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A Basic Review on Identification, Isolation and Applications of Stem Cells

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Abstract: ***Development of stem cells therapy and the mechanical isolation, cultivation and characterization is the thrust of today's translational research. This paper discusses a basic review in the form of an outline of different types of stem cells and lineages and their cultivation derived from various sources along with their application. A short note on limitations of each is also mentioned.***

Keywords: Stem Cells, Embryonic stem cells, Adult stem cells, Cancer stem cells, Scaffold, Bioreactors, Growth factors

1. Stem Cells

Stem cells are distinct group of pluripotent cells distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division. Second, they can be induced to become tissue- or organ-specific cells with special functions. Stem cell research has already generated new possibilities and stimulated development of new strategies for increasing our under- standing of cell lineages and differentiation.

Stem cells are pluripotent cells capable of self-renewal and multilineage differentiation [1].They can be identified from three main sources: Embryonic stem cells, Adult stem cells, Induced pluripotent stem cells[2].When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

1-Embryonic stem cells (ESC)

They are pluripotent cells derived from embryonic stage. These cells have not undergone complete differentiation and retain the capacity to divide into any of the three germ layers:, Ectoderm, Endoderm, Mesoderm[3]

Source, identification and isolation

Mouse ES cells were first derived by Evans and Kaufman in 1981; Martin in 1981 [4, 5] but it was not until 1998 that derivation of human ES cells were first reported by James Thompson from the undifferentiated inner cell mass (the early stage of embryonic development after fertilization) of human blastocysts that were produced through in vitro fertilisation.[6]

Currently ICM is isolated by using mechanical dissection[7, 8], laser dissection, [9, 10] and immune surgery procedures[11, 12]. In another method, hESC can be isolated from ICM by microdissection of human blastocysts using fine needles.

Application

1. HESC differentiates into numerous types of cells including osteoblasts, cardiomyocytes, hepatocytes, neurons, and endothelial cells.
2. HESC is useful for allogenic cell transplantation research as well as clinical trials for treatment of disease such as spinal cord injury, cardiovascular disorder and diabetes. [13, 14]
3. Esc research makes better understanding of mechanism of cell differentiation which ultimately leads to discovery of novel treatments for disease such as myocardial infarction. [15, 16]
4. ESC could be induced to differentiate into different type of cells that could be used for therapeutic intervention such as regenerative transplantation. It can be transformed into hepatocytes, retinal ganglion cells, chondrocytes, pancreatic progenitor cells, cone cells, cardiomyocytes, pacemaker cells, eggs, sperms which can be used in regeneration of tissue and treatment of diseases in tissue specific manner[17, 18]

Limitation

Embryonic stem cells have both moral and technical problems; because these cells will later develop into a human being, taking these cells will require destruction of an embryo. Technically these cells are difficult to control and grow and they might as well form tumor after their injection [19].

2-Adult stem cells

Pluripotent cells which resides in specific location of each tissue in a specialized microenvironment known as the stem cell niche, also called as Somatic Stem Cells or Post Natal cells. They are more restricted in their differentiation capacity when compared with Embryonic stem cells.

The two main types of adult stem cells are Haemopoietic stem cells (HSC) and mesenchymal stem cells (MSC).

Examples of Haemopoietic- Transplanted human umbilical cord blood mononuclear cells improve left ventricular function angiogenesis in myocardial infarction.[20]

Examples ofMesenchymal-Friedenstein et al. described in the 70s the approach ofusing adherentfibroblastic cells that were drawn from thebone marrow [21] and their capacity to differentiate into several mesenchymal tissues.[21]

MSCs are multipotent stromal stem cells that can be harvested from many different sources and differentiated

into a variety of cell types, such as preosteogenic chondroblasts and osteoblasts

Several types of Specialised adult stem cells have been identified like dental stem cells (DPSC- They are present in peri vascular niche within the dental pulp tissue.)[22], periodontal ligament stem cells (PDLSC)[23], stem cells from human exfoliated deciduous teeth (SHED)[24], dental follicle progenitor cells (DFPC)[25], and stemcells from apical papiila (SCAPs)[26].

Source

Adult human dental stem cells were first identified and isolated in 2000 by Gronthos et al.[27] from normal impacted third molar's pulp. Mesenchymal stem cells were first recognised in bone marrow by Friedenstein et al in 1970s.

Application

- Adult SC maintains connective tissue by differentiating into several cell lineages such as; osteoblasts, chondrocytes, adipocytes, and myoblasts [28 29].
- They can differentiate in vitro into odontoblasts, adipocytes, neural cells, osteoblasts, chondrocytes and myoblast.
- They play a major role in tissue maintenance, renewal, and repair following stress and injury impacts.[30]Adult stem cells has been extensively used in Cancer treatment.
- Provides signalling molecules and growth factors that enhance functions of other cells through paracrine mechanism. ADSC were characterised as clonogenic and highly proliferative, being able to form in vitro calcified sporadic nodules.[22]

3-Induced pluripotent stem cells (ipsc)

IPSCs are modified ESCs or can be defined as embryonic stem cell -like cells derived from the reprogramming of adult somatic cells by the introduction of specific pluripotent associated genes. Like ESCs, IPSCs can proliferate extensively in culture and can give rise to three germ cell layers; endoderm, mesoderm, ectoderm.

Source

IPSC were initially derived by Shinya Yamanaka et al in 2006 from fibroblasts by transduction of genes encoding transcriptional regulators of stem cells.[31]

IPS cells can be obtained from multiple oral mesenchymal cells: SCAP, DPSCs and SHED, TGPCs, buccal mucosa fibroblasts, gingiva fibroblasts, and periodontal ligament fibroblasts [32]. It is expected that oral cells can be an ideal iPS cell source, which can be applied in regenerative procedures for periodontal tissue, salivary glands, missing jaw bone, and tooth loss [33].

Application

Duan et al. described that making the combination between iPS cells and enamel matrix derivatives can enhance periodontal regeneration and the cementum formation of the periodontal ligament and alveolar bone [35].

Other studies suggested that the ability of iPS cells to differentiate intoameloblasts and odontogenic mesenchymal cells is promising in tooth bioengineering [34, 36]

Isolation of stem cells

Isolation of embryonic stem cells- ESCs are isolated from the inner cell mass of embryonic blastocyst in the early pre-implantation stage after in vitro fertilization.

Isolation of adult stem cells-

Adult stem cells can be easily isolated from bone marrow, having variety of techniques like antibody based cell sorting[37], low and high density culture techniques[38, 39], positive negative selection method40, frequent medium changes 41 and enzymatic digestion approaches.[42]

A standard method for isolation of ADSC has been described by Pittenger et al in 1999[43].

Gronthos et al were the first one to isolate the Dental pulp stem cell (DPSC's) in 2000[21].

The processes of extraction of few human third molars are done in the in-vivo method.

After the extraction they are split to further extract the Pulp tissue to isolate the Dental pulp stem cell we have three methods:

1. Digestion of the pulp by collagenase /dispase enzymes and culture of the released stem cells,
2. Culture of undigested pulp pieces leading to cellular outgrowth
3. Digestion of pulp tissue by fixing them

The minimum essential medium alpha modification (a MEM) medium supplemented with 20% fetal bovine serum (FBS) in humid 37 degree Celsius incubator with 5% Co_2, is used to culture the cells.

The reverse transcriptase polymerase chain reaction is used to study the markers of stem cells.

Limitations of isolation

1. Inner cell mass can be obtained only from fresh or frozen human embryos [44].
2. It requires establishment of reproducible methods for therapeutic interventions. [45, 46]
3. The isolation of stem cells has raised many ethical concerns, among them these two have gained specific prominence: 1- whether stem cell research is ethically problematic because it entails the destruction of human embryos and 2- what kind of control embryo donors should have over the stem cell lines derived from their embryos.[47]

4. The success of obtaining good quality HESCs depends on the quality of blastocyst and isolation procedures and culture conditions.

Scaffold

A scaffold is an artificial structure capable of supporting a 3-D formation. Matrices of scaffold is used to fill void, provides structural support and tissue forming cells along with the growth factors.

Examples: high pressure co2 foamed scaffold, injectable scaffold, novel custom scaffold.[49]

Here broadly two main approaches are utilised – first, scaffolding can be used as a cell support device upon which cells are seeded in vitro; cells are then encouraged to lay down matrix to produce the foundation. The second approach involves using the scaffold as a growth factor/delivery device. This strategy involves the scaffold being combined with growth factors, so upon implantation cells from the body are recruited to the scaffold site and form tissues upon and throughout the matrices. These two approaches are not mutually exclusively and can be easily combined.[48]

Role of scaffold

1. Scaffold architecture can modify the response of cells and subsequent tissue formation (Ripamonti, 2004).
2. Composition, topography, and architecture of scaffolds are able to interact and influence cell behaviour, so the manner of combination of cell type and scaffold should be carefully matched.
3. Nano to microscale topography has been demonstrated to affect cell behaviour by modification of cytoskeleton arrangement (Meredith et al 2007).
4. Different cell types react to different materials for example: different scaffold materials produced different levels of glycos-amino glycans in tissue engineered cartilage (Freedet et al 1993)

2. Ideal Properties or Favourable Properties

1. With use of molecular weights, cross links, and side chains, material can be produced with tailored made properties.[49]
2. Scaffold materials are very potent drug delivery device.[49]
3. They are biodegradable surgical component which makes them ideal for use in tissue engineering.[49]

Scaffold design and manufacture

The source of cells is also an important choice for scaffolds, as is the culture regime used (Francioli et al 2007). There is wide range of cell types which can be used by combining them with scaffold to produce tissue engineered construct.

Polylactic acid (PLA), a biodegradable polymer has become widespread, but the manner in which these polymers are processed and the additives used at the time of manufacture allows the final properties of the scaffold. These scaffolds can be further modified using growth factors, zonation of materials and plasma polymerisation deposition [49].

Scaffold has been produced for individuals via custom three dimensional (3D) printing using laser stereolithography techniques. This allows the scaffold to be built from computed 3D information derived from patient scanner from computer simulations (Antonov et al 2004).). The process is similar to rapid prototyping procedures whereby layers of particles are selectively sintered using a directed laser; these fused particles are further layered and sintered until several to several hundred layers have been bonded together, producing a custom 3D scaffold. Scaffolds may also be printed to include the cells using systems such as the fusing gel and cell bead system (Jakab et al. 2004). Certain tissues, such as muscle, may require different material properties as this tissue needs flexibility as a fundamental part of its mode of action. To address this, modification of a flexible polymer, poly (1, 8-octanediolco- citric acid) (POC), to make it more suitable for culture of muscle cells has been developed (Yang et al. 2005; Hidalgo-Bastida et al. 2007).

Growth factors

Tissue engineering requires interaction of cells with scaffold along with growth factors through incorporation of appropriate physical and cellular signals for the regeneration of tissues and it includes modifying factors like such as biologically active proteins and DNA. They are naturally occurring regulatory molecules, resembles structurally to peptide like hormones, which binds to receptors on cell surface.

They stimulate cell and tissue functions through influencing cell differentiation by changing their biochemical activity and cellular growth, and regulating their rate of proliferation. They act on target tissues in both diffusible (endocrine, autocrine and paracrine) and non-diffusible (juxtacrine or metacrine) manners and regulate a variety of cellular events including cell migration, survival, adhesion, proliferation and differentiation [48]

Growth factors provides biochemical cues for stem cell differentiation and are used to develop novel strategies to treat human diseases by investigating cellular processes which controls development, aging, and tissue regeneration. Modulation of growth factors at the injury site is one of the strategies to stimulate tissue regeneration.[51, 52.]

Some of the clinically approved growth factors are-

- FIBROBLAST GROWTH FACTOR (FGF): for tissue regeneration of skin, blood vessel, muscle, adipose, tendon/ligament, cartilage, bone, tooth, and nerve.
- PLATELET-DERIVED GROWTH FACTOR-BB (PDGF-BB): for diabetic neuropathic ulcers and periodontal defects.
- BONE MORPHOGENIC FACTOR -2 (BMP2): tissue regeneration at site of tibia fracture and non union

- BONE MORPHOGENIC FACTOR-7 (BMP-7): tissue regeneration in tibia non union

Roles of Growth Factors in Differentiation of Stem Cells

Growth Factors	Sub Types	Functions
Activin/Inhibin	Activin A, Activin B, Inhibin	• Mesodermal induction • Neural cell differentiation
BMPs	BMP-2, BMP-2a, BMP-3, BMP- 3b, BMP-4, BMP-5, BMP-6, BMP-8b, BMP-10	Bone formation • Induction of ventral mesoderm
FGF	FGF acidic, FGF basic, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-12, FGF-16, FGF-17, FGF-18, FGF-19, FGF-20, FGF-21, FGF-22, FGF-23	Cell proliferation, differentiation and migration • Embryonic development and angiogenesis
IGF	IGF-I, IGF-II	• Maintenance of pluripotentency, differentiation and proliferation of myeloid cells • Promotion of neural stem cell self renewal, neurogenesis and cognition
TGF- beta	TGF-β1, TGF-β2, TGF-β3	• Maintenance and differentiation of embryonic stem cells and somatic stem cells
Wnt	Wnt-1, Wnt-2, Wnt-7a	• Cell survival, proliferation and polarity • Tissue homeostasis, tissue patterning and cell fate

There are multiple "super families" of growth factors that contain multiple subfamilies of proteins, all with related primary sequences.. Such super families may themselves comprise several subfamilies, each with multiple sub members (each of which is encoded by a distinct gene). For instance, the fibroblast growth factor (FGF) superfamily contains at least 22 distinct members. The TGFβ superfamily contains at least 35 known members that fall into about 10 subfamilies. One of these subfamilies, the bone morphogenic proteins (BMP's) is comprised of at least 15 different gene products. The neurotrophins superfamily contains but 4 members.

"Classical" Growth Factors

- EGF - Epidermal Growth Factor
- FGF - Fibroblast Growth Factor
- NGF - Nerve Growth Factor
- TGFβ - Transforming Growth Factor Beta
- INSULIN & IGF'S (INSULIN-LIKE GROWTH FACTORS)
- PDGF- Platelet Derived Growth Factor

Additional Growth Factor

- FAMILIES WITH ROLES IN DEVELOPMENT
- HEDGEHOG PROTEINS
- WNT'S
- INTERLEUKINS
- SLIT'S
- NETRINS
- EPHRINS
- TUMOR NECROSIS α FAMILY (TNFα'S)

Not a single growth factor can directly differentiate one cell type, each factor has a unique effect that may result from directed differentiation and/or cell selection.

We have learned that there are three categories of growth factors in which they can be divide according to their overall effect:

1-Growth factors (Activin-A and TGFB1) that mainly induce mesodermal cells
2-Growth factors (retinoic acid, EGF, BMP-4 and bFGF) that activate ectodermal and mesodermal markers.
3-Factors (NGF and HGF) that allow differentiation into three embryonic germ layers, including endoderm.

This analysis directs differentiation of human ES cells in culture and indicates that multiple human cell types may be enriched in vitro by specific factors [52]

Growth factors incorporation into scaffold

Growth factors attaches to the surface of scaffold following manufacture through the use of functional group to chemically attach the proteins and/or drugs. (Chen et al 2006) used this method to attach basic fibroelastic growth factors (bGFG) to the surface of alginate beads via an -NH functional group. This scaffold provides a microenvironment permissive for the growth and differentiation of human of human neuronal stem cells prior to their use in tissue engineering procedures. The function of growth factor incorporation can be enhanced by zoning, offering an interesting way of controlling tissue integration and development, which potentially allows the regionalized release of proteins to act on specific cell populations or initiate physiological processes, i.e. angiogenesis, at particular sites throughout scaffolds.. An alternative to growth factor incorporation is to integrate DNA plasmids encoding a gene and mammalianpromoter into the polymer; transfection with the DNA programmes the cells to produce their own growth factors.[48, 50]

Once optimized, changing the inserted gene to alter the growth factor produced would allow a range of factors tobe produced; however, uptake rates and toxicity are still major issues to this promising technique (Heyde et al. 2007).

Bioreactors

A bioreactor is a device that uses mechanical means to influence biological processes. In tissue engineering bioreactors can be used to aid in the in vitro development of new tissue by providing biochemical and physical regulatory signals to cells and encouraging them to undergo differentiation and/or to produce extracellular matrix prior to in vivo implantation.

Applications

Bioreactor technologies can be used to grow functional cells and tissue for transplantation.

It is used in In vitro studies on the regulation effect of biochemical and biomechanical factors on cell and tissue development.
These systems are used to establish spatially uniform cell distributions on three dimensional scaffolds, to maintain desired concentrations of gases and nutrients in the culture medium, and to expose developing tissue to appropriate physical stimuli.

Requirements of bioreactor

The requirements for a FTE bioreactor will vary depending on the dimensions, complexity, and physiological environment of the tissue to be engineered.

The requirement of a bioreactor is to have systems that reliably and reproducibly form, store, and deliver functional tissues that can sustain function in vivo.

The bioreactor needs to provide the appropriate physical stimulation to cells, continuous supply of nutrients (e.g. glucose, amino acids), biochemical factors and oxygen, diffusion of chemical species to the construct interior, as well as continuous removal of by-products of cellular metabolism (e.g. lactic acid).

A bioreactor has to be able to operate over long periods of time under aseptic conditions since maturation of a functional tissue may take up to 3- 4 months.

Designing and processing of bioreactor

Important issue in the design of FTE bioreactors is the monitoring of tissue growth. Minimizing variability of growth conditions does not necessarily result in perfectly uniform growth between batches and, therefore, it is necessary to monitor growth during culture to ensure that the harvest time is optimal for each batch.

The monitoring method is likely to be individualized for each tissue, although the monitoring of glucose uptake has been used successfully in the tissue engineering of different tissues.

- In order to induce cell growth in the third dimension and to support tissue development, it is critical to provide mass transport to and from all cells using dynamic culture systems such as bioreactors
- In static cultures, mass transport is based on diffusion, and generally limits tissue development to thicknesses less than 0.2 mm due to drops in oxygen tension and increased concentrations of toxic metabolites (e.g., acidification).
- In bioreactors, stirring, perfusion, and dynamic loading have been applied to provide convective transport and allow tissue development on a millimeter to centimeter scale (Chahine et al. 2009; Grayson et al. 2010; Grayson et al. 2011; Hofmann et al. 2007; Marolt et al. 2006).

Limitations / Challenges of a bioreactor

Challenges also remain in the area of bioreactor fabrication, operation, and medical regulation. Many of the bioreactor systems implemented up to date are at the development stage, and have been tested in stem cell research studies but not in basic biological studies.

Closer collaboration between fields is expected to widen the opportunities for applying and optimizing bioreactor systems, and help disseminate user-friendly devices that can be easily and robustly operated.

In the meantime, because bioreactors are often custom-designed, and because cells are highly sensitive to changes in their environment, even minute changes in molecular and biophysical cues may have implications on the reproducibility of the bioreactor regulation of cell survival, behaviour, and differentiation. [53]

Scope of Stem cells

There is an unleashed vista when it comes to deliberation of stem cells. This is an ever-growing field of stem cell research where in biological stem cells or cells which inherit the stem like properties or so called stemness are under rigorous exploration.

This paper would be falling short if the cancer stem cells are not mentioned. The cancer stem cells, are a group of clonally derived cells from a tumor specimen which display the following characteristics: (1) self-renewal and proliferation, (2) Differentiation and expression of markers typical of the end terminal cells of an organ (i.e. markers for astrocytes, oligodendrocytes, and neurons in the case of brain tumor stem cells), and (3) tumor recurrence, invasion and metastasis [54]. Major categories of cancers from which cancer stem cells are derived are represented: leukemia stem cells, brain cancer stem cells, prostate cancer stem cells, pancreatic cancer stem cells, head and neck cancer stem cells, and pituitary adenoma stem cells. Currently the cancer stem cells are posing biggest challenge for oncologist as these are resistant to the standard chemotherapy and radiotherapy regimen.

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Cancer Stem Cells in Oral Cavity Squamous Cell Carcinoma: A Review

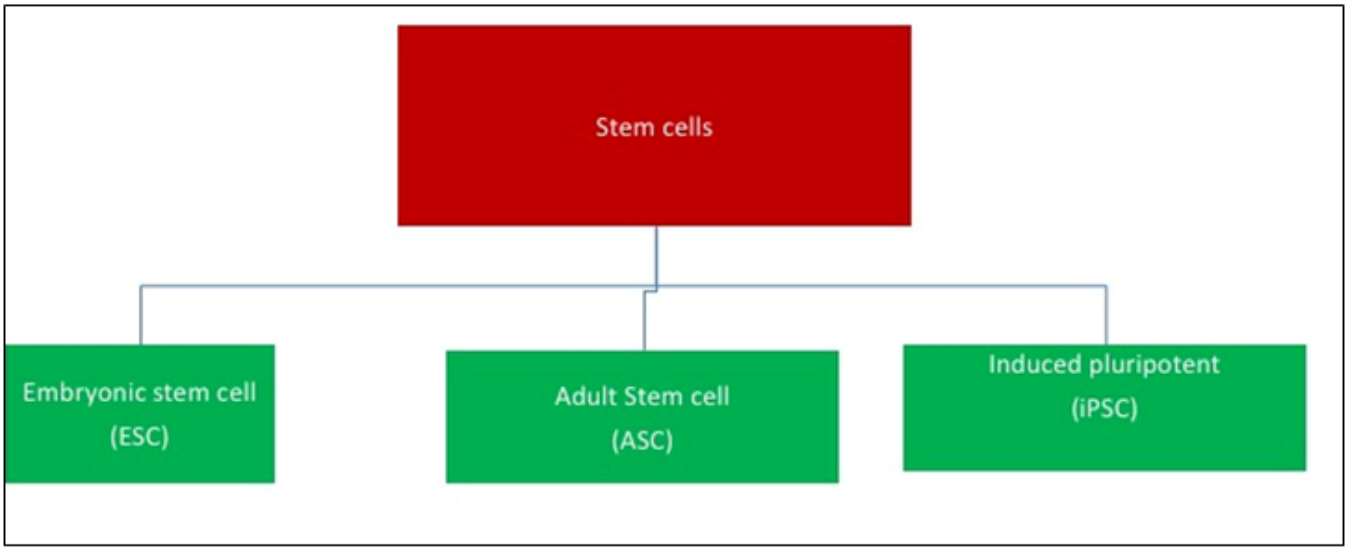

Figure 1: Broad classification of stem cells

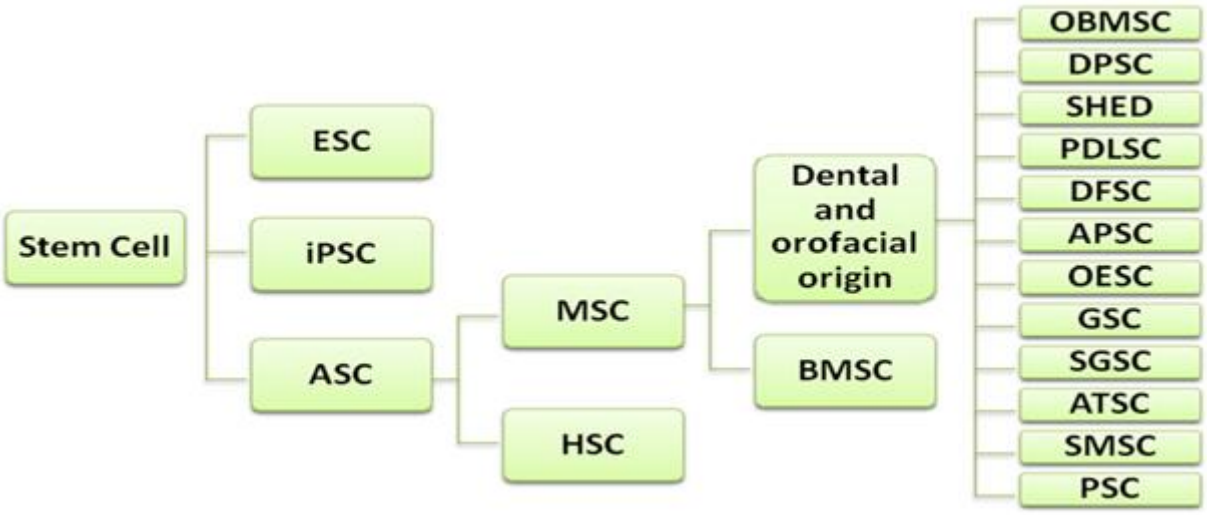

Figure 2: Stem cell lineage