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Stem Cell Therapy: in Ancient, Modern and Futuristic Perspective

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ABSTRACT

The "Stem Cells" have become a great source for repair and neo-regeneration. Recent research has revealed possibility of human use of such cells. Ailments like large Incisional Hernia, Myocardial disease, Diabetes, Parkinson's disease, leukemia etc. can be managed by Cell Therapy. There is confusion in the literature about plasticity, differentiation, trans-differentiation and fusion of cells. This is scientifically and critically addressed. Recent research has revealed that stem cells undoubtedly will play a great role in the management of human diseases. SCT is still a debated modality so far as the safety and efficacy is concerned. Ethical issues of human use of Stem Cells are discussed. The clinical management of damaged neurological and myocardial tissue is observed in certain centers. All this is analyzed in the perspective of available modern research publications and authors own publications along with relevant unpublished data from research work of last 30 years.

KEYWORDS

Stem cell therapy, trans-differentiation, Repair, Tissue engineering, Neo-Organogenesis. Desired Metaplasia

INTRODUCTION

Cell therapy has generated tremendous interest in the minds of common man suffering from terminal incurable illnesses. Human use of stem cell is a big challenge for scientists, clinicians and researchers globally. Thanks to media for awakening the interest of common man on this subject. Failed and functionally compromised organs are treated by extirpation or transplant surgery. This involves removal of diseased, nonfunctional organs and replacing it with functional organs from live or brain death donor. It is well known that these are far from being ideal and perfect. Since the first modality uses only the remaining viable tissue or organ. This modality is not useful when tissue or organ is extensively damaged while the other is plagued with ethical, moral, social and psychological problems, associated with complications of rejection, suppression of host immunity due to use of drugs to prevent rejection. This increases the host susceptibility to infection and even mortality. Its general use is restricted due to non-availability of donor organs and tremendous cost involved in such management. Transplant surgery has generated immoral acts in trade of human organs and stealing of organs from bodies of innocent persons keeping aside the ethics of the medical profession. Growing artificial "neo organs" from stem cells in the laboratory is being explored by international biotech companies considering its business potential. Thus the "spare part surgery" is likely to hit the medical market soon in future [1, 2]. The tissues and organs grown ***in vitro*** for use as spare parts to replace the diseased ones, will need re-vascularization ***in vivo*** to accomplish the ultimate goal and is subject to rejection. On the contrary, re-generative surgery neo regeneration of organs" ***in-vivo*** from autogenous tissue cells is probably the most ideal and ultimate method of management. Since the raw material is from autogenous body tissue and regeneration is in the body, it will, undoubtedly be the cost effective as well. At the same time it will be free from complication of rejection, suppression of host immunity etc. There is no need of organ donor or re-establishing blood supply for implantation in body. Regeneration modality is a first step forward, toward ideal management of human diseases. This is a very cost effective method and useful for all the factions of the society - the rich and poor. The human utility of such biological tissues and organs has been demonstrated. Neo-regeneration of tissues and organs has been published in various science journals and awarded international patents [5]. A myth and misunderstanding is prevailing on the subject especially in the society that SCT can cure all incurable human diseases. Current status of the SCT is discussed in this article. This article scientifically discusses the subject with critical analysis of literature in the perspective of author's own research. At present it is obvious that many more research adventures will be needed before SCT proves an important modality for management of human ailments. Special attention is paid to management of Myocardial infarction, Diabetes, Spinal cord and brain injuries, Parkinson's disease using SCT.

MATERIAL AND METHODS:

A scientific aspect of stem cell and stem cell therapy is critically studied keeping in view of the awareness of the common man. Due to media highlights, a great hope has been awakened for treatment and cure of serious and incurable human ailments. This has given a quantum jump in stem cell research. The search of literature is made from three sources: (1) Ancient literature of Sanskrit speaking civilization (2) available literature of 19th and 20th century and (3) last quarter of 20th and early 21st century till date. Attempt is made to search relevant articles from on line publications. Serious attempt is made to search "human use of stem cells" from literature. Vedic literature studied from the libraries having ancient books and literature. Past literature studied from available literature in National medical library, New Delhi, India, Extensive search of Index Medicus, Quarterly cumulative Index to current Medical literature. A search was also made from the available Medlar and medline services (USA), available in Delhi libraries. Abstracts of selected articles scrutinized. Relevant articles studied in detail. The study was with the intention to understand the current level of stem cell research, stem cell definition, differentiation and trans differentiation of stem cell, cell fusion, stem cell therapy etc. Search was also made to understand the tissue engineering and progress made in achieving the goal. Special emphasis made to find human use of stem cell therapy and success level achieved in this direction. An emphasis made to find the use of stem cells therapy in human beings in clinics and hospitals of National capital territory of Delhi, India, by personal approach and communication.

RESULTS:

Search of Vedic literature revealed knowledge of presence of chromosomes and their 23 numbers as observed in modern literature. Elaborate description of Knowledge of embryology, fertilization of ovum is available. The growth of fetus has been described on nocturnal basis. The description was not on diurnal basis as seen in modern embryological descrip-

tion. (without any explanation for nocturnal development). A chapter mentioning how a human fetus can be grown from cells procured from aborted product of conception was found in literature of "Mahabharat". This is achieved in extra uterine artificial device out side human body. Despite such an advanced description in stem cell use in Vedic literature, its use in treating diseases could not be observed because of the fact that literature searched was not for the management of diseases. Modern literature of 150 years has slowly evolved with description of cell. The knowledge that the cells only grow into cells changed the concepts of our understanding on the subject in modern times. The concepts cherished and taught in medical schools that smooth muscles have no regeneration capacity and cardiac muscles do not regenerate at all got shattered with modern research and developments of early 20th century. Later part of 20th century has witnessed substantial progress in the direction of regeneration of tissues and organs. Last decade of 20th and beginning of 21st century showed a phenomenal development on the subject of stem cell. A new physiological phenomenon has been observed in scrutinized literature. The phenomenon is responsible for neo-regeneration and growth of tissues and organs in mammalian and human body. It is recognized as "Desired metaplasia". Current literature shows a great emphasis on cell therapy for treating human diseases. A substantial progress observed in experimental research but only a few attempts have been made for human use. Attempts at growing tissues and organs in vitro, in the laboratory on biodegradable scaffolds using stem cells has been observed. It is still in experimental stage. Some clinics in and around Delhi claim about use and success in Stem Cell Therapy in Spinal cord injury and myocardial infarction (personal contact and News from AIIMS Delhi) but scrutinized literature could not find any revealed research or published authentication.

DISCUSSION:

The progress in stem cell research in modern time is so fast that some of the issues raised in this article may get out dated by the time it is published. Treatment modalities alternative to transplant surgery, are on constant search globally because it is associated with known unavoidable problems. Availability of off the shelf tissues and organs is still under progressive research. The promising lucrative business potential in "off the shelf tissues and organs" has attracted attention of many multinational companies and the availability of tissue and organ shops in the malls in near future may not be far from reality. This has still to cross many more hurdles. Scientific concepts of stem cell biology is constantly growing since the understanding that "cells only grow to form cells" (Virchow 1855) [6]. In modern times transplantation of organs, replacement of joints, grafting artificial eye lens, implantation of artificial tooth etc have made our life easy and comfortable. Current knowledge of cellular development and cell proliferation of stem cells into unlimited possibilities of forming different tissues has changed our views of life. More and more attempts are being made to grow tissues and organs in vivo and in vitro. In this context concept that the "current living is one of the best time of our life" gets shattered with revelation of knowledge that Vedic period of Sanskrit speaking civilization knew the use of stem cells and could produce normal human beings in extra uterine devices outside the human body [7, 8, 9, 10]. Modern research and scientific development is gradually progressing towards such a goal. During Vedic period fertilization and embryology was well known. There was knowledge about Chromosomes (Gunavidhi = Sanskrit name for chromosomes) and their 23 numbers [8, 9]. Regarding cloning, Rigveda mentions three brothers – named Ribhus, who prepared human clones. There is a mention about development of cow from skin of a cow. Rigveda also mentions the birth of humans (Agasti & Vasistha rishi) born out of an URN (called as Vastivara) from semen of Mitra – Varuna. "Mahabharat" (A treatise on great war of India) describes Draupadi and Drushtdyumna (female and male children of King Drupad) were grown in Urn (special device) using semen of king Drupad. Drona was a clone born from semen of Bharadwaj in Urn known as Drona [8, 9]. A chapter titled "Aadiparv" in "Mahabharat" describing birth of 101 children using cells from aborted embryo of female named "Gandhari" (queen of ruling king Dhritrashtra), after two years of arrested pregnancy. The cells of aborted embryo were reactivated in an artificial device. The dormant cells due to arrested pregnancy were reactivated and rejuvenated in the device for two years. Only then 101 human babies could be born [7]. Discovery of a 7500 BC old city by Archaeological Survey of India, from under sea exploration, off the coast of Gujrat state of India, [11] is a testimony of the statement in the same Mahabharat script about the submerged ancient city (abode of lord Krishna). If it is considered that Mahabharat is not a fiction but is true human history the above stated scientific statement is beyond inerrability. Unfortunately a precious knowledge on use of stem cells in producing children in devices out side human body, cloning and embryology has been lost in the pages of history.

Stem cell therapy using Embryonic Stem Cells is tagged with ethical and legal controversies while Adult stem cells having no controversy has become a reality in modern times. In fact the adult stem cells are the specialized embryonic stem cells with limited differentiation and transformation capability, determined by their link with the three germ layers of germ disc of the developing embryo. A fertilized egg up to 8-cell stage is morula. It has totipotent cells

capable of producing any cell type of the adult body. These cells become more specialized to form germ layers. Germ layer cells retain capacity to form tissues and organs developed from that germ layer. Cells from embryo up to the formation of germ layer disc are ESC while primitive cells retained in developed adult tissues and organs of body, are ASC. These cells are responsible for repair and replacement of cells lost due to normal wear and tear during life.

The capability of embryonic stem cells to form unlimited cell varieties has generated scientific research interest but is associated with ethical, social, political and legal problems (Table I). In embryological development of fetus the single celled fertilized ovum divides and re divides to form basic cells – Embryonic Stem cells. These cells further divide to form multiple clones of cells to form germ disc of three germ layers. The germ layer cells further specialize to form tissues and organs. Each germ layer destined to form specific tissue or organ. Ectoderm forms protective tissues like Skin, Brain and nerve tissue. Mesoderm forms majority of tissues Muscular, osseous, Cardio-vascular, kidney, ureter etc. Endoderm cells forms Gastro-intestinal system, liver, Pancreas etc. [12]. Therefore differentiation to form these tissues is encoded memory into these cells genetically. In other words embryonic stem cells (ESC) and adult stem cells (ASC) are the same cells with different levels of specialization. Many recent research activities in fact differentiate ESC in vitro into desired tissue cells that is differentiated cell derivatives of ESC (like-adult stem cell), before their use for colonization [13]. Therefore, logically, the ESC and ASC share the same hierarchical plate-form. Author of the present article has used ASC in autogenous peritoneal membrane of mesoderm germ layer and used it in human beings. A new technique for repair of large incisional hernia based on regenerative methods has been published in 1991 and 1999 in World journal of Surgery. It has also been published in text book of operative surgery "R Maingot's Abdominal operations" in 1997 [3]. Another human utility for the management of "complex genitourinary rectal fistula" published in Indian J of Urology in 1997 [4]. A new physiological phenomenon of "Desired Metaplasia" was discovered [5]. (US Patent with effect from 1995) This phenomenon in fact is also applicable to ESC. Colonized ESC in a tissue system undergoes desired Metaplasia (DM) and transforms into cells of local tissue of exposure. This is possible due to tissue environment, functional need of the tissue of exposure, local cytokine signaling and Cell Surface Receptor response of the cell (for details see detailed work elsewhere).

If one looks into the cell differentiation pattern described by various researchers (table II) and correlate it with the prin-

ciples of embryology, it can be concluded that the cells follow a standard pattern for differentiation into other cell lineages. Sweeney's experiment, on metaplasia of oral mucous membrane of dog [14, 15, 16], can be quoted as Glaring example. Sweeney et all removed skin from over the cartilage of dog's ear (pinna) and grafted oral mucous membrane in its place. After two months the composite graft of ear cartilage with grafted oral mucous membrane, shifted and colonized with tracheal tissue after removal of segment of trachea. In first place mucous membrane showed squamous differentiation and on second part of the experiment, squamous change disappeared and ciliated columnar change was observed. Author considered the change as metaplasia. According to the author of the present article if the changes observed are co-related to the embryological principles it is clear that these changes are within the laws of embryology. Oral mucous membrane is derived from two germ layers (1) stomatodeum derived from ectoderm and (2) Fore-gut derived from endoderm. Therefore oral mucous membrane when grafted in place of excised

skin of pinna that is exposed to environment of ectoderm it showed squamous change. When composite graft exposed to tracheal (endoderm) environment, endoderm cells got proper stimulus and responded to form columnar epithelium. In tracheal region the stimulus of ectoderm environment disappeared and hence the squamous change disappeared. Environment and functional need is responsible for such desired transformation and hence the name "Desired Metaplasia". This explains desired metaplasia is due to proper environmental stimulus to which the cells are exposed. It also explains germ layer cells respond to same germ layer stimulus. Maintenance of change depends upon the continuous stimulus sustained by the exposed environment of germ layer where exposed. Updated Table 2 explains the same pattern described above [17-35]. Failed experiments tabled in previous publication in IJEB indirectly authenticate the same principle (for convenience of reader Table 2A is presented again). Therefore it can safely be concluded that differentiation of a stem cell follows definite pattern and change taken place in colonized cells needs perpetuation of proper stimulus [36]. On analysis of Sweeney's experiment [16] on metaplasia of oral mucous membrane, it can be concluded that the changes were "Desired metaplasia" and not metaplasia as described by Sweeny et all.

Capacity of ESC to differentiate in vitro is retained only under specialized conditions and chemical stimulus [37]. Similarly ASC, when removed from their anatomical abode and exposed to different environment and stimulus of functional need of a tissue system in vivo, the cells undergo appropriate differentiation to regenerate new cells suitable in that region [38 – 41]. At niche, these cells specialize to form cells suitable for that tissue for maintenance, repair and replacement of cells lost in normal wear and tear. Therefore cell plasticity depends upon proper suitable environment and stimulus (chemical and functional need). Even if stimulus for change is present needful change can only take place when the cells are prepared to accept the stimulus and can undergo change. Therefore the cell Surface receptors must accept the stimulus and be able to respond to that stimulus. Stem cells of a germ layer positively respond to the environment of tissues of the same germ layer [42]. Stimulus of external environment has more influence on the stem cells than genetic coding and memory of the cell for "desired plasticity" of the cell for therapeutic use [43]. Confusion and debate on plasticity, trans-differentiation is observed in literature. Trans-differentiation potential of Adult Stem Cell is controversial [41, 44] (vide infra – General consideration Section B) . Proper environmental exposure, chemical stimulus and appropriate cell surface receptor response is most essential for differentiation potential of stem cell otherwise cell opts apoptosis or change may take place in an abnormal manner and may result into abnormal cell formation and result in tumor formation. **This is an important consideration that SCT be used with caution.**

General Consideration about Therapeutic Use:

Even though a lot of progress has been made in last decade still there are many practical problems in therapeutic use of stem cells. It is certain now that embryonic and adult stem cells both have capacity to grow into tissues and organs. Stem cell therapy is still under evolution phase. To understand more about factors which are responsible for successful SCT can be listed as follows–

- Detection and identification of stem cell suitable (morphological, proliferation and functional suitability) for therapy of diseases of concerned tissue and organ.
- Differentiation and trans-differentiation of selected stem cell in vivo and in vitro.
- Selection of cells as per embryological principles from contiguous embryonic regions during embryo development.
- Harvest and culture stem cell in laboratory.
- Stem cell banking.
- Dealing with political, social problems in using stem cells, and animal activists for use of experimental animals.

A. Identification and Detection:

Detection needs definition and till date the definition is largely functional. Unfortunately the detection is retrospective i.e. after a cell shows its functional capability [45 - 48]. Global efforts are on for detecting specific characters of stem cell (like cell markers etc). Some of the known markers are Oct –3/4, Nanog, Tdgfl, Utfl, Lin-28 as regulatory factors. Signaling pathways like LIF, BMP, Wnt, Nodal etc. Theoretically cellular trans-criptome (all the R N As in a cell type) should be able to define molecular phenotype [37, 41]. Further, details about markers identifying stem cells, is beyond the scope of this article.

B. Differentiation and Trans-differentiation:

Cells to be used for colonization in SCT must be effectively and properly differentiated into particular cell type of that tissue and organ cells (morphological and functional suitability). This is because different varieties of human ailments of different tissues and organs need properly differentiated cells suitable for that tissue. The diseases range from chronic diseases like Diabetes, Parkinson's damaged neurons, degenerative damages resulting in liver and Kidney failures, Traumatic nerve, spine and brain injuries, damaged infracted heart muscle due to ischemia, Muscular dystrophy, improperly developed bones etc.

Some researchers have posted practical and useful guidelines for cell plasticity [39, 40]. The strict criteria may not be satisfactorily suitable to demonstrate the plasticity (for details of criteria reader is referred to appropriate reference) [41]. Some researchers believe that the trans-differentiation is not true trans-differentiation but in fact cell fusion of labeled stem cell with local cell [40, 41]. Pancreatic tissue cell forming liver cell, Bone marrow cells forming cardio-myocytes is considered trans-differentiation [41, 42]. In this context the attention of the reader is drawn to publications of the present author in I J E B and ASAIO Journal [5, 36, 43]. Detailed explanation is discussed in these publications. By differentiation and trans-differentiation the primitive cells (stem cells) in a tissue or organ transforms itself into suitable cells for repair or regeneration of cellular loss due to normal wear and tear during life of an organism in vivo. Similar changes are possible in vitro if suitable environment is provided and such changes are needed for effective repair in SCT. Genetic coding and memory in a cell is normally responsible for differentiation of stem cell into cells of tissues and organs developed from the same germ layer to which the cell belongs [35, 43]. Tissue repair, regeneration or neo-regeneration of tissue and organs is achieved by cellular proliferation, differentiation and trans-differentiation of primitive cells in tissues or cells colonized in tissue or organs. In vivo this is possible by desired metaplasia if principles of embryology are observed (vide hypothesis published elsewhere).

Trans differential potential of Adult Stem Cell is controversial [41, 44]. Tissues and organ development in Fetus is the result of specialization of cells of germ layers of germ disc of developing embryo. Whole body is formed from three germ layers.

Many tissues and organs are formed from a single or by combination of more than one germ layer. As stated earlier each layer has a coded memory to form certain tissues. Therefore cells of a tissue or organ developed from a germ layer can be grown from cells of that germ layer. It is the property of that germ layer stem cell due to encoded memory in that cell. Formation of liver cell from pancreatic progenitor cell is the memory coded in that cell as both the tissue cells have developed from the same germ layer endoderm. Similarly Bone marrow cells forming cardio-myocyte, is again an encoded memory in these cells, as both tissue cells have developed from germ layer mesoderm. Numerous examples are analyzed in previous publications and now updated (Table 2). Interestingly in an abnormal environment of chronic peritonitis, similar property of the cell is exhibited. Stem cells of peritoneum, developed from germ layer mesoderm, forms abnormal tissues in the region of peritoneum in abdominal cavity when exposed to abnormal environment of chronic peritonitis or radiation etc. This change is recognized as metaplasia. This is well known to all the surgeons dealing with chronic peritonitis patients and confirmed by the pathologists. On embryological analysis, all transformed metaplastic tissues in abdominal cavity are derived from germ layer mesoderm [5, 36, 43] and so are the cells of the peritoneum. Therefore, cell formation of such tissues (Pancreatic tissue forming hepatocytes, Bone marrow cells forming cardio-myocytes etc), logically will be questionable to label as trans-differentiation. In other words if a stem cell of a germ layer forms cells of another germ layer it can be considered as trans-differentiation or trans germ layer differentiation. How it is possible is not known at present. Intra germ layer cell formation is possible by synthesizing their own protein molecule, as a result of coded memory in RNA [43, 45]. Thus are capable of differentiation on a different pathways and can proliferate, limited to the germ layer to which these cells belong.

Another confusion and debate prevails on trans differentiation and cell fusion [41]. Transformation to different germ layer cell is beyond the capacity of RNA memory in the cell. To synthesize totally a new protein molecule of different germ layer for differentiation and proliferation, it must take the help of cell in different germ layer tissue surrounding for example by cell fusion. In this way the cell can trans differentiate into cell of that new germ layer and able to synthesize protein suitable for differentiation and proliferation in that tissue or organ as the case may be. Therefore cell fusion should not be the hindrance in trans-differentiation but is a nature's way to synthesize needed protein molecule for formation of that tissue in new environment. In authors opinion cell fusion is the way by which trans-differentiation is effected (author's hypothesis based on the laws of embryology). In fact fusion is universal phenomenon in nature. Fusion of two elements forms totally a new substance with radically different properties. Common example is Hydrogen gas with oxygen forms water in an environment at a particular temperature. Water has totally different properties in comparison to hydrogen and oxygen. Laws of nature though intricate but have uniform applicability in nature. Therefore fusion is the way of nature to achieve desirable, effective and needful change for suitable repair or regeneration. It activates the capacity to synthesize new protein molecule essential to form cells in the region of exposure. This is responsible for effective repair, regeneration or neo-regeneration.

C. Selection of cells as per embryological principles from contiguous embryonic regions during embryo development.

As per the hypothesis of the first author, during embryo development Stem Cells of tissues of a germ layer which grow in contiguous region during embryonic growth, posses and share similar growth potential and property. In other words the cells of tissues and organs developed

in contiguous regions during embryonic development, retain capacity to from cells of each

other tissues and organs. To explain further, Aponeurosis of Abdominal wall and peritoneum, Fallopian tube, uterus and peritoneum, Ureter and peritoneum, grow in contiguous embryo regions. Both are developed from the same germ layer mesoderm. Similarly Rectum and urinary bladder, Bile duct and Duodenum grow in contiguous embryo regions and both have

developed from germ layer endoderm. Such adult stem cells can be directly used for repair, or neo-regeneration. With this hypothesis author has grown tissues and organs [5].

D. Harvest and culture:

Procurement of suitable cells, proliferation of cells in-vitro with the help of chemicals and growth factors and their immune reaction to host tissue / organ is a big challenge in SCT. With successful identification and detection of stem cell in adult tissues and understanding use of chemicals and stimulating agents it will be an easy task in future. In author's research embryological principles are utilized for identification of suitable stem cells for effective neo-regeneration of tissues and organs [43].

E. Stem Cell Banking:

Preservation of stem cells, developing stem cell lines (human and mouse) and use of cord blood stem cells are being worked out and some centers have even opened banks to preserve cells for future use. It is an entrepreneur's venture to stalk cord blood at the time of birth which can be used if needed for the same person in future for 20 years. How far cells can be successfully preserved (morphology and function) for long period, and can prove useful in future. This will be decided by SCT in future using preserved cells. But certainly it can be useful for the research purpose. Such banking must be carefully planned and bodies like Indian Council of Medical Research (ICMR) and other government bodies should take prior cognizance of all the set-up to avoid problems to innocent public. It must be strictly guarded and controlled. It should be the responsibility of such organizations.

Dealing with political, social problems, unethical attitude in using stem cells and SCT, and interferences by animal activists for use of experimental animals.

Restrictions Imposed on research is a big problem and hindrance in progress of stem cell research and establishing standards for SCT in management of human ailments. At the same time some centers have started commercial use of SCT without any released or published standard data. Some institutions have established commercially viable cord blood banks for use after 20 years. All these activities need effective control and guidelines by government bodies, Indian council of medical research (ICMR), Medical council of India (MCI) and Ministry of health. Use of experimental animals has become difficult due to newly framed ethical norms, at the same time restrictions on number of animals for experiments, the research data may not be statistically appropriate. Publications of author have been denied due to small number of animals used. Author of this article has received numerous suggestions from

suffering patients with incurable terminal illness, offering their own body for experimental purpose due to restrictions in use of animals. This desperate need of suffering humanity has to be taken into cognizance by policy makers and animal activists.

Stem Cell Therapy:

Use of stem cells for repair, regeneration or neo-regeneration in experimental and clinical research has been on increase in recent years. This is due to our understanding about the plasticity of stem cells. Plasticity is the ability of tissue specific primitive cells to transform to

new cells of other tissues. In fact this transformation of cells is not new. Metaplasia is transformation of tissue stem cell under abnormal environment of chronic infection, inflammation, chemical irritation, tobacco smoke or irradiation etc. in

an anatomical abode. This word metaplasia (abnormal tissue formation) is responsible for our ignorance about plasticity of cells. The transformation of one type of tissue specific stem cells to other type of tissue cells has been explained in previous publications [1, 5, 36, 43, 49]. The metaplasia is due to the ability of stem cells of a tissue. It is limited to form cells of tissues of germ layer origin to which these cells belong. The tissue cells formed are abnormal in that anatomical abode hence the term metaplasia. But it is the property of that cell to form as coded in memory of that cell as per the laws of embryology[36].

Embryo developed after in vitro fertilization or aborted embryos in India are the ample source of the ESC. Research activities for repair of tissues and organs using stem cells in experimental biology / medicine is for a long time now. Human use is restricted to allogeneic or matched donor from Adult tissues. Adult Stem Cells are used in Bone marrow transplant (Leukemia), peritoneal cells for repair of Large Incisional Hernia [49, 50] and management of Complex Genito Urinary Rectal fistula [4]. Recently attempts are being made to treat myocardial infarction and spinal injuries. In fact stem cell therapy is facing problems in finding, harvesting, culturing and obtaining proper progenitor cells for tissue and organ repair all over the world. Critical address on these problems will definitely decide the current use of this therapeutic modality in clinics in recent years.

Cells for repair and therapy can be obtained from various available sources:

1. Self - Autologus,
2. Same Species - Allogenic,
3. Different Species – Xenographic,
4. Cell lines – Primary or immortalized cell lines,
5. Donor derived Adult stem cells.

These cells can be cultured, can proliferate, can be manipulated though limited. This has encouraged their use in treatment. Mouse and progeny derived from human embryonic stem cells are being experimented in animal models. In 1996 Klug et all used cardiomyocyte differentiated from mouse embryonic stem cells which survived in graft in mice [51, 52]. Interestingly based on author's hypothesis human use was demonstrated by using peritoneal stem cells for regeneration of abdominal wall aponeurosis for the treatment of large incisional hernia in 1991 (15 years study). This method was developed by using neo-regenerative surgical techniques with the help of the pluri-potent stem cell layer of peritoneum. This fact was not revealed at that time (in 1991) until more tissues and organs were regenerated using similar techniques. This was later revealed in 1999 in world journal of surgery after it was patented (US international Patent) with effect from 1996 [5, 49, 50]. This surgical technique has been published in Text Book of R. Maingot's Abdominal Operations in 1997 [3].

The following examples indicate that current research out come is indicative of progress in positive direction.

*Isolated endothelial cells from human embryonic stem cells after enzymatic dissociation were seeded on biodegradable scaffolds and later implanted sub-cutaneously in mice, demonstrated development of micro-vessels. Similar success story repeated in rhesus monkeys [2, 37,

*Neural cells derived from embryonic stem cell survive and differentiate in developing mouse

brain. Partial improvement in mouse model with Parkinson's disease after use of progenitor cells derived from mouse embryonic stem cell and partial recovery in spinal cord injury in rodents, has encouraged attempts for such therapy [53-55]. **Long term survival and function of neural cells derived from human embryonic stem cells when used is yet to be confirmed. Such therapy on clinical application showed no significant benefit but such study has proved survival of transplanted cells[37, 41].**

*Improvement in experimentally induced diabetic mouse by using mouse embryonic stem cells and observation of SC differentiating into functional islets cells [56-59] is highly encouraging. But similar possibility in human ESC and their derivatives is yet to be achieved with long-term functional capability[37].

SCT using human ESC for Heart, Nerve and brain, diabetes etc need in-vitro differentiation into specific progenitor cells and after purification used in vivo. These cells then must transform into functional cells. This is yet to be evolved. Such therapy needs in-vitro differentiation, Transplantation of differentiated stem cell progeny, Tissue engineering, Stimulation of cells by growth factors etc.

AVAILABLE MODALITIES FOR STEM CELL THERAPY:

Both ASC and ESC can be used for research and therapy. Different modalities currently available are:

1. Colonization of stem cell membranes developed from different epithelial lining based on hypothesis on principles of embryology (published elsewhere). It is well known that almost all the lining epithelia of various body cavities have stem cells including endothelial lining in brain [60]. These epithelial lining can be developed into transplantable membranes for colonization and used for regeneration and repair of tissues and organs in vivo [1, 3, 5, 36, 43, 49, 50]. Human use has been demonstrated, patented and published (for repair of incisional hernias and complex genito urinary fistula).
2. Local or systemic administration of specific stem cells or progenitor cell (Homing of SC). ASC it is possible but not for ESC as ESC need be cultured and differentiated into tissue specific cells i.e. cultivation in vitro [37, 41]. Numerous reports of experimental studies have been published
3. Transplantation of Differentiated stem cell progeny. Differentiated and selected cells of specific use transplanted in tissues and organs where repair or regeneration is needed. The danger of epigenetic and genetic modifications is involved in such procedures. It is possible in mice at present but not in humans [48, 49].
4. Tissue engineering. Stem cells are seeded in artificial scaffolds, cultured in vitro and then tissues and organs are transplanted in vivo. This needs re-vascularization in the body. Bone Cartilage, muscle are grown in this manner [2, 61-63]. The tissue engineering envisages use of differentiated progeny of ESC, ASC, or Fetal SC for repair and regeneration of tissues and organs. This is a multidisciplinary field. It includes knowledge of life sciences, medicine and engineering. Cellular growth is facilitated by Biomaterials, Bioreactors and understanding of stem cell biology.
5. Endogenous stem cell stimulation. Induction / augmentation by stimulating patients own stem cells by using growth factors. Bone Marrow stem cells can be mobilized by using stem cell factor and granulocyte colony stimulating factor. In myocardial infarction stem cells reach damaged site and promote repair. In experimental study in diabetic mice such procedures are useful [53].
6. Therapeutic cloning can be reproductive or therapeutic. Nuclear transfer technique is therapeutic while replacement of nucleus in procured oocyte is reproductive cloning. Formation of sheep - Dolly, from nuclear replacement is reproductive cloning [64]. Pluri Potent Embryonic Stem Cell obtained from culture of nuclear replacement can be a source of autologus tissue graft for transplantation [64-68]. Till date such attempts are experimental level and under development phase. It needs more research before it can be used for therapy in human beings. It is considered expensive, ethically questionable, outcome of reprogramming and epigenetic changes are still unknown at the same time it is subject to potential rejection [37].

In present scenario SCT is facing problems such as:

1. Availability of efficient Human Stem Cells (Adult and Embryonic)

2. Knowledge of Tissue specific functioning capacity of cultivated transplanted stem cells
3. Tumorigenicity of used cells after in-vitro manipulation of cells.
4. Immune compatibility of Stem Cells with host tissue cells and their rejection.
5. Ethical problems.
6. Legal problems in use of stem cells.

SPECIFIC THERAPIES:
For repair and regeneration of damaged tissue or organ it is important that the dead cells be replaced and newly generated cells proliferate multiply and organize to restore structure and function of the damaged tissue or organ. In healthy bodies, this is constantly achieved in skin and blood during lifetime. If cells are terminally differentiated to form specialized tissues, healing is generally by scar tissue formation for life to continue to full term. In modern medicine after achieving the knowledge and know how that the body's building blocks – stem cell and their biological behavior, regeneration and repair of tissues and organs is being attempted.

Myocardial Infarction.
Distant stem cells are available in body for the rescue of damaged myocardium, but of limited value. The mortality and morbidity after ischemic heart disease continues. In experimental animals numerous innovative measures have been attempted to regenerate damaged myocardium due to ischemia or cryo-injuries. Various cells have been used to repair and regenerate myocardium like, Fetal cardiomyocytes, Skeletal myoblast, Fibroblasts, Embryo

endothelial cells, Smooth muscle cells, Bone marrow cells etc. Bone marrow cells are non-immunogenic due to autogenous source [69, 70 - 72]. A variable success rate has been reported using such cell therapies. But at present the subject is debated and there is no consensus on the promising form of therapy for myocardial injury. Mechanically inactive scarred myocardium is transformed into active contractile one is basically not clear. It is difficult to find cell population / cytokines to manage myocardial infarction by SCT and the results in literature are varied [41]. Applications used in SCT are at site injection (intra-cardiac muscle), Intra coronary infusion. Complications faced are ventricular tachycardia, arrhythmias and

even death [73]. In conclusion SCT for Myocardial Infarction (MI) is a new modality with many unanswered questions and issues. **Recommendation of SCT for MI is controversial and debated. It needs many more experiments and research outcome [41].**

Diabetes:
Available current data suggests the capacity of mouse embryonic stem cell (mESC) and human embryonic stem cell (hESC) to form useful cell types but functional capacity in vivo is uncertain on longterm basis [37]. Even though the experimental research on mice reveals control of experimentally induced diabetes using mESC, but the human use has to be evolved [56, 74-75, 75A]. **Current limitations in SCT in diabetes must be over come like immunogenicity, therapeutically incorrect insulin levels, engineering of Pancreatic islet cells in vitro need further evolution of ESC for tissue specific function.**

Brain, Spinal cord and Neuro-Repair:
Development of nerve cell from ESC, which is differentiated in vitro has prompted research for the management of neurological damage due to infarct and neuro-degenerative diseases [37, 52]. Partial recovery in experimental mice model of Parkinson's disease and development of dopaminergic neurons has raised great hope in the management of Parkinson's disease. But hESC derived neural cells can survive and function is yet to be confirmed [37, 53, 55]. **Survival of cells is encouraging but SCT needs utmost caution and judicious attempts for clinical use of SC in therapy to avoid irreparable damage of SCT and a powerful, useful tool in the management of difficult human ailments may get lost.**

SCT in ophthalmology:
Stem cells are considered to be present in limbus, conjunctiva, ciliary body etc. Limbal stem cell deficiency manifestations have been well documented. Limbal deficiency is managed by debridement, amniotic membrane transplant, cadeveric transplant. Limbal epithelium enriched with stem cells has been used at L V Prasad Eye Institute (LVPEI), Hyderabad, India. The in vivo survival of transplanted cells is interesting and encouraging. **Though the clinical success is evident but long term follow up and other studies are warranted to understand pathology of transplanted cells** (personal communication from the team at LV P rasad Eye Institute, Hyderabad, India) [76, 77].

Ischemic Limb Disease:
Umbilical cord blood derived Stem Cells used in Buerger's disease and Ischemic Limb disease in animal models [78]. Similar therapy has been tried in four men (humans), showed improvement clinically and on follow up angiography in four weeks time.

Stem Cells and tissue Engineering:
Single tissue phenotype or composite tissue constructs to repair multi cell lineage tissue and organs represent beginning of in vivo and in vitro technologies. Tissue engineered constructs like articular condyles, composition of synovial joint, Epiphyseal growth plates, Ligaments or

tendons, Cranial sutures are being attempted in animal models. Biomaterial scaffolds are essential to grow tissues in vitro for ultimate use in vivo [79].

Future Perspective:
Considering the outcome from recent animal and experimental research this therapy undoubtedly holds a great future in clinical management of intractable diseases like: Diabetes, Myocardial infarction, Spinal cord and brain injuries, Parkinson's disease, Muscular Dystrophy etc. At present this therapy is facing certain complex problems like How to Detect, Harvest, culture and out come of transplanted cells in host tissue at the same time wide variety of human diseases like- Diabetes from damaged cells of Langerhans, Parkinson's disease due to destruction of Dopaminergic neurons in substantia nigra.

Modern research and development on stem cells has shown the capacity of cells to form tissues ranging from bone, nerve, cardiac muscle etc. Seeded stem cells in bio-degradable artificial scaffolds can transform into tissues and organs like external ear, nose, tooth etc. possibility of growing more complex tissues and organs in vivo and in vitro, like kidney, liver, pancreas, heart in future can not be denied.

Theoretically by nature each individual cell of an organism has the capacity to form and grow into an organism but due to specialized function acquired during differentiation and proliferation, this capacity is lost. **However by manipulation this capacity can be revived. Nuclear manipulation for cloning is one such example and the other is by providing proper environment and functional stimulus to properly selected cell as per author's hypothesis.** This can be explained by another example of Bengal Gram seed. If Bengal gram seed is kept in a container it will remain there for years together without any change. On the contrary if it is provided with water and moisture it will sprout in few days. If these seeds are thrown on ground in Monsoon season, it will sprout. On rocky ground it will sprout, but will die soon for the want of water and nourishment. But on a fertile ground it will become plant, bear flowers produce fruits and develop seeds for next generation, to accomplish the destined task of the nature. Therefore each cell is an individual organism itself and can be manipulated unfortunately at present the knowledge is not available to accomplish this goal.

From the currently available concepts of stem cell biology

and the progress made in repair and regeneration of tissues and organs using stem cells, all over the world SCT holds a great future. However present century will be facing basic challenges, which must be answered. Increased aging population Globally it is more relevant in 21st century. It is clear from the Vedic literature that there was freedom for scientific use of stem cells [4], use of human cloning and marriages of cloned individuals, capable of producing healthy progeny [4, 5, 6]. How ever major tasks ahead are the following "ten commandments":-

1. Recognition of stem cell prospectively that is Identification and definition of stem cell.
2. Understanding and more relevant knowledge of stem cell biology.
3. Understanding, optimization and control at will, of differentiation, trans-differentiation of stem cells.
4. Knowledge of signals that induce proliferation of stem cells – Cell Surface Receptors,
5. chemical Inducers, inhibitors (inhibition of cellular proliferation is important to avoid unchecked growth of cellas in tumor. Production of new protein molecule for perfect Cell division and proliferation suitable for repair and regeneration in target tissues and organs.
6. Identification of factors that guide cellular movement during embryo development and their use in repair and regeneration of tissues and organs. This will guide us for homing of injected cells at site of needed repai or regeneration.
7. Overcoming of Religious, political and governmental restrictions for stem cell use. At the same time control of frantic Human Clinical Use to avoid maligning of reputation of S C T.
8. Development of "Host tissue friendly" donor stem cells that is development of non-immunogenic stem cells by genetic modifications (availability of stock of stem cells from IVF ovum from host sperm or ovum or Cell nucleus replacement by nucleus of host cell).
9. Understanding of in vitro growth, culture and factors that stimulate and enhance differentiation of cells for transplantation and therapeutic use.
10. Freedom for experimental study on animals within the framework of Ethical committees irrespective of use of number (for proper stastistics) and of size big and small animals (relaxation on both sides – researchers and animal activists).
11. Stem cell banks or Mass production of suitable therapeutic stem cells is the need of the century. Preservation of cord blood and charging public should be with caution and perfection.

Human ESC research and human use needs certain ethical principles [80] like:

- Respect to human dignity,
- Individual autonomy: Consent, privacy, confidentiality of personal data etc.
- Justice and beneficence – improvement and protection of health
- Freedom of research
- Proportionality (no alternative modality available)

CONCLUSION:

Though a substantial progress has been made in understanding the stem cell and its usefulness in treating diseases but its use is facing difficulties. Stem cell therapy is not free from potential hazards. Adult autologus stem cell membranes developed from lining epithelium can be safely used. ESC use needs more research and development before it can be recommended in human beings. Stem cells are in fact a very important tool in managing many hither to intractable and untreatable human ailments. Vast majority of precious embryonic stem cells from aborted and in vitro fertilized embryos are being wasted in India and many countries because of ethical, religious, social, political and cultural reasons. If the descriptions in Rig-Veda and Mahabharat are believed, it is high time that conscience be reached and useful therapeutic modalities be developed and used shading all controversies what so ever.

Table 1.
Comparison of Embryonic Stem Cells and Adult Stem cells.

Serial numbers	Embryonic Stem Cells	Adult Stem Cells
1. Origin	From growing embryo	From developed tissues
2. Differentiation Potencial	Excellent. Forms any tissue or organ cell	Limited. Forms tissue or organ cell limited by its germ layer origin
3. Ethical & Legal** Problems	Yes. Attached as it destroys embryo. Fate of processed SC is unknown.	No
4. Formation of heterogenous cell populations	Yes. Possible as SC are tempered to differentiate to suit tissues & organs	No. if embryological Laws are followed
5. Tumorigenecity	Yes. More so when genetically modified	No. But possibility is as good as in any normal cell of developed body
6. Functional Development	May not be normal	Normal. if embryological Laws are followed
7. Immune Rejection	Yes	No if autogenous cells are used properly as per laws of embryology
8. Human Use	Under exploration Scientifically.	Demonstrated, Patented and Published [3a,29, 30]. Bone marrow transplant is in use.
9. Physiological Phenomenon of Desired Metaplasia	Yes if colonized with tissues in vivo	Yes if colonized with tissues in vivo
10. Use in Stem Cell therapy	Needs to be converted into needed progenitor cell.	Properly selected cell can be used directly (as per author's hypothesis)

**(US stem cell project sued. 10 out of 40 patients died after processed SC use in 1998-99. St Luke's hospital, Community Blood Center, Kansas city, USA reported in Kansas City Star – AP news. TOI – Times International,. Tuesday, October 10, 2006, p.32)

Table 2. Cell differentiation to different lineages
(Up dated from Ind. J. of Exptl. Biol. 40: 1331-1343: 2002)
Blood Cells to other lineages:

Sr No.	Author, Year,	Differentiation to different tissues	Relation to germ layer	Remarks
1.	(a)Eglitis-Mezey,1997 [12] (b)Kopan, 1999 [13] (c) Mezey 2000 [14].	Neurons , Glial tissues.	Ectoderm derivative	Blood cells from wandering mesoderm Nerve, Glial cell from ectoderm
2.	Gussoni, 1999 [15].	Skeletal muscle	Mesodermal derivative	Both mesoderm derivatives
3.	Asahara 1999 [16]. Kalka, 2000 [17].	Neovascularization	mesoderm derivative	Both developed from wandering mesenchyme.
4.	Petersson 1999 [18] Quesenbery 2005 [19]. Lagasse 2000 [20]. Lakshmipathy 2005 [21].	Hepatic oval cells Hepatocytes Hepatocytes hepatocytes	Endoderm derivative	wandering mesoderm to endoderm

5.	Orlic,D, 2001. [22].	Myocardium	Mesoderm derivative	Both mesoderm derivative
6.	Pittenger 1999 [23]. Prokop 1997 [24]. Yoo, 1998 [25].	Cartilage, bone, fat	Mesodermal derivative	Both developed from mesoderm germ layer
7.	Till, J.E. 1961 [26].	In spleen build Myoerytheroid colonies	Mesoderm derivative	Both derived from mesoderm
8	Krause 2001 [27].	Pneumocytes	Endoderm derivative	wandering mesoderm to Endoderm cell

B. CONVERSELY: Other cells to blood cells

9	Bjornson 1999 [28].	SC of neural tissue can form Blood Cells	Ectoderm to wandering mesenchyme	Suggests compatibility (see text)
10	Jackson, 1999 [29].	SC of muscle tissue can form blood cells	Mesoderm to wandering mesenchyme	Suggests compatibility (see text)
11.	Young, 1999 [31].	Form skeletal muscle fat, Cartilage & Bone	Mesoderm influence on deeper skin SC	Conversion into mesoderm tissues

OTHER EXAMPLES OF TRANS DIFFERENTIATION (from IJEB)

Sr. no.	Tissues	Metaplastic tissues formed	Remarks
12.	Retinal cells of Eye Ball	Neuronal Cells	Both Ectodermal origin
13.	Iris and Retina	Mutually form one to another	Both Ectoderm derivative
14.	Adrenal Medullary Chromatin Cells	Sympathetic Neurons	Both Ectodermal derivatives
15.	Fibroblast Cells	Form Fat, Smooth muscle, cartilage	Both Mesoderm Derivative.

Inference: The tissue trans differentiation in literature cited above follows a set pattern of embryology. PPSC derived from a germ layer of Germ Disc forms tissues derived from the same germ layer

Reserve SC of Dermis Note:

The embryological relation to germ layer of germ disc of developing embryo and remarks are as per the hypothesis of the author of the present article. (Bone marrow SC are derived from wandering mesenchym). The embryological relation has not been mentioned by any authors referred in table.

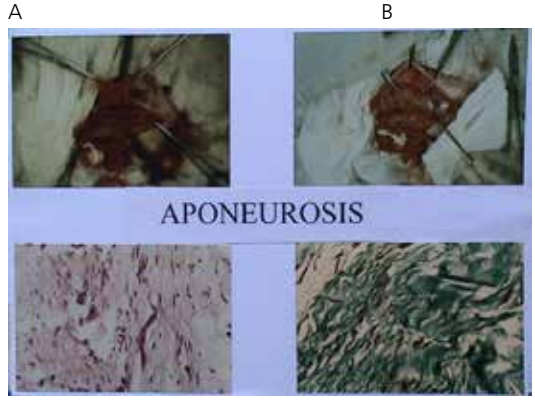
TABLE 2A: GROUP-B. "CELL COLONIZATION ATTEMPTS" AND THEIR EMBRYOLOGICAL CORELATION. (Author's unpublished experiments.)

Sr. No.	Donor Tissue	Recipient Tissue.	Germ layer Origin	Relation in Developing Embryo.	Histological Changes & Period	Results & Remarks.
1.	Free Peritoneum	Skin of Inner part Of pinna	Peritoneum mesod. In origin. Skin an Ectodermal tissue.	Both fr. Different Germ layers Having different Function	Fibrosis & Scarring. Loss of cells & cell death 3 months	No neo-formation of Tissues. Cell death Resulting into Fibrosis and Scarring
2.	Peritoneum	Mucosa of Isolated Small Intestinal Ileal pouch.	Peritoneum mesod. & mucosa of small intestine endoderm in origin	Two different Germ layer with Different tissue Forming capacity	Sloughing Infection Cell death.	Celldeath. No tissue formation
3.*	Tunica Vaginalis	Vas Deferens	Both mesodermal In origin	Tunica grows fr. Active growth of Peritoneumin Gubernacular Mesenchyme	Testis Develops at Mesonephros &invaginates tunica	Mnd forms Duct system of testes. Both are mesod. But Are not Neighbors Graft survived.
4.	Peritoneum Fr anterior Abdominal Wall near mid line.	Ureter	Both mesodermal In origin	Anterior abd wall Peritoneum grows With paraxial Mesod.	Ureter fr mnd Postero lateral Wall peritoneum & NGC grow together	Graft survived No neo formation of Ureter. Both mesod But not neighbor. Cell potential may be Lacking
5.	Peritoneum	Urinary Bladder	Both mesod in origin	UB develops fr. Cloaca (hind gut)	Both are not Neighbors in Embryo	No neo formation of Bladder tissue Graft survived
6.	Peritoneum Fr posterior Abdominal Wall	Aponeurosis Of abdominal Wall	Both mesod origin	Paraxial mesod Elongates & Peritoneum grows With it & fuse Ventrally in mid Line to form wall Of abdomen	Not neighbors In embryo Development	Graft survives but No Tissue formation of apneurosis seen No cell death No fibrosis
7.	Peritoneum	Profunda Femoris Artery	Both mesod in origin	Artery develops Fr. Wandering Mesenchyme Peritoneum fr. Lat plate mesod	Sites of Development of Two tissues are Different in Embryo	Peritoneal graft Survived as Membrane. No cell Death observed. No fibrosis

*** Research funded by Indian Council of Medical Research.**
Fr = from. NGC = nephrogenic cord. UB = Urinary bladder. Mesod = mesoderm.

Fig. 1. Regeneration of abdominal wall aponeurosis **

A B



C D

Gross view of anterior abdominal wall dissection showing isolation of peritoneum membrane (A - Top left).

Peritoneum membrane Colonization in aponeurotic tissue system (B - Top Right). Histology: H E Stain. Aponeurotic tissue stained eosin. (C - Bottom Left).

Histology: Masson's Tri Chrom stain (MTS). The aponeurotic tissue in stained green (D - Bottom Right).

**From World J. of Surgery [33].

This regenerative surgery technique used to repair large incisional hernia. World J of Surgery [34].

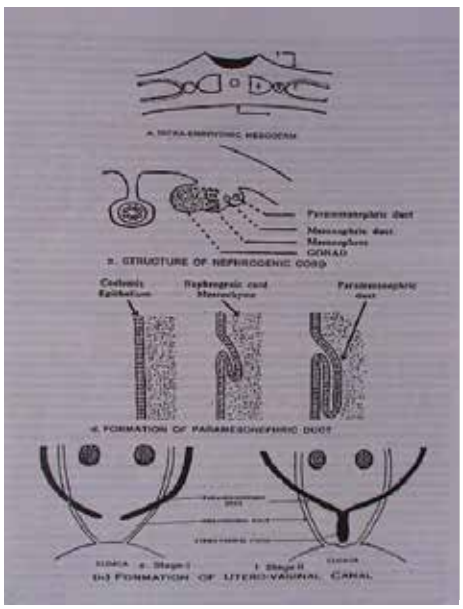


Figure 2A.
Germ Disc Showing A. Paraxial, B. Intermediate Cell Mass and C. Lateral plate Mesoderm (Diagram on Top), Lateral plate mesoderm has a cavity known as coelomic cavity. It is lined with coelomic epithelium.

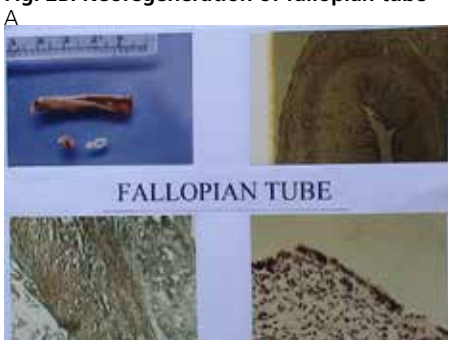
Intermediate Cell Mass mesoderm forms Nephrogenic cord (NGC) Different components of NGC are shown. (2nd from top). The coelomic epithelium (Peritoneum) lines the Nephrogenic cord.

Paramesonephric Duct formed from cells of coelomic epithelium, invaginating the mesenchyme. Coelomic epithelium in abdomen is known as peritoneum (3rd from top).

In developing embryo Paramesonephric ducts form Two Fallopian Tubes (Right and left) Two tubes unite in the center below in mid line to form Uterus (Bottom).

Fig. 2B. Neoregeneration of fallopian tube

A B

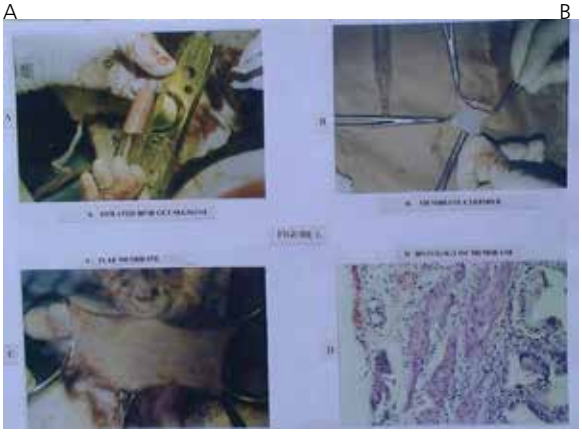


C D

Gross view of mesodermal membrane (peritoneum) after colonization, 3 months post operative period. Long slit to show inner lining of tube. Transverse section of tube is compared with freshly prepared tube from peritoneum membrane (in white) for comparison (A - Top Left). Low power (6.3 X 10 magnification) microscopic histology of the same H E stain (B - Top Right). Histology – 10 X 10 magnification - (Masson's Tri Chrome stain) smooth muscles in eosin stain and fibrous tissue in green (C - Bottom Left). Histology to show Cilia in H E Stain in high power (10 X 40 magnification) microscope. (D - Bottom Right).

Figure 3. Preparation of membrane from intestine containing Stem cell of submucosal crypts

A B



C D

A segment of intestine isolated and carefully dissected to isolate submucosal membrane - Rectosigmoid colon for Urinary Bladder / Duodenum for Bile duct regeneration.(Fig.A.)

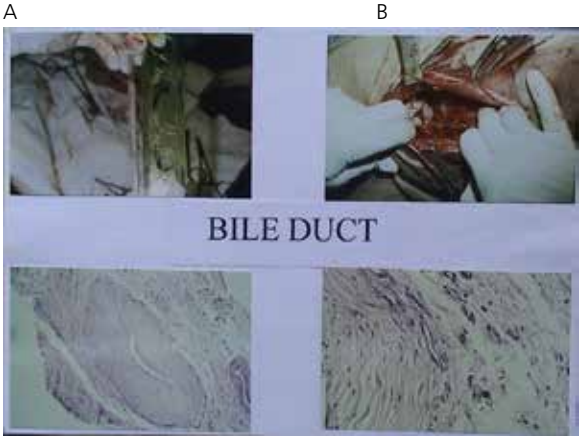
Cylinder of Submucous membrane isolated containing endodermal crypt stem cells, after meticulous dissection (Fig.B).

Gut cylinder converted into flat membrane for urinary bladder graft.(Figure C).

Histology of submucous membrane showing crypt Bases. Endoderm stem cells are present in these crypts in annular rings.

Fig.3B. Rregeneration of Bile Duct

A B



C D

Tube prepared from membrane developed from submucosa of dudenum (endoderm stem cells) (A - Top Left).

Constructed Tube colonized in bile duct tissue system ***in vivo*** (B - Top Right). Histlogy of grafted membrane: H E stain (6.3 X 10 magnification) Low power (C - Bottom Left) and high power 10 X 10 magnification (D - Bottom Right).

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