an effective therapy in case of eye disorders.

In the current write up, the process of regeneration and role of stem cells in the process of regeneration will be elaborated. Work done and the research going on in AIIMS, with their future prospective will also be discussed.

Materials and Methods:

All the studies were initiated after the approval from Institute Ethics Committee (IEC) & Institute Committee-Stem Cell Research and Therapy (IC-SCRT).

1. Clinical Research:

With the bone marrow mononuclear (MNC) cells various clinical trials are going on at various levels. We have three multi-centric trials in collaboration with various multispecialty tertiary hospitals in India. Various double blinded randomized controlled trial studies are also going on. Stem cell clinical trials in AIIMS are going on for dilated cardiomyopathy, coronary artery disease, paediatric diseases, limb ischemia, macular hole, stroke and spinal cord injury.

Apart from these Bone Marrow MNC involving Stem Cell Trials we have been able to translate the bench side research into translational research with significant success in the following instances:

1. 1 Stem Cell Clinical Trials in Heart Diseases:

a. Application of Stem Cell Technology for Dilated Cardiomyopathy:

The ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) Trial was the first clinical trial involving stem cells in Dilated Cardiomyopathy in India. The study included patients between 15 and 70 years of age with idiopathic dilated Cardiomyopathy with normal coronary arteries, an ejection fraction (EF) of< 40%, and no other severe co morbidities (e.g., chronic renal failure). The study design was an open-label, randomized

trial in which 85 patients were enrolled (23).

b. Application of Stem Cell Technology for Coronary Artery Disease:

Although the myocytes that are lost during Myocardial Infarction (MI) cannot be regenerated, a small population of muscle cells in the region of viable myocardium may replicate and prevent heart failure. At our institute, 43 patients underwent combined CABG (coronary artery bypass grafting) and stem cell transplantation between February 2003 and October 2006. Their mean age was 51.6 ± 6.5 years (range, 42- 62 years). Eighty-six percent had a documented previous MI, 43% had hypertension, 36% were chronic smokers, and 14% had diabetes. The basal New York Heart Association class ranged from 2 to 4 (mean, 2.9 ± 0.7). All patients had akinetic areas with 86% of the akinetic areas occurring in the anterior wall. The basal LV ejection fraction was 33% ± 16%. These patients received 2 to 4 grafts (mean, 2.8 ± 0.6). Additional procedures were Dor's procedure (n = 2), LV clot removal (n = 1), and post-MI ventricular septal defect closure (n = 1) (24).

1.2 Stem Cell Clinical Trials in Pediatric Diseases:

Stem Cell Clinical trial was done in challenges faced in the management of bilateral multicystic kidney disease (MCKD). The tibial bone marrow tap was dry. Repeated blood transfusions and erythropoietin injections were given. At the age of 1 year, the bone marrow tap was attempted again. Ten milliliters of bone marrow was collected under local anesthesia, from the patient's tibia (autologous). Autologous bone marrow mononuclear stem cells were injected into the aorta at the level of the renal arteries, applying pressure over the aorta below the renal arteries during injection, to direct the stem cells into the renal arteries. Repeated renal biopsies at the time of stem cell transplant were taken (25).

1.3 Stem Cell Clinical Trials in corneal defects:

a. Limbal stem cell transplantation:

Stem Cell clinical trial has been done with cultivated epithelium was transplanted in patients with total or partial limbal stem cell deficiency. Limbal tissue specimens were from cadaveric corneo- scleral rims, live related donors or contra lateral eye of the patients. Harvested tissue was cultured on denuded human amniotic membrane (dHAM) using various techniques to stabilize the dHAM (26).

b. Assessment of Central Retinal Function after Autologous Bone Marrow Derived Intravitreal Stem Cells Injection in Patients with Retinitis Pigmentosa using Multifocal ERG :

Patients with RP with visual acuity (VA) =1.90 were included. All patients underwent mf-ERG testing (61 hexagons) prior to intravitreal stem cells injection. Mf-ERG was repeated at end of 1st month,

3rd month and 6th month post injection. First order kernel mf-ERGs were analyzed (amplitude and implicit time of n1 and p1) (27).

1.4 Hair Follicle Outer Root Sheath Cells in the Treatment of Vitiligo:

We have also undertaken the first of its kind in the world- translational research by using the extracted non cultured hair follicle outer root sheath cell suspension (EHF-ORS-CS) in the treatment of vitiligo (28).

2. Pre- clinical Research:

2.1 Effect of bone marrow-derived mononuclear cells on nerve regeneration in the transection model of the rat sciatic nerve:

Materials and Methods:

Bone marrow from 24 adult male Wistar rats was aspirated and MNCs were isolated by Ficoll- Paque gradient separation method. For transplantation, 4 million to 8 million BM-MNCs were suspended per millilitre of normal saline. MNCs were transplanted between the approximated ends of the sciatic nerve. In the control group, the transected nerve ends were repaired with two epineural microsutures using 10-0 monofilament nylon and fibrin sealant only. On day 30 and day 60, Phases I and II respectively, the right sciatic nerve was re-explored and transected and the distal end was labelled with a thread. It was fixed in 3% gluteraldehyde. Nerve segments were processed for histopathological analyses for neurofilament, CD34, S100 and leukocyte common antigen markers (29).

2.2 Dose-dependent facilitation of peripheral nerve regeneration by bone marrow–derived mononuclear cells: a randomized controlled study:

Materials and Methods:

The right sciatic nerve of 60 adult female Wistar rats (randomized into 2 test groups and 1 control group, 20 rats in each group) underwent transection under an operating microscope. The cut ends of the nerve were approximated using 2 epineural microsutures. The gap was filled with low-dose (5 million BM-MNCs/100 ml phosphate-buffered saline [PBS]) rat BM-MNCs in one group, high-dose (10 million BM-MNCs/100 ml PBS) rat BM-MNCs in another group, and only PBS in the control group, and the approximated nerve ends were sealed using fibrin glue. Histological assessment was performed after 30 days by using semi quantitative and morphometric analyses and was done to assess axonal regeneration, percentage of myelinated fibres, axonal diameter, fiber diameter, and myelin thickness at distal-most sites (10 mm from site of repair), intermediate distal sites (5 mm distal to the repair site), and site of repair (30).

2.3 MSC differentiation onto 3D biocomposite scaffolds and transplantation in rat model:

Materials and Methods:

3D biocomposite scaffolds of chitosan by freeze drying method and the bone formation was studied in three study groups, i.e. only scaffold, scaffold+ undifferentiated MSCs and scaffold+ osteoinduced MSCs, when transplanted in the subcutaneous pockets in Wistar rats. Except the only scaffold's group, other two groups were seeded with $1X10^5$ cells/20µL of solution were seeded and finally, cells were induced for osteocytes. On the completion of 14 days of incubation, scaffolds were transplanted in the subcutaneous pockets in Wistar rats. X-ray and histological studies were carried out at 2nd, 4th, 6th, 8th and 12th week of transplantation of scaffolds. All surgical procedures were performed under aseptic conditions using dual access animal handling station [Unpublished data].

3. Basic Research:

3.1 Differentiation of bone marrow stem cells into cells of neuronal lineage:

Materials and Methods:

Bone marrow from 5 patients (Age range- 13-58 yrs) was aspirated and isolated by plating bone marrow directly onto the culture vessel and expanded in LG-DMEM supplemented with 10% FBS at 37ºC/5%CO_2. Cells were characterized by the presence of various surface proteins (CD105, CD90, CD29, CD73, HLA I & II and CD 34/45) using flow cytometry. Cells of 3rd passage were used for the experimental purpose. Different induction cocktails were used to differentiate BMSCs into neuronal cells. 12 days differentiation protocol was followed for four different differentiation media (NIM1: FGF2, NIM2: FGF8+ SHH, NIM3: ATRA, NIM4: SHH+FGF8+ATRA) with neurobasal media, B27 supplement, EGF, L-glutamine and antibiotics in common. The results were evaluated on the basis of RT-PCR, q-PCR, immunoflorescence and flow cytometric analysis of neuron specific markers NF, NSE, β- tubullin III, astrocytic marker GFAP, etc. Dopamine level secreted in the media by the DA cells was quantified by ELISA. The functionality assay of the neurons was done by calcium ion signaling, using confocal microscopy (31).

3.2 Differentiation of bone marrow stem cells into cardiomyocytes:

Materials and Methods:

BM-MSCs from bone marrow were isolated by direct plating and expanded in LG-DMEM supplemented with 10% FBS at 37ºC/5%CO_2. All the experiments were performed using 3rd passage cells after their characterisation. They were checked for the presence of vimentin and Fibroblast Specific Protein (FSP) (Immuno fluorescence (IF) assay); Surface markers -CD105, CD90, CD29, CD73, HLA I & II and CD 34/45 by flowcytometry. After characterization, the cells were differentiated *in vitro* into cardiomyocytes using 2 induction media (1) 5-Azacytidine, 6uM for 24 hours and then kept in expansion media for 30 days (2) TGFβ1, 10ng/ml for 14 days.

Morphological changes were observed and documented. After differentiation, cells were characterized by Reverse Transcriptase-PCR (RT-PCR), Immunofluorescence (IF), Flow cytometry and qPCR studies for cardiac specific markers –Myosin light chain -2v (Mlc-2v), Cardiac Actin (CA), Connexin 43 (Cx43), GATA4, Cardiac Troponin I (cTnI).These cells were positive for vimentin, CD105, CD90, CD29, CD73, HLA I and negative for FSP, HLAII and CD34/45 (32).

3.3 Differentiation of Epidermal Stem Cells (EpiSCs) into Melanocytes, Keratinocytes and Neurons:

Materials and Methods:

Healthy pigmented skin tissues were collected from the patients undergoing surgery for vitiligo treatment in the Department of Dermatology, AIIMS and from children coming for circumcision in the Department of Pediatric Surgery, AIIMS. Healthy pigmented skin tissues were harvested by two different methods (i) skin blister and (ii) foreskin. Healthy pigmented hair tissues were harvested by the following three methods of biopsies (i) Follicular Unit Extraction (FUE), (ii) Scalp Tissue and (iii) Follicular plucking. Hairs were trimmed (shaft removed) and the root was treated with 0.25% trypsin-0.05% EDTA thrice for an incubation of 30 min each. Cells obtained from each digestion were pooled and filtered with 70µm cell strainer. The final cell pellet was suspended in DMEM-HG medium supplemented with 10% FBS, added with choleratoxin, glutamine, FGF2, insulin and adenine and plated on the culture vessel and expanded further. Skin biopsies were treated with dispase with overnight incubation at 4°C. After the incubation period is over, epidermis is peeled off and dermis was discarded. Epidermis was chopped and trypsinized for 15- 20 min using 0.25% trypsin-0.05% EDTA. Final cell suspension was passed through a 70µm cell strainer and the resulting cells were suspended in Epidermal stem cell culture medium. Cells from both the sources were characterized using flowcytometry for CK15, CK19, and β1-Integrin markers. Undifferentiated cells were assessed for doubling time and proliferation assay (MTT Assay).

EpiSCs were differentiated into melanocytes using differentiation induction media consisting of 50% MCDB 201 media, 40% Ham's F12, nutrient mix, supplemented with 10% fetal calf serum, 2mM/ml L-glutamine 10-4 mol/L L-ascorbic acid, 10 nM/ml phorbol 12-myristate 13-acetate, 10ng/ml cholera toxin, 20ng/ml fibroblast growth factor, 100 IU/ml penicillin and 100 mg/ml streptomycin. Differentiated cells were characterized by Fontana Masson staining, IF staining for HMB45 and S100, RT- PC for TYR and Melan A genes, qRT-PCR for MITF and TYR genes. Functional assessment of the melanocytes was done by L-DOPA staining.

The media used for the keratinocyte differentiation was of following composition- DMEM, and

Ham's F12 medium in a 3:1 ratio supplemented with 10% FBS, 10 ng/ml Epidermal Growth Factor, Ca Cl2, Dihydroxyvitamin D, hydrocortisone, Adenine, Insulin, 5µg/ml Transferrin, Triiodothyronine, Penicillin and Streptomycin. Keratinocytes were characterized by IF staining for K1/K10, RT-PCR for K1/K10 and Involucrin genes and qRT-PCR for K1/K10 gene (33).

Neuronal differentiation induction media consisted of neurobasal media containing penicillin and streptomycin, supplemented with bFGF, EGF, B-27 supplement and L-glutamine. Post induction, differentiated cells were characterized by IF staining for Neurofilament, Nestin and Tyrosine hydroxylase and qRT-PCR for TH and NF genes.

3.4 Standardizing the technique of *ex vivo* culture of limbal epithelial stem cells (LESCs):

Materials and Methods:

Limbal tissue specimens were isolated from cadaveric corneoscleral rims, living related donors, or contralateral eye of the patients. Harvested tissue was cultured on denuded human amniotic membrane (dHAM) using various techniques to stabilize dHAM. The optimization of *in vitro* culture conditions was achieved by modifications in culture media. The LESCs were cultured in both types of media for 2 weeks, and growth patterns were observed. Expanded cells were further characterized by Immunocytochemistry (K3/12, K19, and ABCG2) and reverse transcriptase polymerase chain reaction (K12, Cx43, Pax6, ABCG2, p63, and K19) (34).

3.5 Improved reprogramming efficiency of disease specific iPSC using immortalized human foreskin fibroblast feeder cells:

Materials and Methods:

In vitro-culture and expansion of fibroblasts cells were established using explant method. $1x10^5$ Fib cells were infected with hSTEMCCA (Human Stem Cell Cassette) lentiviral vector in the presence of 5ug/ml Polybrene. Next day, cells were plated onto mouse and I-HFF feeder at a density of $1x10^4$ cells onto the 6- well plate. After 24 hours, the medium was switched to reprogramming medium (ESC media and I-HFF conditioned media in 1:1 ratio).

Induced colonies were picked up based on human ES cell colony morphology and live staining for TRA-1-60 marker at days 16-24 post infection. The iPSC lines were assessed on the basis of morphology, expression of pluripotent makers by Immunoflorescence (Oct4, Sox2 and Klf4) & RT-PCR (Oct4, Sox2, c-myc, Nanog and Klf4). The in –vitro pluripotency and ability to differentiate into three germ layers was assessed by embryoid body formation. The experiments were performed using fibroblast cells from two different patients in triplicates [Unpublished data].

3.6 Maintenance of human Embryonic Stem Cells in feeder and bFGF free culture system using conditioned media from immortalized human Foreskin Fibroblast Cells:

Materials and Methods:

The secretion of TGF- β and IGF-II from the mitomycin-C treated I-HFF supplemented with various exogenous bFGF concentration (0, 2, 5 and 10ng/ml) was assessed by ELISA. The KIND -1 hES cell lines were gradually adapted to grow in feeder free system on geltrex coated culture dishes using CM at varying concentration of exogenous bFGF supplementation (0,2,5 and 10ng/ml). The hESC line grown in feeder free culture with CM was assessed on the basis of morphology, expression of pluripotent makers at 1st, 3rd, 5th and 10th passage by Immunoflorescence and flow cytometry. Any karyotypic abnormalities were also assessed at 20th passage [Unpublished data].

Results and Conclusion:

1. Clinical Research:

1.1 Stem Cell Clinical Trials in Heart Diseases:

a. Application of Stem Cell Technology for Dilated Cardiomyopathy:

In the initial 6 months of the ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial, we found that 76% of our patients who were NYHA functional class III showed an improvement in EF by 5.4%. This improvement manifested after 1 month, which is too early to be explained by formation of fresh myocytes. This benefit was predominantly due to improvement in end-systolic volumes while the end diastolic volumes remained the same. This is similar to a number of other studies that also showed an improvement in EF of about 4% to 6%, and also with no change in end-diastolic volumes. This would suggest that stem cells do not cause any change in the remodelling process but improve myocardial cell function.

b. Stem Cell Clinical Trials in Pediatric Diseases:

An improvement seen in urinary parameters, with fall in urea from 260 to 163 mg/dl, fall in creatinine from 1.9 to 1.5 mg/dl and fall in serum K from 6.6 to 3.6 was noted at first month following stem cell transplant. A marked reduction was seen in the doses of medicines needed to keep the child metabolically stable. The baby kept struggling but succumbed at the age of 17 months and 15 days due to end stage renal failure. Post mortem bilateral trucut renal biopsies demonstrated presence of glomerular sclerosis, primitive appearing renal tubules, thick walled blood vessels and scattered blastemal cells that were not demonstrated earlier. However, though the tissue obtained was little so it was difficult to confirm regressive changes, there was an improvement in the fibrosis as appreciated histologically.

1.2 a. Stem Cell Clinical Trials in corneal defects:

After transplantation, epithelial transparency increased in all patients. Reduction or absence in superficial corneal vascularization was observed in 80% patients. Conjunctivilizaion of the cornea seen preoperatively was found to improve in all patients after the ex-vivo cultured limbal stem cell transplant.

b. Assessment of Central Retinal Function after Autologous Bone Marrow Derived Intravitreal Stem Cells Injection in Patients with Retinitis Pigmentosa using Multifocal ERG :

Thirty patients (26 male and 4 female) aged 18 to 58 years (mean 35.9) were included in the study. Visual acuity pre- injection ranged from 0.48 to 1.9 log MAR (mean 1.25289 ± 0.5324). At 6 months of follow-up there was no statistically significant change in the best corrected visual acuity after stem cells injection (p = 0.785 Friedman test). At 6 months follow-up period mf-ERG p1 wave amplitude within 2° from fovea (ring 1) showed improvement (p-value 0.014). The p1 wave latencies also showed reduction in the implicit times (p-value 0.03). The maximum mean value of p1 wave amplitude was observed at 3 months of injection. The increase in P1 wave amplitude was maximal in ring 1. The change observed was statistically significant in ring 1 (p-value 0.014).

1.3 Hair Follicle Outer Root Sheath Cells in the Treatment of Vitiligo:

The mean ± SD repigmentation was 65.7 ± 36.7%. Overall, nine of 14 patients achieved > 75% repigmentation. Mean percentage repigmentation was significantly higher in patients with ± 1 year stability than those with < 1 year stability (P = 0.02).

2. Pre- clinical Research:

2.1 Effect of bone marrow-derived mononuclear cells on nerve regeneration in the transection model of the rat sciatic nerve:

Histological assessment of the nerve was performed 30 days and 60 days after the operation and regenerative changes were compared between the two groups. The recovery after nerve anastomosis was far better in the test group at both 30 days and 60 days. There was a statistically significant difference in axonal regeneration, remyelination and myelin thickness at sites 5 mm and 10 mm from the site of repair of the nerve. Schwann cell proliferation and degenerative changes were more prevalent in the controls.

2.2 Dose-dependent facilitation of peripheral nerve regeneration by bone marrow–derived mononuclear cells: a randomized controlled study:

The recovery of nerve cell architecture after nerve anastomosis was far better in the high-dose BM-MNC group than in the low-dose BM-MNC and control groups, and it was most evident (p < 0.02 in the majority of the parameters [3

of 4]) at the distal-most site. Overall, the improvement in myelin thickness was most significant with incremental dosage of BM-MNCs, and was evident at the repair, intermediate distal, and distal-most sites ($p = 0.001$).

2.3 MSC differentiation onto 3D biocomposite scaffolds and transplantation in rat model:

Scaffolds removed from back of the rats for 2, 4, 6, 8 and 12 weeks after implantation were immediately photographed and assessed for opacity and formation of any bone like tissue radiographically. Retrieved implants were fixed in 10% formalin and taken to G.B.Pant Hospital for histopathology. Tissue blocks were sectioned at 5um thickness, positioned on glass slide and stained by hematoxylin and eosin (H&E). The stained sections were observed under microscope and analysed to evaluate host tissue response in terms of inflammation, fibrosis, necrosis, vascularisation, scaffold degradation and tissue ingrowths in scaffolds.

3. Basic Research:

3.1 Differentiation of bone marrow stem cells into cells of neuronal lineage:

Induced BM-MSCs revealed neuron like morphology and expressed cellular markers suggesting neuronal differentiation with all the inducing agents. However, upon quantitative analysis through q- PCR, cells induced with FGF2 were found to show maximum expression of tyrosine hydroxylase (TH). ELISA revealed the highest level of dopamine secreted by the cells in the culture media, induced with FGF2 alone. The presence of TH was observed in the cells when induced with other inducers but with significantly lesser expression as compared to that observed in FGF2 alone. We conclude that BM-MSCs can be coaxed to differentiate efficiently into dopaminergic neurons in the presence of a very simple media cocktail containing only one main inducer and thus contribute towards cellular therapy in Parkinson's and other related disorders.

3.2 Differentiation of bone marrow stem cells into cardiomyocytes:

Differentiation studies revealed the expression of Mlc-2v, CA, Cx43, GATA4, cTnI by RT-PCR and Mlc-2v and cTnI by IF in both treatment groups. The cells after completion of the differentiation protocol were checked for (Transcription factors) TFs TBX5 apart from GATA4 and NKX 2-5. Also, these were checked for BAF chromatin remodelling complex, BAF60C through RT-PCR. It was found that all these were expressed in the BM-MSC derived cardiac cells. However, qPCR results revealed that the TFs NKX 2-5, GATA4, TBX5 and chromatin remodelling complex, BAF60C are expressed at significantly lower levels when compared to adult beating cardiomyocytes.

3.3 Differentiation of Epidermal Stem Cells (EpiSCs) into Melanocytes, Keratinocytes and Neurons:

The new method of extracting the stem cells from outer root sheath of the hair follicle is a very effective one. The EpiSCs isolated by this method can be differentiated successfully into melanocytes and keratinocytes, as shown by IF, RT-PCR and qRT-PCR results. These cells also have the tendency to differentiate into neuronal like cells, supported by the results of IF, RT-PCR and qRT-PCR.

3.4 Standardizing the technique of ex vivo culture of limbal epithelial stem cells (LESCs):

Stabilization of dHAM was successfully achieved using coverslips. Histopathological analysis showed multilayer formation and immunostaining, and reverse transcriptase polymerase chain reaction data confirmed the expression of both stem cell markers (K19, p63, and ABCG2) and differentiation markers (K3, K12, and Cx43). Patients who had undergone limbal stem cell transplantation showed a stable ocular surface with improved visual acuity over a long-term follow-up period.

3.5 Improved reprogramming efficiency of disease specific iPSC using immortalized human foreskin fibroblast feeder cells:

We found that the average days of appearance of colonies was 16 on human feeder in comparison to 24 on mouse feeder. The total number of colonies obtained from two independent experiments at the end of reprogramming period was 41 and 23 on human and mouse feeders respectively. The average reprogramming efficiency of iPSC on human vs. mouse feeders were 0.1 % and 0.05 % respectively as demonstrated by TRA 1-60 Live staining. Disease specific DMD-iPSC generated in this manner displayed ES cell like morphology, expressed stem cell markers TRA 1-60 and TRA 1-81. These iPSC lines exhibited endogenous expression of pluripotency markers like OCT-4, Sox2, Klf-4, cMYC and Nanog. The iPSC lines derived using both the feeder cells were able to spontaneously differentiate into cells of all three germ layers as characterized by Immnunofluorescence and RT-PCR assay.

3.6 Maintenance of human Embryonic Stem Cells in feeder and bFGF free culture system using conditioned media from immortalized human Foreskin Fibroblast Cells :

ELISA results confirmed that the level of both TGF- β and IGF-II secretion was comparable at all bFGF treated group versus no exogenous bFGF added. The cells cultured in the CM in the feeder free conditions even after 20 passages, showed typical hESC morphology and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, alkaline phosphatase and normal karyotypes in all groups compared to positive control. Flow cytometric analysis for TRA1-60 and SSEA-4 surface marker expression shows the increasing trend but the difference was negligible among different groups. (From 0-, 2, 5,10ng/ml and positive control

TRA1-60: 0- 75.6% ± 3.86, 2- 76.87% ± 5.64, 5- 77.28% ± 5.21, 10- 78.1% ± 5.83 and 81.6% ± 3.53, SSEA-4: 0- 81.47%±4.27, 2- 82.9%±3.86, 5- 82.73%±3.80, 10- 84.07%±5.72 and 82.73%±3.80 respectively). There was no difference in the expression of pluripotency-related genes (OCT4, SOX-2, c-MYC, Klf-4 and NANOG) in test groups as compared to positive control as revealed by semi quantitative RT-PCR.

Discussion :

Stem cells, the foundation of all life forms has remarkable potential which gives rise to different cell type right from the early life to the adult form. These stem cells are termed as the embryonic stem cells. These embryonic stem cells have the highest plasticity. The major drawback of embryonic stem cells is that they generate the tumor of all the three germ layers; teratoma, when injected into nude mice. This limits their potential to be exploited into the clinical application. Tissue derived stem cells are there in almost every organ right from hair follicle to the linings of the intestine. These stem cells reside in the discrete pockets inside the tissue/ organs called as stem cell niche. Stem cell niche provides a sort of protective environment for stem cells which helps maintain the homeostasis of the cellular damage to the tissue/ organs due to wear and tear. These tissue- based or adult stem cells are the oldest known stem cells in the clinical practice. The autologous bone marrow derived stem cells are into clinical trials at different centres in India and abroad.

AIIMS is one of the major centres in India to have involved in clinical trials of autologous bone marrow derived stem cells. The stem cell clinical trials in which AIIMS is involved include myocardial infract, dilated cardiomyopathy, critical limb ischemia, cerebral palsy, stroke and macular hole. Apart from this we are also involved in clinical trials involving tissue derived stem cell in ocular surface reconstruction and vitiligo treatment. Clinical trials at AIIMS are still at infancy. Limbal stem cell therapy has been done routinely at AIIMS for various kinds of ocular diseases. We are at par with the clinical trials that are being carried out all over the world.

As far as the pre- clinical studies are concerned, we are working on bone regeneration, using biocompatible scaffolds. Cell based therapy of peripheral nerve injury, using stem cells is also being studied at pre- clinical level at AIIMS. Our study reveals that local delivery of BM-MNCs (which can be isolated easily from bone marrow aspirates) into injured peripheral nerve increases the rate and degree of nerve regeneration, but in a dose dependant manner.

Moving further to the basic research, at this level, we are working on the differentiation aspect of stem cells from various sources and the molecular mechanisms involved in the same. An in-depth study of the factors responsible for and affecting the differentiation of stem cells is required to develop any kind of cell based therapy regime for the degenerative diseases. This knowledge helps in better

understanding the cell behaviour in- vitro. The pre- clinical studies give an outlook to the cellular and molecular mechanisms that may be occurring in the *in vivo* conditions. A balance between the *in vitro* and *in vivo* behaviour of stem cells is mandatory to initiate any kind of clinical trial.

AIIMS has taken the lead in all the aspects of stem cell research, starting from basic research to the clinical trials. We, at AIIMS, are aimed to develop the best cell based therapy that can be taken from bench top to bed side to the patients. The stem cell technology is advancing at a very high pace from the humble beginning of autologous bone marrow stem cells into clinical practice to the arena of iPSCs. We need to move step by step and with great caution. Potential is huge and great, we may have touched the tip of the iceberg but true story remains to unfold.

Future Scenario:

Stem Cells and tissue engineering hold a great potential in the field of regenerative medicine. Scientists, now days, are trying to develop organs by using stem cells on organ prints or moulds made up of biodegradable composites. One such example is the Vacanti mouse, developed by Charles Vacanti, who injected cow cartilage stem cells, seeded on a biodegradable biopolymer and implanted under the skin. Scientists are focusing more on tissue engineering as the biodegradable polymers hold a lot of benefits like, better stem cell delivery and homing, added drugs can be transplanted together, act as a matrix and support for stem cells to grow and lastly, as they are biodegradable, there is no issue of them being hazardous to the body. With the help of tissue engineering, scientists have also been successful in creating trachea lumen model. Apart from the use of stem cells in tissue engineering, these are also being explored for the treatment of deafness, blindness, diabetes type I, bone regeneration, blood supply, Parkinson's disease, Alzheimer's disease, Huntington's disease, myocardial infarction, etc.

Stem cells will be available as off-the-shelf product in the near future for treating various unmet medical needs. The stem cell technology is advancing at a very high pace from the humble beginning of autologous bone marrow stem cells into clinical practice to the arena of iPSCs and tissue engineering. Generation of induced pluripotent stem cell (iPSC) lines and their differentiated derivatives will promote patient specific & disease specific drug development. Tissue engineering will omit the need of donors for organ transplantation, hence reducing the risk of organ rejection and HLA matching. The major concerns of teratoma formation by these cells have to be overcome before their clinical application. Due to their pluripotent nature, iPSCs might change the entire scenario in drug development and will open avenues for personalized medicine using stem cells. We need to move step by step and with great caution. Potential is huge and great, we may have touched the tip of the iceberg but true story remains to unfold.

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