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Stem cells: Time for regenerative medicine

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Abstract
Stem cells are biological unspecialized cells that can differentiate into any specialized cells of the body. Regenerative medicine refers to the science of medicine which discusses the algorithm of healing by itself. Stem cells have been used to treat bone marrow diseases for decades but the potential of the stem cells in regenerative medicine has not been understood properly to date. The science of stem cells in treating the most dreadful diseases of the era sounds fascinating but it might be possible with stem cells. Stem cells are the most significant breakthrough in treatment in Central Nervous System disease, diabetes, bone disease, heart disease, renal injury, blood pathology, sterility, liver cirrhosis, and various others which have a prognosis of inevitable death. The various questions on its efficacy, tumorigenicity, preservation, side effects are still in the womb of time which needs further detailed research and clinical trials. It has been concluded with the obstacles and promising hope to treat the most dreadful diseases of the planet.

Keywords: cell therapy, induced pluripotent stem cells, regenerative medicine, stem cells, stem cells bank

Introduction
The idea of a miracle cure and body healing them holds a particular fascination. Stem cell research brings regenerative medicine a step closer, but many of the ideas and concepts remain controversial to the reader. This review will enlighten the various aspects of stem cells and their clinical application in the future.

The term "stem cell" appeared in the scientific literature as early as 1868 in the work of the eminent German biologist Ernst Haeckel. In demonstrating the evidence for Darwinian evolution, Haeckel developed phylogenetic trees and used the term "Stammzelle" (stem cell) to describe the ancestor unicellular organism from which he presumed all multicellular organisms evolved. Stem cells are biological unspecialized cells that can differentiate into any specialized cells of the body. For a cell to be stem cell should fulfill two criteria of self-renewable (the ability for numerous cell division while maintaining the undifferentiated mass) and potency (differentiate into any type of cell). There are two types of stem cells i.e. totipotent stem cells and pluripotent stem cells. In plants, every cell is totipotent and can give rise to the whole plant but in animals or humans, the only zygote is the totipotent stem cell which can rise to the whole body and is limited for some time after fertilization which can changes into a pluripotent cell as embryonic stem cells. ESCs are derived from the inner cell mass of the blastocyst stage of the preimplantation embryo [1].

In adults, there are adult stem cells which are multi or unipotent cells. Mesenchymal stem cells skeletal stem cells, neural stem cells, skin stem cells; epithelial stem cells are adult stem cells. Mesenchymal Stem Cells are derived from mesodermal progenitor cells. They can be isolated from different tissues, including Bone Marrow, Peripheral blood, adipose tissue, dental pulp, and a variety of fetal tissues, such as amniotic membrane, amniotic fluid, placenta, and cord blood, and Umbilical Cord [1].

The adult cells can be reverted to differentiate in any other specialized cells called induced pluripotent cells. Recently, Yimlamai and Christodoulou *et al*. [2019] discovered in a mouse model that mature hepatocytes are highly plastic and can revert (dedifferentiate) to a stem cell-like state [1, 2]. Figure 1 describes the changes in the potency of stem cells in human body development.

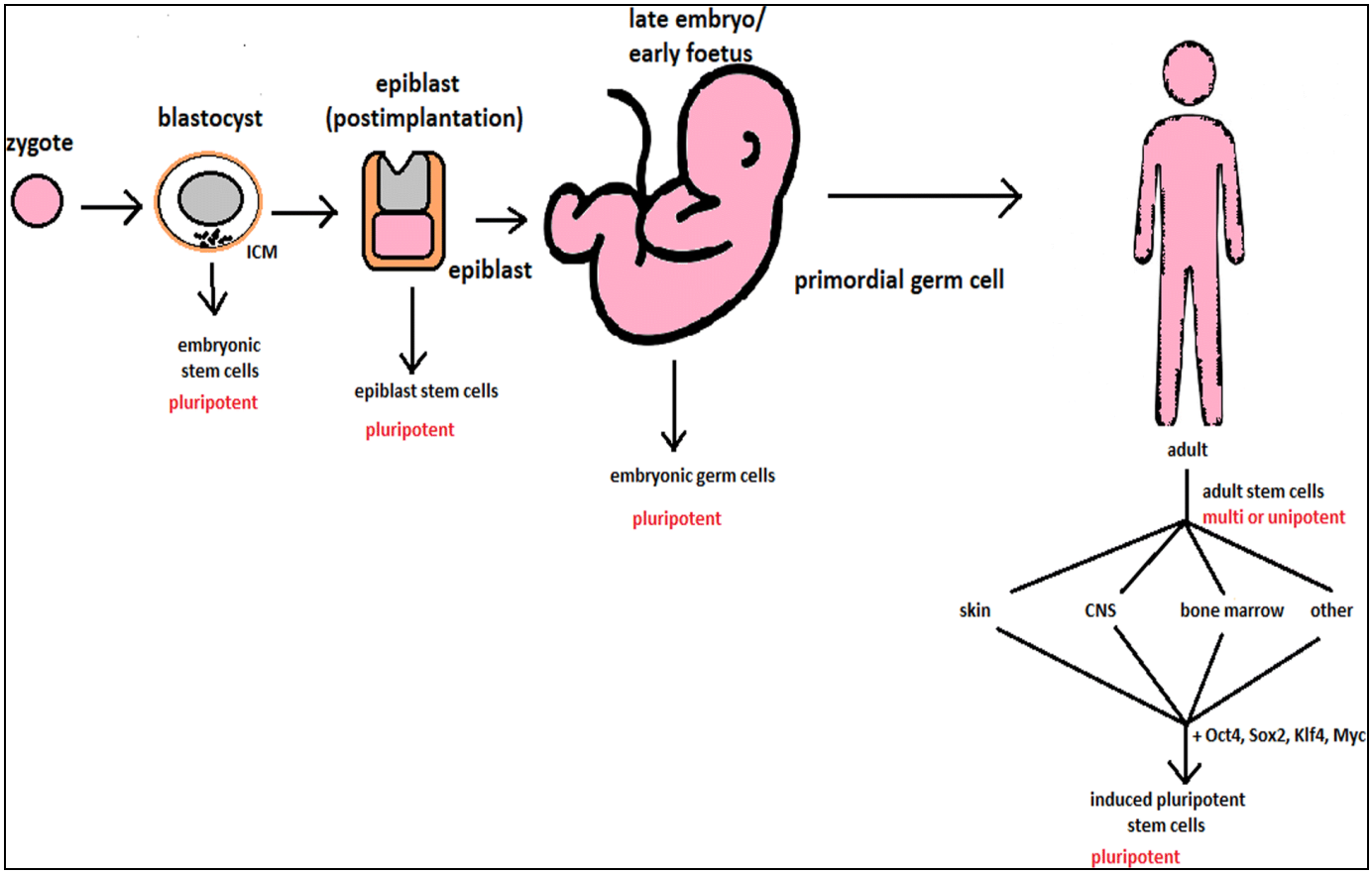

Fig 1: Changes in the potency of stem cells in human body development. Potency ranges from pluripotent cells of the blastocyst to unipotent cells of a specific tissue in a human body such as the skin, CNS, or bone marrow. Reversed pluripotency can be achieved by the formation of induced pluripotent stem cells using either octamer-binding transcription factor (Oct4), sex-determining region Y (Sox2), Kruppel-like factor 4 (Klf4), or the Myc gene[1].

Derivation of stem cells

Growing cells in the laboratory are known as cell culture. Human embryonic stem cells (hESCs) are generated by transferring cells from a preimplantation-stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as a culture medium. The cells divide and spread over the surface of the dish. In the original protocol, the inner surface of the culture dish was coated with mouse embryonic skin cells specially treated so they will not divide. This coating layer of cells is called a feeder layer. The mouse cells at the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Researchers have now devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time cells from the preimplantation-stage embryo are placed into a culture dish. However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or subculturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation. [3]

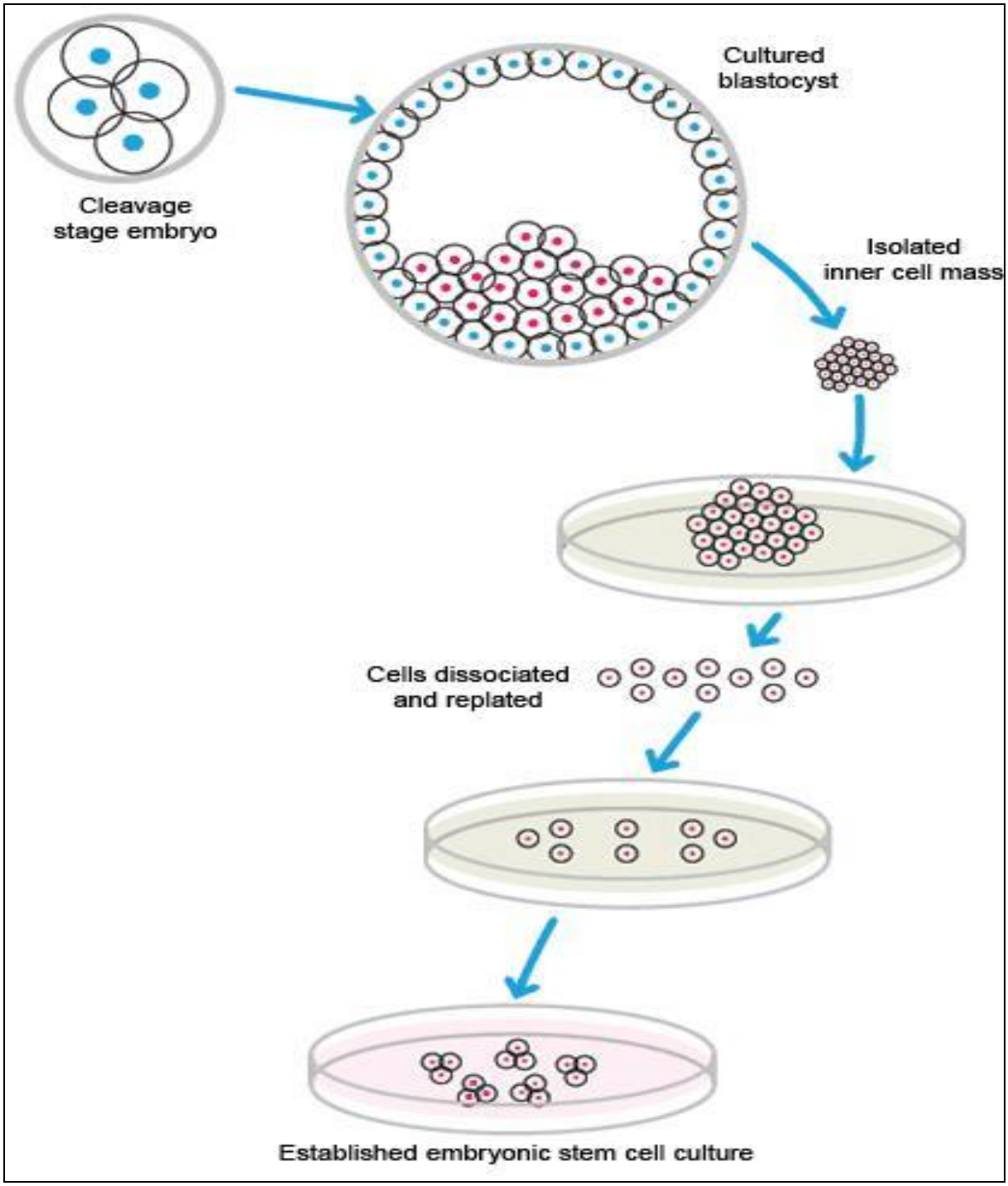

Fig 2: Culture of embryonic stem cells

Stem cells can also be derived using a variety of methods, from classic culturing to laser-assisted methodologies or microsurgery. Human embryonic stem cell differentiation must be specified to avoid teratoma formation. The various methods of acquiring EBs, such as bioreactor culture [4, 5], Hanging drop culture [6], or micro well technology [7]. These methods allow specific precursors to form *in vitro* [8]. The essential part of these culturing procedures is a separation of inner cell mass to culture future human embryonic stem cells [9].

Rosowski *et al*. [2015] described that particular attention must be taken in controlling spontaneous differentiation [10]. When the colony reaches the appropriate size, cells must be separated. The occurrence of pluripotent cells lasts for 1-2 days. The procedure used in culturing is the separation of inner cell mass to culture future human embryonic stem cells, cell passaging, enzymatic dissociation, trypsin utilization [1].

Higashioka *et al*, [2017] generated Myogenic-mutated human iPS cells using CRISPR/Cas9 technology. Myogenin is known to function as an essential myogenic transcription factor during the terminal differentiation stage. Myogenin gene-knockout mice display deficiency of differentiated skeletal myofibers, while there are residual myofibers in the mutant mice possibly due to functional compensation by MYOD1 and/or MRF4. Interestingly, in the paper, the authors found that human induced pluripotent stem cells can differentiate into skeletal muscle without myogenin activity *in vitro*, indicating similar compensation mechanisms by MYOD1 and/or MRF4 for myogenic differentiation of human-induced pluripotent stem cells [11].

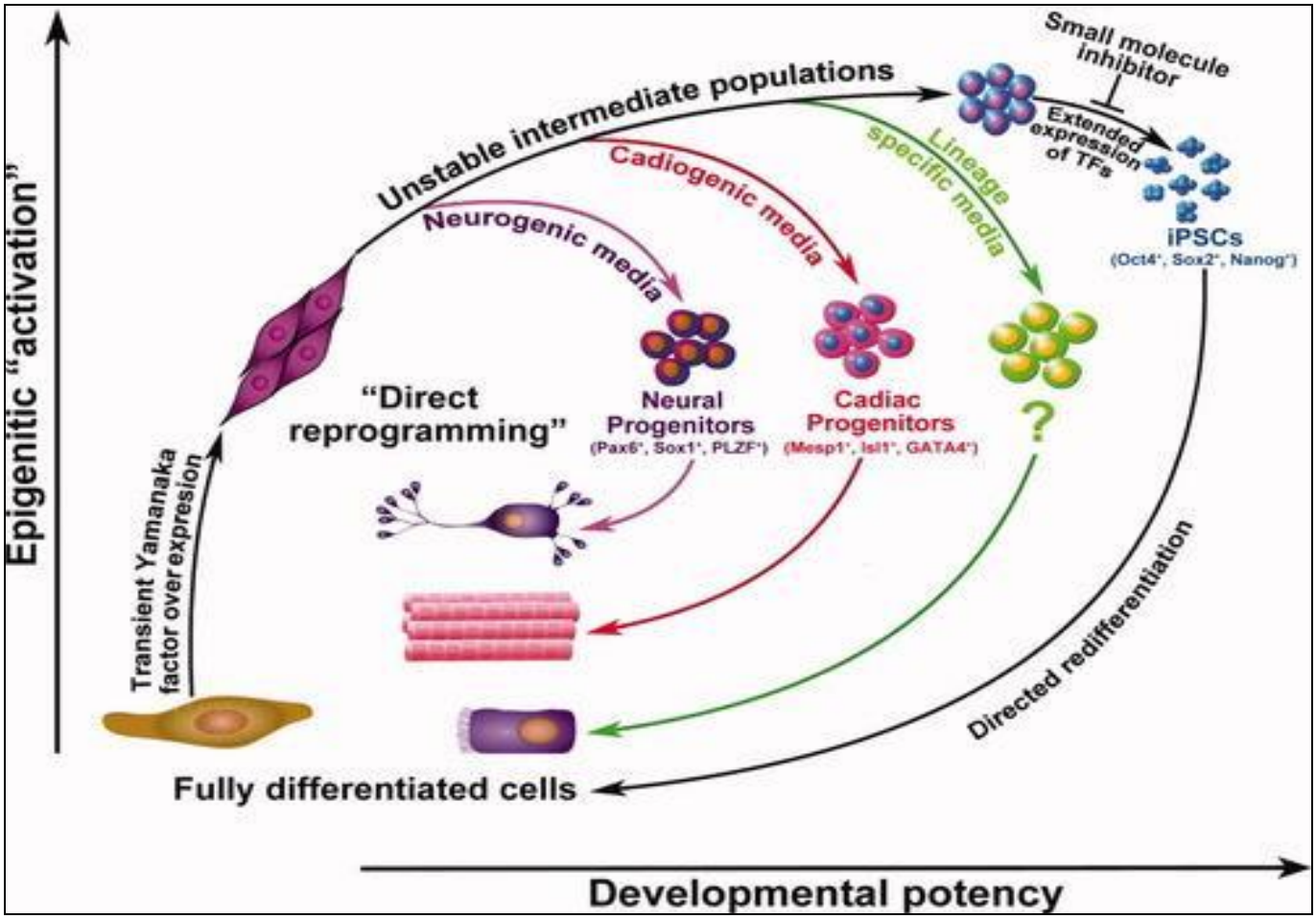

Fig 3: The model of direct reprogramming. Transient overexpression of reprogramming factors in fibroblasts leads to the rapid generation of epigenetically activated cells (unstable intermediate populations), which can then be coaxed to relax back into the various differentiated state(s), ultimately giving rise to fully differentiated cells entirely distinct from the starting population. Aside from restricting iPSC formation by drastically limiting Yamanaka factor expression, the reprogramming process can be made to overwhelmingly favor transdifferentiation by using small-molecule modulators of signaling, for example, Janus Kinase inhibitor that prevents the establishment and maintenance of pluripotency. Using empirically determined media and culture conditions, neural, cardiac, and possibly other lineage-specific cells can be obtained. Importantly, progenitor populations belonging to these lineages are generated in the process and can perhaps be isolated [12].

Besides, a series of genetic studies elucidated the requirement for miRNAs in the maintenance of embryonic stem cells self-renewal and pluripotency. Several reports revealed that a core regulatory circuitry of transcription factors controls the self-renewal and pluripotency properties of embryonic stem cells. Oct4, Sox2, and Nanog Function as central regulators to the transcriptional control hierarchy that determines the fates of embryonic stem cells. Moreover, miRNAs, including miR-134, miR-296, miR-470, and miR-145, have been demonstrated to modulate the pluripotency of embryonic stem cells by repressing the expression of Oct4, Sox2, and Nanog[13-15]. Q *et al*, [2012] also reviewed the various new technologies for obtaining stem cells.

Stem cells as Regenerative medicine

Leland Kaiser introduced the term "Regenerative medicine" in 1992. He forecasted that "a new branch of medicine will develop that attempts to change the course of chronic diseases and in many instances will regenerate tired and failing organ systems" [15].

Today in medical science we use allopathic, ayurvedic medicine and surgery to halt the progression or cure the diseases. Moreover, we cannot completely cure diseases. The results are not satisfactory and as a result, we are left with compromised body parts. The properties that stem cells are bearing could be helpful in the scenario of tissue damage, inflammation, and infection associated with these organs implicating the power of mesenchymal stem cell's therapeutic potential: versatility and applicability. The way stem cell research is going is giving hope to eliminate various degenerative diseases which are considered incurable now. K.Ye *et al*, [2018] demonstrated that B7-H1 expression on endometrial regenerative cells (ERCs) was up-regulated by IFN-γ in a dose-dependent manner, and it was required for ERCs to inhibit the proliferation of peripheral blood mononuclear cells (PBMCs) *in vitro*. This study provides a theoretical basis for the future clinical use of human stem cells.

Stem cells and wounds

When wound healing does not occur, the wound may become chronic and need additional interventions [16]. Mesenchymal stem cells are very versatile and promote pro- and anti-inflammatory responses, along with angiogenesis [17]. New studies have shown that mesenchymal stem cells home to sites of injury and provide therapeutic impact [17]. Several studies have suggested that mesenchymal stem cells home to regions of injury by specific trafficking to chemokine ligand7 (CCL7) [18, 19]. Once the mesenchymal stem cells reach the point of injury, the mesenchymal stem cells exit the

vasculature in the connective tissue stromal region [32]. The mesenchymal stem cells respond to the specific tissue while at the same time contribute to the secretion of biomolecules [20]. This exquisite interaction between the tissue and the MSCs defines the efficacy, potency, and overall therapeutic impact of the MScs. In a research study, Stoffand his colleagues found that human MScs injected near the site of injury in immunocompetent rabbits improved tissue function and reduced the amount of scarring [21]. Further, Stoff found that there was no evidence of rejection of the mesenchymal stem cells. In another study by Falanga *et al*, [2007] Mesenchymal stem cells were placed directly on the site of injury resulting in wound improvement [22, 23]. Raquel Guillamat-Prats and Antonio Artigas in their review article "Current Status of Stem Cell Therapy for Sepsis and Acute Respiratory Distress Syndrome" discussed the various potential of stem cell therapy in treating acute respiratory distress syndrome and sepsis. The table below depicts the same [24].

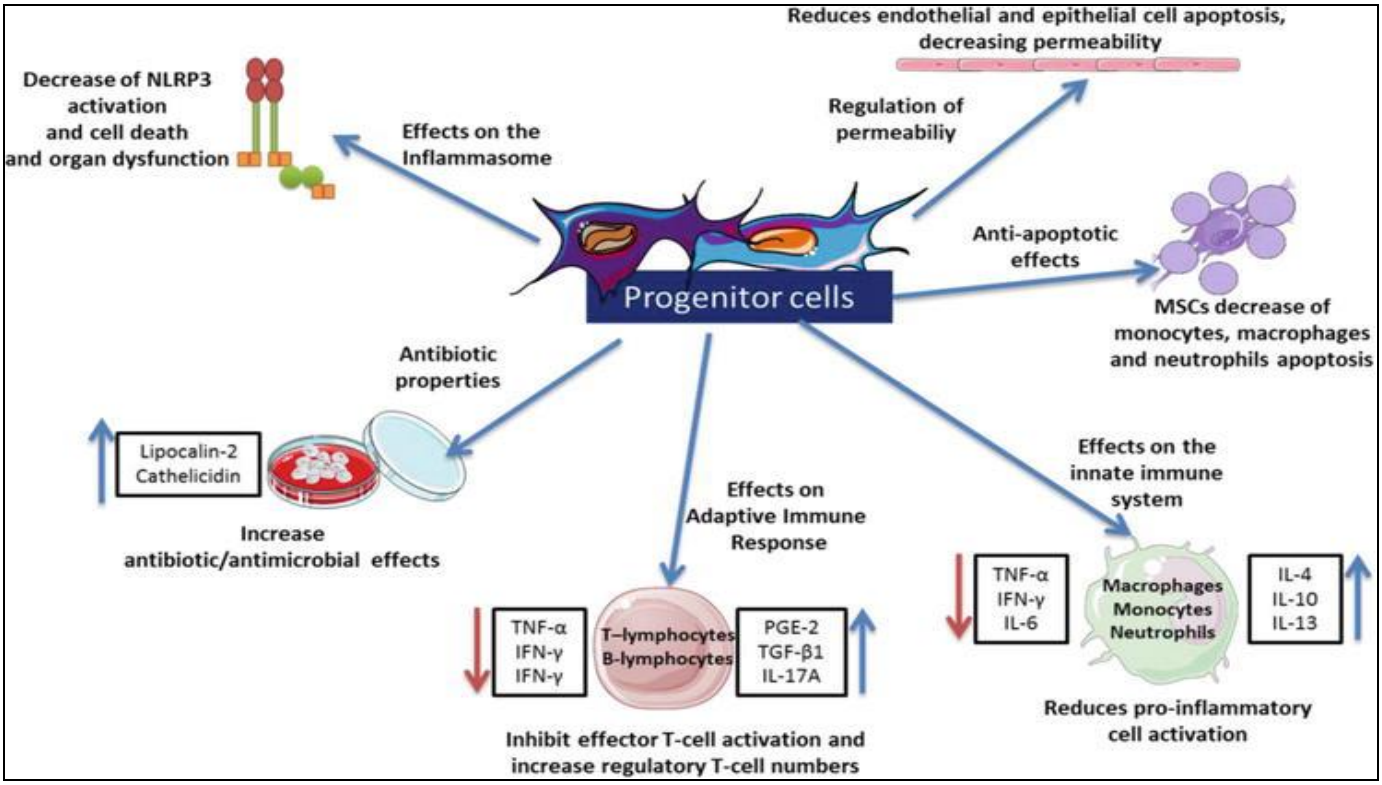

Fig 4: Potential mechanisms of the cell therapies

Stem cells and Blood pathology

Treating blood pathology is the most successful aspect of regenerative medicine which has been practiced for 50 years in the form of bone marrow transplantation. Adult stem cells or bone marrow stem cells are also known as mesenchymal stem cells in aplastic anemia [25, 26]. *In vitro*, many studies have reported the promotional effects of mesenchymal stem cells for the expansion of hematopoietic stem cells (HSCs) [27-29]. Many investigations on the feasibility of the clinical use of mesenchymal stem cells have mushroomed in the 1990s. However, Lazarus and colleagues were among the first to inject autologous cultured mesenchymal stem cells into human subjects intravenously and assessed their safety for cell-based therapy. It was demonstrated that the infusion of autologous MSCs into 15 human subjects with hematological malignancies was a safe treatment with complete remission [30]. Koc *et al*,[2000] first reported that co-infusion of autologous bone marrow stem cells at the time of stem cell therapy can lead to rapid hematopoietic recovery [31]. Accordingly, 46 patients received allogeneic blood stem cells from HLA-identical siblings, and most patients had prompt hematopoietic recovery without significant side effects [32]. The beneficial effects of stem cells on engraftment may relate to their supportive role in hematopoiesis. Bacigalupo *et al*, [2005] demonstrated that bone marrow stem cells from patients with severe aplastic anemia were deficient in the ability to inhibit T-cell proliferation and cytokine release, indicating the lack of mesenchymal stem cells immunoprotection in the bone marrow [68]. We found that bone marrow stem cells derived from children with severe aplastic anemia exhibit poor potential of proliferation and differentiation [34]. Due to the possibility of bone marrow stem cell insufficiency, we transplanted umbilical cord stem cells in 2 children with severe aplastic anemia [35]. Both achieved faster hematopoietic engraftment without infusion-related toxicities.

Stem cells and orthopedics

According to a report by the Surgeon General and the World Health Organization, Osteoporosis is the most common cause of fractures. Roughly 10 million individuals over age 50 in the United States have osteoporosis of the hip. An additional 33.6 million individuals over age 50 have low bone mass or "osteopenia" of the hip and thus are at risk of osteoporosis and its potential complications later in life. So, there is great hope that stem cell therapy could halt the progression and cure the diseases since the various trials of stem cell therapy are giving promising results. Since 1991 various pieces of research on stem cells are going on to promote the healing and development of bone and cartilage cells. Gan *et al*, [2008] developed a controlled release codelivery system of MSCs encapsulated in dextran/gelatin hydrogel with TGF-β3-loaded

nanoparticles for Nucleus pulposus regeneration. Treatment of nonunion, osteonecrosis, enhancing spinal fusion, filling bone cyst, filling bone defects, and various other osteochondral talar defects, high tibial osteotomy has been successful through stem cell therapy which has raised the curiosity of regenerative medicine among orthopaedician [36].

Stems cells and cardiac abnormalities

Scientists are also busy developing the cardiac muscle cells so that they can implant in the heart and can treat ischemic cardiac diseases. Researchers have found that the SKCas (calcium-activated potassium channels) are involved in cardiac pacemaker-cell development from embryonic stem cells and morphological shaping of neural stem cells [37]. Mesenchymal stem cells have also been used in ischemic cardiomyopathy with promising results [38]. However, the benefits of mesenchymal stem cell treatment may be due to the paracrine effects of mesenchymal stem cells instead of their capacity to differentiate into cardiomyocytes after injection. Zaruba et.al (2009) described a small-molecule-based regenerative strategy for myocardial infarction by enhancing the recruitment of endogenous bone marrow stem/progenitor cells to the heart through inhibition of CD26/dipeptidyl peptidase IV *in vivo* via a chemical compound, ultimately increasing the formation of new blood vessels and improving heart functions. In ischemic heart tissue, stromal cell-derived factor 1α (SDF-1α) is the major chemokine attracting endogenous endothelial progenitors expressing SDF-1α receptor (C-X-C chemokine receptor type 4, CXCR4) homing to the heart. However, SDF-1α is sensitive to several proteases (including CD26) cleavages. The authors demonstrated that combined administration of granulocyte-colony stimulating factor (functions to mobilize stem/progenitor cells, including endothelial progenitors, from bone marrow) and a CD26 inhibitor Diprotin A intraperitoneally enhanced recruitment of CXCR4-positive stem/progenitor cells to the myocardium and improved myocardial function by increasing neovascularization, leading to increased animal survival [39].

Stem cells and liver diseases

Talking about liver diseases patients with liver cirrhosis need liver transplantation which is very difficult due to lack of donors and HLA incompatibility and graft vs. host diseases. In recent years stem cell therapy solutions are being explored for the treatment of multiple liver diseases. Stem cell therapy has shown favorable results in improving the functions of the cirrhotic liver. Y. Zhang *et al*.2018 revealed the therapeutic effect of human umbilical cord mesenchymal stem cells on acute liver failure in rats. Dong *et al*, [2019] characterized intestinal micro ecology during mesenchymal stem cell-based therapy for mouse acute liver injury. Pang *et al*, [2019] demonstrated a potential antitumor effect of dendritic cells fused with cancer stem cells in hepatocellular carcinoma.

Stem cell therapy and renal diseases

Despite the various supportive treatments in renal diseases, renal diseases have become a public health concern. The fate of a chronic kidney is only death if kidney transplantation is not done in time but also the risk of rejection is extremely high. In this scenario, stem cell therapy can be a promising regimen. One study reported that ESCs, transplanted adjacent to injured kidney rats with 5/6 nephrectomy, slowed the progression of the disease due to the release of paracrine factors [40]. Several recently published studies on renal differentiation of Amniotic fluid stem cells make it tempting to speculate that these stem cells could once be considered as a new promising source for cell-based therapies to repair kidney injury and warrant further investigations into this direction [41]. Research focuses on understanding whether induced Pluripotent Stem Cells, obtained from patients with chronic kidney diseases, could be a suitable tool for deriving renal precursors that could efficiently regenerate their kidneys [42, 43].

Stem cells and fertility

Qiuwan *et al*, [2015] provided important evidence that human amniotic epithelial cell (hAEC) transplantation could effectively improve ovarian function by inhibiting cell apoptosis and reducing inflammation in injured ovarian tissue of mice, and it could be a promising strategy for the management of premature ovarian failure or insufficiency in female cancer survivors [44]. For the time being, we can only wait to see the complete cure of infertility in a few decades.

Stem cells and diabetes

In the case of type 1 diabetes, insulin-producing cells in the pancreas are destroyed due to an auto immunological reaction. As an alternative to transplantation therapy, it can be possible to induce stem cells to differentiate into insulin-producing cells [74]. Through experiments performed by the Zhao Laboratory, it was found that peripheral blood insulin-producing cells could be isolated and preserved for future insulin production because they can hinge onto a polystyrene petri dish, and they showed transcription and insulin production at protein and mRNA levels. This technology would allow patients to generate their insulin-producing cells [45]. This treatment would eliminate the hazard of rejection by the immune system, shorten the time to transplant due to the shortage of donors, and would have no ethical issues [46, 47] and could be therapy to cure diabetes in the future.

Stem cells and neurodegenerative diseases

The patient of neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease has a lot of expectations from stem cell therapy since it can change the life of the sufferer. The discovery of neural stem cells (NSCs) has nullified the previous idea that adult CNS was not capable of neurogenesis [48, 49]. Neural stem cells are capable of improving cognitive function in preclinical rodent models of AD [50-52]. Awe *et al*.2013 clinically derived relevant human induced pluripotent stem cells from skin punch biopsies to develop a neural stem cell-based approach for treating Alzheimer's disease [53]. Neuronal degeneration in Parkinson's disease (PD) is focal, and dopaminergic neurons can be efficiently generated from hESCs. Parkinson's disease is an ideal disease for induced pluripotent stem cells -based cell therapy [54]. However, this therapy is still in an experimental phase. Brain tissue from aborted fetuses was used on patients with Parkinson's disease [54]. Although the results were not uniform, they showed that therapies with pure stem cells are important and achievable therapy.

There are various other aspects of stem cells. If stem cells could not treat the disease also the drugs can be tested against it which can provide appropriate results, safety and can save millions of dollars that are invested to prove its efficacy. Stem cells are being used to study normal human development. A better understanding of the inner workings of living

organisms leads to earlier detection, better diagnosis, and injury. It is also being used in the regeneration of cells and tissue which will be a boon for the patient losing their body parts. For example, the transplantation of healthy retinal pigment epithelial cells to the eye to regenerate those lost in macular degeneration is now being tested in clinical trials. S. Yamanaka and T. Yoko in their review article "Current Bioengineering Methods for Whole kidney Regeneration" 2015 summarized recent research involving the use of renal stem cells and renal bioengineering to regenerate functional whole kidney de novo.

Obstacles in stem cells therapy

Despite many potential therapeutic benefits and promising results of Stem Cell therapy or regenerative medicine, there is still some darkness where light has no reach yet. Most of the results are obtained *in vitro* or from animal trials. Very few clinical trials have been done. There has been a dispute on the role of stem cells in tumor modulation. A few studies have supported that mesenchymal stem cells may suppress tumor growth [54, 55] while others believe that mesenchymal stem cells may contribute to tumor protection via anti-apoptotic effect, tumor progression, metastasis, and drug-resistance of cancer cells [54-57]. Massive *in vitro* expansion for producing enough stem cells is also a risk factor for malignant transformation. Researchers are still not successful in composing the vital bone pieces in larger volume or even whole bones. The problem of vascularization in tissue engineering is not yet solved, inhibiting the translation of tissue engineering methods into the clinic [58].

Wrathall *et al*, [2017] focused on Spinal cord injury is devastating because so many cells and their widespread connections are lost even after a mild traumatic injury, and the natural replacement of these cells and their essential connections are insufficient. Challenge is still to differentiate the stem cells into hepatic lineage which is stalled at the stage of hepatocyte-like cells (HLCs) failing to achieve fully mature hepatocytes [59].

The major hurdle in doing the research is also an ethical problem. The various ethical issues in using embryonic stem cells have halted the progression but induced pluripotent cells and umbilical cord stem cells can be the answer for these questions since the umbilical cord is thrown after the birth of the baby. The traditional use of bone marrow stem cells which is a painful procedure has been replaced with umbilical cord stem cells. The umbilical cord contains a significant amount of mesenchymal stem cells which are easily collected and cultured and these cells have greater expansion capability, faster growth rate expresses the lower level immunogenicity and superiority for clinical application [60-64]. This is the reason the bio banking of the umbilical cord is at its peak for use in the future to the same person. The risk of getting a viral infection in recipient due to feeder layer which is made from is lowered due to various newer technology that is used to culture the stem cells and increase the efficacy of therapy [65], In recent years, many new isolation and culture technologies have been developed to obtain stem cells for their wide application prospects in disease mechanism and its treatment including suspension technology [65] and SB431542 inhibitor differentiation method [66]. Stem cell therapy can be questionable whether organ or tissue regenerated from will work in association and there is no graft vs. host diseases. This therapy should be turned to take host stem cells only where there is no chance of rejection and direction of research to self-destruction if they become teratomatous.

Despite many challenges, clinical trials and research is adding bricks every day towards the cure of many life-threatening conditions. Rebecca S. Y. Wong also reviewed the stem cells therapy in his article 'Mesenchymal Stem Cells: Angels or Demons?'[2011] that the use of mesenchymal stem cells for disease treatment in general: the vast number of clinical trials and the abundance of published literature suggest that mesenchymal stem cells treatment is feasible. Ongoing translational research may bring new hopes to sufferers of many diseases which can lead to improvement or cure.

Conclusion

Stem cell research is going on for decades and has come to the state where it will change the face of medical science at any time in the future. Stem cell therapy will not be able to halt the progression of the diseases only but also will cure them completely. The promising result of the clinical trials obtained in a few decades is much fascinating but still needs further detailed investigation, complete control on differentiation and self-destruction before forming teratoma should be achieved before its direct application on the patient. Moreover, the Umbilical cord should be preserved in stem cell banks so that we can fight against various diseases and accidents.

Stem cells can only be an option in treating the diseases in the future so everyone should be aware of the potential of their use in any form of the disease. The present generation should preserve the crude form of stem cells like the umbilical cord, amniotic fluid, and bone marrow cells of their baby which are thrown thinking as a biological waste for the time being since it will completely erase the complication of rejection. The most significant breakthrough in treating neurodegenerative disorders like Parkinson's disease, Alzheimer's disease, dementia, etc. will be stem cells if the current limitation of stem cell therapy will be overcome. The government and private pharmaceutical companies should work together to overcome the various obstacles of stem cell research and invest some capital in establishing stem cell banks. Technology should be made to produce a large scale of stem cells and store them for a longer time to enhance their rate of viability and efficacy in therapy and research.

In a nutshell, we have much hope on this therapy and researches results are positive and clinical trial on human are also positive up to an extent which could help our body to heal itself and revolutionize the whole concept of health care and the day will come when wheeled chairperson just stand and can hug his/her loved one, which is not so far.

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