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Pluripotent Stem Cells – Potential Uses in the Pharmaceutical Field

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ABSTRACT The cells can divide and differentiate into several types of cells in an organism. It is not able to differentiate as the extraembryonic tissues, which are termed as the 'pluripotent cells'. The transcription factors such as, *SOX-2, C-MYC, OCT-4* and *KLF-4* are involved in the maintenance of pluripotency. Pluripotent stem cells (PSCs) can self-renewal cells, which may be either induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs). Pluripotent cells can be used in the treatment of diseases like cancer, cosmetics and the healing of skin burns. The current review majorly focuses on the PHCs along with their history, their molecular markers and major transcription factors in a brief manner. The techniques including sensors and assays used for the assessment of pluripotency have also been discussed.

INTRODUCTION

'Pluripotent cells' can able to undergo self-renewal and it has the inherent ability to form various types of cells in any organism. This property of the cell is referred to as pluripotency, whereas, the property of a cell that can develop into a whole new organism and is differentiated into several types of cells including the extraembryonic tissues is called the totipotency. Pluripotency is the characteristic of the inner cell mass of the blastocyst, which later develops into the embryo. Every organ and tissue contains pluripotent cells, which decreases the ageing of the individual. Pluripotency is the most important characteristic of all embryonic stem cells (ESC), which is derived from the inner cell mass of the pre-implantation embryo (blastocyst). It can rise to all tissue lineages of the three primary layers of germ cells during the process of differentiation and is named pluripotency. Due to this property, ESC holds a great guarantee for essential studies in the regeneration of tissue and therapies associated with cell replacement. Pluripotency is maintained by its vital property including self-renewal (Zakrzewski et al. 2019).

The self-renewal property allows the PSCs to replicate them without losing the ability to differentiate, thus controlling the plasticity of the transcription profile of the pluripotent cell (Chen and Daley 2008). During the replication of PSCs, both asymmetric and symmetric cell divisions can occur. The symmetric cell division occurs within ESC that undergoes self-renewal *in vitro* and asymmetric cell division occurs in tissue stem cells that provide one daughter cell and one differentiated cell *in vivo*. Micro RNA (miRNA) also plays a significant part in the regulation of cell pluripotency. The transcriptional factors can cause differentiation and reprogramming in these cells. The stem cells commit to one cell lineage and lose their potential to commit to other cell lines during differentiation. Similarly, when cells are reprogrammed, differentiated cells are returned to their pluripotent state (Pei 2008).

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Adult cells can also serve as the source of PSCs, which are obtained by the process of re-differentiation in adult somatic cells by reprogramming the adult cell using the transcriptional factors. This induction of adult stem cells is known to be induced pluripotent stem cells (iPSCs) and it is now widely under research. It is used in the field of regeneration of tissue, which helps repair genetic defects, biomarker discovery, regenerative medicine, better toxicity testing, developmental and epigenetic signaling, etc., (Romito and Cobellis 2016).

Objectives

The review summarizes the benefits of PSCs in the field of medicine that provide a quality evaluation for the assessment of the pluripotency in the human being. The quantity of PSCs in a person's body may reflect the ageing process, and when isolated, it can be used to treat many life-threatening diseases. Hence, the assessment of PSCs based on their pharmaceutical properties and their possible usages are discussed briefly in this review (Fig. 1.).

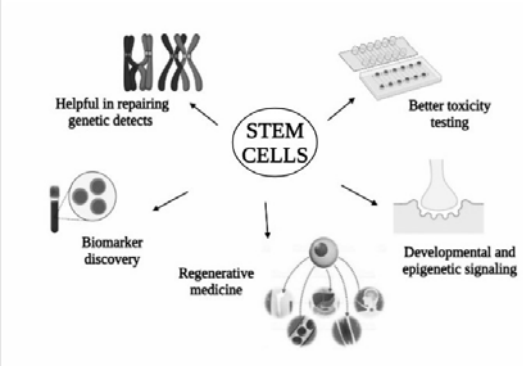

Fig. 1. Applications of pluripotent stem cells
Source: Authors

MATERIAL AND METHODS

Since this is a review, the literature survey has been done using the following database and keywords to identify the opt literature.

Databases like PubMed, SCOPUS and google scholars are used to find the present literature by using the keywords 'hPSCs - Human Pluripotent Stem Cells'; 'iPSCs - Induced Pluripotent Stem Cells'; 'ESCs - Embryonic Stem Cells'; 'PSC - Pluripotent Stem Cell'; 'TCSCs - Tissue Committed Stem Cells'; 'VSEL - Very Small Embryonic-Like Stem Cell'; 'Techniques, like "lateral flow biosensor, multiphoton tomography, surface-enhanced Raman scattering assay, alkaline phosphatase assay, immunostaining, real-time polymerase chain reaction, pluritest® assay, scorecard assay, teratoma formation assay, tetraploid complementation assay, chip assay and flow cytometry analysis".

OBSERVATIONS AND DISCUSSION

Induced Pluripotent Stem Cells (iPSCs)

The iPSCs are derived from the epiblast layer of implanted embryos, which are the peripheral layers of the blastula and also from the somatic cells. Their pluripotency is persistent, from completely pluripotent cell stages, which subsequently end with less potent states: multipotent, oligopotent and unipotent cells (Omole and Fakoya 2018).

History of Stem Cells

Till and McCulloch in 1961, identified the clone forming activity in the spleen of irradiated mice by injecting it with the bone marrow cells, and also they discovered the hematopoietic stem cells, which they called blood-forming cells, which is published in Nature (Becker et al. 1963). This is further discovered to be the self-renewal property of the colony-forming cells (stem cells) by Siminovitch in 1963. Table 1 shows a few historical events in the field of stem cell investigation.

History of Induced Pluripotent Stem Cells (iPSCs)

Takahashi and Yamanaka (2006) have developed the mouse iPSCs using a combination of four reprogramming transcription factors, which may include 1. Octamer-binding transcription factor-3/4 (*OCT-4*), 2. Sex-determining region Y-box 2 (*SOX-2*), 3. Kruppel like Factor-4 (*KLF-4*), and 4. c-Myc in 2006 and they are abbreviated as the "OSKM factors". Human iPSCs are de-

Table 1: The history of stem cells

S. No.	*History*	*Discovery*
1	Alexander Alexandrowitsch Maximow 1908	Proposed the term "Stem Cell" and the existence of HSC (Konstantinov and Igor 2000).
2	James Thomson 1998	Discovered the human ESCs (Thomson et al. 1998).
3	John Gearhart 1998	Obtained the germ cells from the tissues of fetal gonads prior to forming pluripotent stem cell lines (Shamblott et al. 1998).
4	Shinya Yamanaka 2006	iPSCs mice (Takahashi and Yamanaka 2006).
5	Shinya Yamanaka and Kazutoshi Takahashi 2007	Human iPSCs are developed by reprogramming the human fibroblasts (Omole and Fakoya 2018).
6	Mari Dezawa 2010	Discovered Muse cells are stress-tolerant, self-renew and associated with pluripotency (Kuroda 2010).
7.	Okita and co-workers 2011	Epi programming system optimized (Okita et al.2011).
8.	Zhou and co-workers 2012	Next-generation iPSCs generated from urine (Zhou et al. 2012).
9.	Hou and co-workers 2013	Complete chemical induction of mouse fibroblasts into iPSCs (Hou et al.2013).
10.	Lei and Schaffer 2013	Poly-hydrogel 3D culture for human PSC differentiation (Lei and Schaffer 2013).
11.	De Bonis and co-workers 2014	The role of SIRT1 is determined in genomic stability and telomere elongation of iPSCs(De Bonis et al.2014).
12.	Sousa and co-workers 2014	Adult stem cells are generated from multiple organs (Sousa et al. 2014).
13.	Liu and co-workers 2015	The role of ASCI1 is illuminated in the direct change of astrocytes into the nerve cells (Liu et al.2015).
14.	De Soure and co-workers 2016	Scalable Xeno-free microcarrier bioreactors (De Soureet al. 2016).
15.	Mandai and co-workers 2017	iPSC obtained from retinal cells are transplanted in a woman who is suffering from advanced macular generation (Mandai et al. 2017).

veloped in 2007 by reprogramming the cells of the human fibroblast (Omole and Fakoya 2018).

Molecular Markers of Pluripotent Stem Cells

The molecular markers are used for evaluating cellular potency. Transcription Factors (TFs) play a vital role in the maintenance of the pluripotency of the stem cell. The four reprogramming factors (*OCT-4, SOX-2, KLF-4* and *C-MYC*) are related to the pluripotency status of several cell populations and induce somatic cell reprogramming (Singh et al. 2016). Transcriptional factors like *OCT-4* and *NANOG* are the initially identified and most important transcriptional factors, which maintain the pluripotency and hence are called the master regulators. Along with this, *SOX-2* has the same role, where all three are regulating each other and also control the gene expression of transcriptionally active genes. The studies have proved the transcriptive characteristics of *OCT-4*, which shows its exclusive pluripotent nature (Boyer et al. 2005).

The stemness is an exclusive property of PSCs that refers to the general molecular process of the core stem cell characteristics of the self-renewal and the formation of differentiated progeny (Chen and Daley 2008; Singh et al. 2016). *SOX-2* plays a vital role in determining cell fate and pluripotency in regulation. *KLF-4* is another transcription factor that aids in the maintenance of *OCT-4* and *SOX-2* are binding with the promoter region of *NANOG* and this plays an important responsibility in pluripotency maintenance. *C-MYC* gene is accountable for regulating the chromatin environment, which may be enhancing the proliferation of the cells (Singh

et al. 2016). *SOX*-2, *OCT-4* and also *NANOG* set key transcription factors for the development and differentiation and are transcriptionally inactive in embryonic stem cells (Fig. 2.). In addition to the transcription factors, *SOX*-2, *OCT-4* and *NANOG* are required for maintaining pluripotency, *ESSRB, DAX1, SALL4, TCL1, NAC1, TBX3, ZFP281* and *RIF1* genes are also involved in the same function. All these together regulate each other and form a complex network of transcriptional regulation in embryonic stem cells (Chen and Daley 2008).

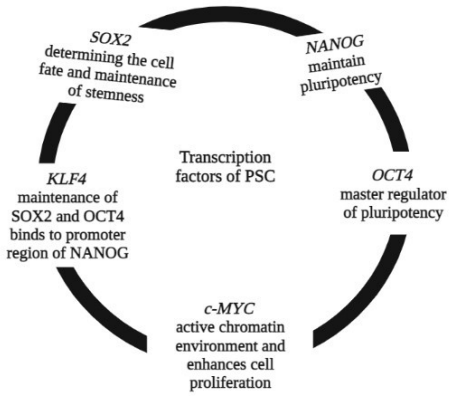

Fig. 2. Major transcription factors that maintain pluripotency
Note: PSC - Pluripotent stem cells; SOX - (SRY); Sex determining region Y-box 2; Klf4 - Kruppel-like factor 4; cMYC - Myelocytomatosis oncogene; Oct-4 - Octamer-binding transcription factor; NANOG - Nanog homeobox

Very Small Embryonic Like Stem Cells (VSELs)

The VSELs are one of the derivatives of the epiblast stage of PSCs. These are the quiescent stage stem cell populations that get deposited in almost all the organs in the embryonic developmental stages of life. They are small in size, about 3-5µm with a large nucleus cytoplasmic ratio with a large quantity of euchromatin in the large nucleus. The mesenchymal stem cell markers including $CD29^-$, $CD90^-$and $CD105^-$ and are negative; they don't express Major Histocompatibility Complex I (MHC class) and Human Leukocyte Antigens (HLA-DR). These cells later serve as tissue committed stem cells (TCSCs). The VSELs and TCSCs aid in tissue regeneration, renewal, and homeostasis in any organ. These cells decrease ageing (Bhartiya et al. 2013).

In early gastrulation, VSELs are deposited for developing the tissues or organs that endure in adulthood and play a vital role as a back-up population of PSCs in the turnover of TCSCs (Ratajczak et al. 2011). VSELs are recognized in the murine adult bone marrow, which is named as muVSELs cells it is not hematopoietic in nature and have the nuclear-cytoplasmic ratio, small, scattered euchromatin and also express the primordial germ and pluripotent embryonic cell markers (Sovalat et al. 2016). Murine VSEL cells overexpress pluripotency genes (*OCT-4, NANOG* and *SOX-2*) both at mRNA and protein levels. It also maintains a demethylated *OCT-4* promotor. Human very small embryonic-like (HVSEL) cells are expressed as surface markers like *CD34*, *CD133* and *CXCR4* which are the characteristics of progenitor populations and adult stem cells, which are the stem cells along with the *SSEA-4* antigen on the surface of embryonic stem cells (Danova-Alt et al. 2012).

Multilineage-differentiating Stress-enduring Cells (Muse Cells)

The muse cells are non-tumorigenic PSC and extremely resistant to cellular stress. This cell express *SOX-2, OCT3/4, NANOG* and *TRA1-60* intrinsically in every connective tissue. It has asymmetric division and low telomerase activity. When these cells are transplanted into the host organism does not undergo tumorigenesis. Muse cells are highly capable of residing in damaged tissues and are involved in continuous differentiation into the cells of damaged tissue, bringing about tissue repair and useful restoration of its function. The part of these cells profoundly preserved the components of cells and related to cell survival as well as recovery, in reaction to stress to cells and intense damage (Simerman et al. 2016).

Muse cells may proffer knowledge interested in the atomic and evolutionary bases of independent tissue recovery and illustrate the cellular and molecular components that avoid warm-blooded animals from recovering appendages and organs (Simerman et al. 2016). Muse cells can tolerate the stress and are able to form M-

clusters in suspension culture, which express pluripotency markers, self-renewal by itself, is not exceptionally higher in its proliferation activity and don't appear to create teratomas in the immunodeficient mouse testicles. It is capable of distinguishing into endodermal, mesodermal and ectodermal cells (both *in vitro* and *in vivo*) and is proficiently confined from gullible tissues as cells positive for *SSEA-3* and *CD105* (Kuroda 2010).

Muse cells renewed modern skeletal muscle cells (matched box 7-positive cells dystrophin and the human dystrophin) in the degeneration of muscle in mice when injected intravenously. Muse cells harbour the capacity to home into the liver in fulminant hepatitis in mice renewing unused cells and also contributing to the repair of tissue. The muse cells injected locally may be accelerated and significantly heals the skin ulcers in type 1 diabetes-induced mice. The fraction rich in muse cells coordinates into the dermis of the mice, separating into vascular endothelial cells, keratinocytes fibroblasts and dermal fibroblasts. Notably, muse cells are not found in the functional regions around the skin ulcers (Kinoshita et al. 2015). Muse cells are used in regenerative medicine and can be effectively derived from the skin biopsies of patients as SSEA-3(1) cells by identifying them with FACS or magnetic-activated sorting. These cells have the major advantage of being non-tumorigenic (Uchida et al. 2016).

Techniques to Assess Pluripotency

The pluripotency of cells for the protection of primaeval stages and repression of differentiation has several molecular mechanisms that regulate gene expression responsible and these functional assays can give stringent quality measurement tools for the assessment of the pluripotency (Singh et al. 2016).

Lateral Flow Biosensor

A lateral flow biosensor helps to detect the human stem cells. Stage-Specific Embryonic Antigen-4 (SSEA-4) is coated with the gold nanoparticles, an antibody that is specific to the human stem cell surface antigen, whereas, an antibody against other human pluripotent stem cells (hPSC) surface antigen (SSEA-3), in the nitrocellulose membrane, is immobilized on the test zone. Gold nanoparticles are coated by an antibody bound to the target cell and form nanoparticles and stem cell complex, and this complex is captured on the test zone by another antibody immobilized to form a red line for visual recognition (Wu et al. 2013). In this biosensor system, SSEA-3 and SSEA-4 are utilized to mark the hPSCs. SSEA-3 is coated with magnetic beads for the enhancement of hPSCs that is antibody specific, and the carboxyl-modified tDNA sequence is conjugated with an antibody specific SSEA-4 which is used as a template for strand displacement amplification.

The single-strand DNA is amplified and detected using a lateral flow biosensor. The lateral flow biosensor has a strip reader that detects a minimum of 19 human embryonic stem cells. This approach distinguishes hPSCs from the various types of cells. The lateral flow biosensor will have vital applications in regenerative medicine including determining the effectiveness of iPS cells from somatic cells (Wu et al. 2015).

Multiphoton Tomography

The high-resolution multiphoton fluorescence lifetime imaging tomography (MPTflex) is an optical non-linear imaging technique, that uses the near-infrared (NIR) picojoule femtosecond laser and is provided with a 360° scan/detector head, which is mounted on an adjustable mechano-optical articulated arm for concurrent intra-tissue fluorescence and second harmonic generation detection is employed. The multiphoton microscope is used to observe the proliferation of cells, migration of cells, and differentiation of stem cells with high interest. Multiphoton imaging can conquer this difficulty because it is based on two-photon excitation of intrinsic fluorophores, which includes nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), porphyrins, melanin, flavins and elastin. Additionally, second harmonic generation images can be get from certain biomolecule structures like muscle protein myosin and collagen. Multiphoton tomography is label-free and non-invasive so it is the more advantageous technique to detect iPSCs (Uchugonova 2017).

The photo destruction and impact on cellular metabolism due to the source can be avoided

in multiphoton microscopy, which is the non-invasive imaging of live cells that is carried out by the sub-femtoliter absorption volume of NIR and by utilizing high numerical aperture objectives. Multiphoton excitation is suitable for the excitation of natural fluorophores, which are the autofluorescence by maximum absorption within the UV/blue range, such as flavins adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) or the flavoproteins. And so, MPT is used in tracking the induced pluripotent colonies which are cultured *in vitro* (Uchugonova 2017).

Surface-Enhanced Raman ScatteringAssay

The surface-enhanced Raman scattering based assays can identify a very small number of undifferentiated hPSCs. The stem cell surface markers, like SSEA-5 (stage-specific embryonic antigen) and TRA-1-60 (tumour-related antigen) are targeted to detect the stem cells. The Surface-enhanced Raman scattering can detect 1 in 10^6 cells, which is around 15,000 fold greater than the flow cytometry assays. Thus, surface-enhanced Raman scattering assays can be used to detect the quality of hPSCs in preclinical and clinical applications (Han et al. 2016).

Alkaline Phosphatase Assay

This assay is used to distinguish PSCs from the feeder and parental cells by screening earlier and by using alkaline phosphatase staining. PSCs are upregulated by placental alkaline phosphatase (Lewandowski and Kurpisz 2016).

Immunostaining

Pluripotent status and homogeneity in cell culture can be assessed phenotypically by this method. It distinguishes, selects and isolates reprogrammed colonies and is easy to handle. The glycolipid antigens, including SSEA3 and SSEA4 as well as keratan sulfate antigens such as TRA-1-60 and TRA-1-81, can determine pluripotent cells (Lewandowski and Kurpisz 2016).

Real-time Polymerase Chain Reaction (RT-PCR)

The undifferentiated marker of expression of a gene can be analyzed by quantitative technique and transgene silencing efficacy can be detected. The set of pluripotent genes and proteins can be detected (Lewandowski and Kurpisz 2016).

PluriTest® Assay

PluriTest® assay is a computer-based bioinformatics assay. The transcriptome of a test cell line can be compared with the transcriptome of various other cell lines that are found to be pluripotent. This test can be done in a short time with a lesser number of cells, considering the early stages while developing a pluripotent cell line. PluriTest® does not access the differentiated cells and can identify only the undifferentiated cells, however, it does not evaluate the differentiation ability of differentiated cells. This could generate two kins of summary scores from the global gene expression profiles, they are a pluripotency score, which can compare and identify the gene expression similarities from the fed samples to the known human PSC data; and another one is novelty score, which can detect the expression of gene patterns that are not related to the human PSC (Allison et al. 2018).

ScoreCard Assay

The ScoreCard assay is q-PCR based quantitative assessment of functional pluripotency of hPSC. It includes the assessment of the effects of culture conditions, small molecules, and also knockdown of key regulators for the transcriptional process. They use the impressions of hPSC gene expression to enumerate the differentiation ability of hPSC lines. A wide range of applications includes assessing different culture conditions, quantifying perturbations of lineage regulators, and screening small molecules. The ScoreCard method is a faster, higher reproducible evaluation of functional pluripotency when compare with the teratoma assay and has a wider range of applications than other existing genomic tools and assays. The weighted Z-method is the enhanced version to carry on statistical analysis that combines information from multiple genes in a weighted, assay-dependent approach whereas also considering the dependencies among the genes. It has a wider range of applications and is more advanced than the Score-

Card assay by providing the statistical measure of functional pluripotency (Tsankov et al. 2015).

Teratoma Formation Assay

Teratoma formation assay is one of the most commonly used to test pluripotency in humans and other mammals. A tumour cell developed exhibiting the characteristics of three germ layers by this test the pluripotency is confirmed. The teratoma assay assesses human PSC pluripotency and is regarded as the 'gold standard' assay. It is regularly performed but does not give quantitative data on lineage differentiation. The more definitive analysis for gene expression by the teratomas (Allison et al. 2018; Tsankov et al. 2015).

Tetraploid Complementation Assay

Tetraploid Complementation Assay is used to confirm pluripotency and developmental aspects of the ESCs and iPSCs. This assay uses a tetraploid embryo mixed with normal 2n ESCs from a different organism that subsequently develops into the fetus. Fetal cells are obtained from the ESCs and the tetraploid cell gives rise to extra-embryonic tissues (Singh et al. 2016).

Chip Analysis

ChIP-seq analysis can reveal epigenetic changes and chromatin signatures in pathways by validating transcriptomic profiling reliable with changed signaling. Epigenomic profiling is utilized to identify the downstream targets in the mutated genes (Parrotta et al. 2017). The *OCT-4, SOX-2* and *NANOG* are often cooperated with other transcription factors to alter gene repression or activation in ESC revealed by Genome binding investigations on other ESC factors ChIP-sequencing (ChIP-seq; Allison et al. 2018).

Flow Cytometry Analysis

Flow cytometry analysis is used to identify the pluripotency markers by using the direct conjugated antibodies method (Martí et al. 2013). It provides an efficient, rapid approach to separating, purifying and evaluating the properties of PSCs. A faster, as well as efficient way of isolating, purifying and studying the characteristics of PHCs, is flow cytometry. The principle of a flow cytometer in differentiating the PSCs is based on the size and complexity of the cells along with their fluorescent properties (Ho et al. 2011; Schinzel et al. 2011; Wu et al. 2013). The flow cytometry functions by using the antibodies that can recognize targets on the PHCs and it may be suggested because it is a powerful method for high-throughput validation of PHC lines (Riordon and Boheler 2018).

CONCLUSION

Various transcription factors are involved to maintain the pluripotency in the circulating PSCs in the blood and tissues. The VSELs and muse cells along with other PSCs have the property of pluripotency are widely used in regenerative medicine for various treatments and have wide applications. Different methods and techniques to detect stem cells and their biomarkers are discussed in this review. This review concluded that different techniques can be used to detect stem cells, thus they can be applied in various fields.

RECOMMENDATIONS

The PSCs are a significant tool in finding new pharmaceutical approaches for fatal human diseases. Human disease modelling, the discovery of drugs and cell therapy are some of the major applications of PHCs. The PSCs are recommended as it avoids the percentage of being rejected during the transplantation process by the immune system as the tissue be able to be created with the cells of the same individual and the PSCs are grown via *in vitro* approach, hence the endanger of any human being is negative.

LIMITATIONS

The applications-oriented to the clinical field involving the development of drugs for human disease has not been studied and not presented in detail in the current study, which adds to the limitations of the study.

ABBREVIATIONS

ESCs - Embryonic Stem Cells
FAD - Flavins Adenine Dinucleotide

HSC - Hematopoietic Stem Cells
hPSCs - Human Pluripotent Stem Cells
HVSEL - Human Very Small Embryonic-like
iPSCs - Induced Pluripotent Stem Cells
MHC - Major Histocompatibility Complex
miRNA - micro RNA
MPTflex – Multiphoton Fluorescence Lifetime Imaging Tomography
muVSELs - Murine VSELs
NIR - Near-Infrared
NAD - Nicotinamide Adenine Dinucleotide
NADPH - Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NA - Numerical Aperture
PSC - Pluripotent Stem Cell
SSEA-4 - Stage-Specific Embryonic Antigen-4
TCSCs - Tissue Committed Stem Cells
VSEL - Very Small Embryonic-Like Stem Cell

CONFLICT OF INTEREST

There is no conflicts of interest.

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