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Stem Cells – Emerging Concepts in Dentistry

Pooja Prasad[1]*, Rajiv Desai[1], Shivani Bansal[1], Pankaj Shirsat[1] and Praveen Raipure[2]

[1]*Department of Oral Pathology, Nair Hospital Dental College, India*
[2]*Department of Prosthodontics, Nair Hospital Dental College, India*

***Corresponding author**

Pooja Prasad, Assistant Professor, Room no.501, Department of Oral Pathology, Nair Hospital Dental College, Dr. A L Nair road, Mumbai Central, Mumbai–400008, India, Email: drprasadopath@gmail.com

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Abstract

Human stem cells have a major effect in the development of human bodies. Acting as an *in vitro* model, human stem cells have a great value in the study of the mechanism of individual development and in the cell replacement and regeneration treatments and gene therapy of tissue deficiency diseases.

This article gives a brief overview on recent advances in the field of stem cell research and its advancement in enhancing the field of tissue engineering especially pertaining to advanced dental treatment modalities; encompassing tooth, bone, cartilage, skeletal tissue and other variants of tissue engineering.

INTRODUCTION

Stem cells are one of the most fascinating and attain a large focus of attention in today's biomedical world. Research on stem cells raises scientific questions as rapidly as it generates new discoveries like many other expanding fields of scientific world. There are many different pros and cons when it comes to discussing the use of stem cells. In order to develop an opinion of whether or not stem cells should be used, one must first understand what they are and how they are used?

The stem cells are defined as clonogenic unspecialized cells capable of both self renewal for long periods and multi lineage differentiation, contributing to regenerate specific tissues [1]. They may be unipotent or multipotent depending on their ability to differentiate along one or more cell lineages [1]. These are primal cells found in all multi-cellular organisms that retain the ability to renew themselves without limit through mitotic cell division and can differentiate into a diverse range of specialized cell types [2].

Research in human stem cell field grew out of findings by Canadian scientists E.A. Mcculloch and J. E. Till in 1960's [3].

The main principles that define stem cell populations are their self renewal property which helps in maintenance of somatic homeostasis and participation in tissue regeneration and repair. It is believed that stem cells form reservoirs of repair cells to replace cells and tissues that degenerate over the life span of the organism by generating replacements for cells that are lost through normal wear, injury or disease. It is now generally accepted that self-maintaining stem cells exist in all the renewing tissues of the body [4].

It has the potential to repair and regenerate dental tissues like dentin, tooth, bone, cartilages, skin, adipose tissues, and glands. The research of stem cells in scientific and therapeutic potential in oro-facial diseases is yet not reached its pinnacle but future is flamboyant in regenerative dentistry [5,6].

PROPERTIES OF STEM CELLS

Stem Cells are very unique cells and have an amazing ability to develop into several distinct cell types in the body, thus, aiding as a repair system for the body. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function (i.e. a muscle cell, a red blood cell, a brain cell, etc.) [7]. All Stem Cells have the same three general properties as follows:

- Stem Cells can divide and renew themselves for long periods of time
- Stem Cells are unspecialized
- Stem Cells can divide and become specific specialized cell types of the body

To elaborate it can be discussed under following heads:

Self-renewal - The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.

Unlimited potency - The capacity to differentiate into any mature cell type.

Potency specifies the differentiation potential of the stem cell, thus classifying them according to their potency

- Totipotent stem cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few

divisions of the fertilized egg i.e., Early (1-3 days) embryo is totipotent. These cells can differentiate into embryonic and extra embryonic cell types.

- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into cells derived from the three germ layers. These cells are derived from inner cell mass of blastocyst (5 to 14 days).
- Multipotent stem cells can produce only cells of a closely related family of cells (e.g. hematopoietic stem cells differentiate into red blood cells, white blood cells, platelets, etc.**).** Fetal tissue, cord blood, and adult stem cells.
- Unipotent cells can produce only one cell type, but have the property of self-renewal which distinguishes them from non-stem cells [8].

Stem cells are unspecialized

It does not have any tissue-specific structures that allow it to perform specialized functions. However, unspecialized stem cells can give rise to specialized cells. The process is called differentiation. Differentiation is controlled by an array of internal signals and external signals. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. Adult stem cells typically generate the cell types of the tissue in which they reside. [8,9]

Lineage

To ensure self-renewal, stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell. [10]

SOURCES OF STEM CELLS

The stem cells are obtained from the following tissues [11]

- **Embryonic stem cells** – These are harvested from the inner cell mass of the blastocyst seven to ten days after fertilization.
- **Fetal stem cells** - are taken from the germ line tissues that will make up the gonads of aborted fetuses.
- **Umbilical cord stem cells** - Umbilical cord blood contains stem cells similar to those found in bone marrow.
- **Placenta derived stem cells** - up to ten times as many stem cells can be harvested from a placenta as from cord blood.
- **Adult stem cells** - Many adult tissues contain stem cells that can be isolated.

Characteristics and differences between embryonic and adult stem cells [12,13].

Embryonic Stem Cells	Adult Stem Cells
Derived from leftover embryo in very stage of development.	Derived from few fully developed tissues and organs.
Can proliferate for longer period in laboratory without differentiating	Cannot proliferate for longer period
It can be used to create large number of unlimited stem cells.	It produces only certain types of specialized cells.
As it can produce unlimited stem cells, it can be used for reversing or terminating of the disease.	It can be used for repair of tissues and organs from which they are derived. But on a limited extent.
It can replicate itself outside the body i.e. It can be cultured, thus, can be used in stem cell transplants.	It cannot grow outside the body and method of expanding them is unknown.

STEM CELL LINE

A stem cell line is a family of constantly-dividing cells, the product of a single parent group of stem cells. They are obtained from human or animal tissues and can replicate for long periods of time *in vitro* ("within glass"; or, commonly, "in the lab", in an artificial environment) [14,15].

EMBRYONIC STEM CELLS

Embryonic stem cells (ES cells) were first derived from mouse embryos in 1981 by Martin Evans and Matthew Kaufman and independently by Gail R. Martin who is also credited with coining the term 'Embryonic Stem Cell [16].

Embryonic stem cells are undifferentiated cells that have the ability to form any adult cell. An embryonic stem cell (ES cell) is derived from the blastocyst stage of the embryo. [9]

Properties of an Embryonic Stem Cell [13]

a. *Capable of undergoing an unlimited number of symmetrical divisions without differentiating (long-term self-renewal).

b. Exhibit and maintain a stable, full (diploid), normal complement of chromosomes (karyotype).Pluripotent ES cells can give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm).

c. *,** Capable of integrating into all fetal tissues during development. (Mouse ES cells maintained in culture for long periods can still generate any tissue when they are reintroduced into an embryo to generate a chimeric animal.)

d. *,**Capable of colonizing the germ line and giving rise to egg or sperm cells.

e. Clonogenic that is a single ES cell can give rise to a

colony of genetically identical cells, or clones, which have the same properties as the original cell.*

f. Expresses the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state.

g. Can be induced to continue proliferating or to differentiate.

h. Lacks the G1 checkpoint in the cell cycle. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, ES cells do not require any external stimulus to initiate DNA replication.

i. Do not show X inactivation. In every somatic cell of a female mammal, one of the two X chromosomes becomes permanently inactivated. X inactivation does not occur in undifferentiated ES cells.

j. [*- Not shown in human EG cells. **- Not shown in human ES cells. All of the criteria have been met by mouse ES cells.]

Sources of Embryonic Stem Cells

The isolation and culture of human embryonic stem cells (hereafter referred to as hes cells) and human embryonic germ cells (hereafter referred to as heg cells) can be derived from these sources:

i. Blastocysts remaining after infertility treatments and donated for research [11].

ii. Primordial gonadal tissue obtained from cadaveric fetal tissue or blastocysts generated from donated gametes (oocytes and sperm) [18]

iii. Products of a technique called somatic cell nuclear transfer or, more simply, nuclear transfer (NT) [16,17]

iv. Cadaveric fetal tissue is the only source of heg cells.

Hes and heg cells offer remarkable scientific and therapeutic possibilities, involving the potential for generating more specialized cells or tissue. This could allow the generation of new cells to be used to treat injuries or diseases involving cell death or impairment, such as Parkinson's disease, diabetes, heart disease, spinal cord injury, and hematologic and many other disorders.

v. *In vitro* Fertilization

The IVF technique could potentially also be used to produce blastocysts specifically for research purposes. This would facilitate the isolation of stem cells with specific genetic traits necessary for the study of particular diseases [11].

Potential Uses of Human Embryonic Stem Cells

The most-often discussed is their potential use in transplant therapy i.e., to replace or restore tissue that has been damaged by disease or injury [19].

1. Using Human Embryonic Stem Cells for Therapeutic Transplants:

2. Human ES cells could be used to study early events in human development.

3. Human ES cells could also be used to test candidate therapeutic drugs.

4. Finally, human ES cells could be used to develop new methods for genetic engineering

Adult Stem Cells

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Adult stem cells can be obtained from various tissues of adults or in some cases from neonatal tissues [20].

1. **8.1 Types of Adult Stem Cells**: **Adipose Derived Adult Stem Cells**

Adipose-derived stem cells (ASCs) have also been isolated from human fat, usually by method of liposuction. Human ASCs have been shown to differentiate in the lab into bone, cartilage, fat, and muscle, while ASCs from rats have been converted to neurons [21].

2. **Hematopoietic Stem Cells**

Hematopoietic stem cells give rise to all the blood cell types and are found in the bone marrow [20].

3. **Neural Stem Cells**

Neural stem cells are commonly cultured *in vitro* as so called neurospheres - floating heterogeneous aggregates of cells, containing a large proportion of stem cells [21,22].

Neural stem cells are used in cases of stroke, brain injury, and spinal cord injury. The neural stem cell, when injected into a damaged area of the brain, can restore healthy functioning to that area. Neurological disorders such as Parkinson's disease and Alzheimer's disease have also benefitted from neural stem cell research and therapy.

4. **Mammary Stem Cells**

5. Mammary stem cells provide the source of cells for growth of the mammary gland during puberty and gestation and play an important role in carcinogenesis of the breast [20].

Mesenchymal Stem Cells

Mesenchymal stem cells differentiate into connective tissue, and are found in the bone marrow [20].

Olfactory Adult Stem Cells

Adult stem cells isolated from the olfactory mucosa (cells lining the inside of the nose involved in the sense of smell) have the ability to develop into many different cell types if they are given the right chemical environment.

Olfactory stem cells hold potential for therapeutic applications. Thanks to their location they can be harvested with ease without harm to the patient in contrast to neural stem cells [23].

TESTICULAR STEM CELLS

Multipotent stem cells with a claimed equivalency to embryonic stem cells have been derived from spermatogonial progenitor cells found in the testicles of laboratory mice. Multipotent stem cells have also been derived from germ cells found in human testicles [20].

DENTAL PULP DERIVED STEM CELLS

Another mineralized tissue that has a great deal of similarity to bone is dentin. Although dentin is not turned over throughout life, as is bone, limited dentinal repair in the postnatal organism does occur. It was postulated that a precursor population maintains the ability for limited repair, associated with pulp tissue that has the ability to mature into odontoblasts. Clonogenic and highly proliferative cells have been derived from enzymatically disaggregated adult human dental pulp, which have been termed dental pulp stem cells (DPSCs) that form sporadic, but densely calcified nodules *in vitro* [24].

Human DPSCs and Bone marrow stem cells (BMSCs) were found to have a similar level of gene expression for more than 4,000 known genes represented on the filter. A few differentially expressed genes including collagen type XVIII alpha 1, Insulin-like Growth Factor 2, Discord in Domain Tyrosine Kinase 2, NAD (P) H menadione oxidoreductase, homolog 2 of Drosophila large disk, and cyclin-dependent kinase 6 were highly expressed in DPSCs, while insulin-like growth factor binding protein 7 and collagen type I alpha 2 were more highly expressed in BMSCs [25].

Limitations of Adult Stem Cells

1. However, adult cells are already specialized, their potential to regenerate damaged tissue is very limited: skin cells will only become skin and cartilage cells will only become cartilage.

2. Adults do not have stem cells in many vital organs, so when those tissues are damaged, scar tissue develops. Only embryonic stem cells, which have the capacity to become any kind of human tissue, have the potential to repair vital organs.

3. Another limitation of adult stem cells is their inability to proliferate in culture. Therefore, obtaining clinically significant amounts of adult stem cells may prove to be difficult [20].

ROLE OF STEM CELLS IN DEVELOPMENT & REPAIR OF DENTAL AND OROFACIAL STRUCTURES

Periodontium

Regenerating the periodontium has always been a high priority in craniofacial regenerative biology. Due to the complex structure of the periodontium (consisting of hard and soft tissues: cementum, bone, periodontal ligament, and gingiva), its complete regeneration would require a multipotent cell population [26] demonstrated that transplantations of *ex vivo* expanded autologous Mesenchymal Stem Cell (MSC) can regenerate new cementum, alveolar bone, and periodontal ligament in class III periodontal defects in dogs.

Mandible

Autologous bone grafts have been a 'gold standard' in craniofacial reconstruction. However, donor site morbidity and a limited quantity/supply are still substantial hurdles with this method. Bone tissue engineering can fully replace lost bone tissues through the use of three-dimensional biodegradable scaffold materials carrying osseous progenitor cells and bioactive agents (growth factors, hormones, etc.) [27].

Condyle

The cartilaginous and osseous structures of the Temporo Mandibular Joint (TMJ) can deteriorate because of injuries, rheumatoid arthritis, and osteoarthritis. Tissue engineering of the TMJ can overcome drawbacks of joint replacement such as immunologic rejection, donor site morbidity, transmission of pathogens, or metal loosening [27]

Tooth [28,29]

Duailibi et al [28] reported significant progress toward the creation of tissue-engineered embryonic tooth primordia (tooth buds) using cultured cells. In a mouse model, they tested different mixtures of non-dental-derived mesenchymal cells (embryonic stem cells, neural stem cells, and adult bone marrow cells) with embryonic oral epithelium cells. These mesenchymal–epithelial mixtures were transplanted into the renal capsules of adult mice. All mixtures resulted in the development of a tooth structure and bone. They observed that the host tissues make no contribution to the donor tissue. Moreover, transfer of embryonic tooth primordia into the adult jaw resulted in the development of tooth structures, showing that an embryonic primordium can develop in its adult environment.

In vitro control of the shape of the tissue-engineered dental primordia will be a crucial step to bring this therapy to the clinic [29].

A tooth germ can be created *in vitro* after co-culture of isolated epithelial and mesenchymal stem cells. This germ could be implanted into the alveolar bone and finally develop into a fully functional tooth.

ROLE OF STEM CELLS IN DENTIN REGENERATION

The dentine–pulp complex displays exquisite regenerative potential in response to injury. The postnatal dental pulp contains a variety of potential progenitor/stem cells, which may participate in dental regeneration. A population of multipotent mesenchymal progenitor cells known as dental pulp stem cells with high proliferative potential for self-renewal has been described and may be important to the regenerative capacity of the tissue. Characterization of these cells and determination of their potentialities in terms of specificity of regenerative response will form the foundation for development of new clinical treatment modalities, whether involving directed recruitment of the cells and seeding of stem cells at sites of injury for regeneration or use of the stem cells with appropriate scaffolds for tissue engineering solutions. Such approaches will provide an innovative and novel biologically based new generation of clinical treatments for dental disease [30].

Migration of stem/progenitor cells to the site of injury for differentiation into a new generation of odontoblasts-like cells will be an important event for cell recruitment during regeneration when the vitality of the primary odontoblasts is compromised. Evidence that such migration occurs is provided from the wealth of studies reporting that reparative dentinogenesis and dentine bridge formation occur during pulp capping procedures. Evidence that such migration occurs is provided from the wealth of studies reporting that reparative dentinogenesis and dentine bridge formation occur during pulp capping procedures. Exciting possibilities exist to exploit this aspect of regeneration, both in respect of maximizing recruitment of progenitor cells and also, perhaps through influencing the nature of the cell populations recruited. If the necessary chemotactic signals for specific cell populations can be determined, this could be harnessed for directed recruitment of those cells to provide greater specificity to the tissue response. [30]

THE PULP TISSUE: NICHES OF STEM/PROGENITOR CELL POPULATIONS

Recently, Gronthos et al have attempted to characterize a unique population of postnatal human dental pulp stem cells (DPSCs) [25]. These cells showed capacity for self-renewal and differentiation into odontoblast-like cells, which formed the dentine matrix with some tubular features *in vivo.* The same group has also identified a potential mesenchymal stem cell population derived from exfoliated deciduous human teeth (SHED) [31], capable of extensive proliferation and multipotential differentiation.

Further attempts to identify a stem cell niche in the dental pulp suggested that the putative stem cell marker, STRO-1 was expressed by dental-derived stem cells using immunomagnetic activated cell selection. It has also been reported that DPSCs express the perivascular cell marker CD146, and a proportion of these cells also positively co-expresses α smooth muscle actin and the pericyte-associated antigen3 G5. These findings concur with co-localization studies of these markers to perivascular cells *in situ*, and it is possible that a population of DPSCs may reside in this perivascular niche within the adult pulp derived from outside the tooth [31].

CONCLUSION

Human stem cells have a major effect in the development of human bodies. Acting as an *in vitro* model, human stem cells have a great value in the study of the mechanism of individual development and in the cell-replacement treatments and gene therapy of tissue deficiency diseases. Scientists theorize that virtually every tissue in the body contains some type of stem cell. From there it is possible to consider the idea of medical and specifically dental cell-based strategies for tissue repair. The current focus on self-renewing stem cells provides the opportunity to understand the process of tissue turnover. This leads us to the cutting edge of stem cell research as it applies to dentistry.

Baby and wisdom teeth, along with jawbone and periodontal ligament, are non-controversial sources of stem cells that could be "banked" for future health needs.

Biological engineering will one day provide the nanotools required switching pain signals off in chronic pain situations or dysregulate the quorum sensing required to form dental biofilms. Biological engineering will also yield a remodeling of the cell surfaces to render malignant cells more susceptible to powerful new and highly specific, therapeutics.

It is opined that scientists have exaggerated the immediacy of the prospects of clinical therapies using stem cells, and that this has led to public misunderstanding. Clinical application is a long way off for at least two reasons. Prior to clinical use of embryonic and fetal stem cells, it will be necessary to thoroughly investigate the malignant potential of embryonic stem cells. In addition, a much more comprehensive elucidation of the immune response is necessary to provide the basis to prevent transplanted stem cells and their progeny from being rejected by the transplant recipient. Although science is moving rapidly, these complexities of malignancy and immunology provide us considerable time to ponder the ethical considerations that would accompany clinical use of embryonic and cord blood stem cells [32].

However in coming times, dental scientists can look forward to the development of an enamel-like bio-material fabricated in the shape of a patient's tooth that needs to be replaced. And beyond this, it just may be possible to one day recreate a tooth bud in the laboratory -- then transfer it into the patient's jaw to develop, grow, and erupt on its own.

Although stem cell research is on the cutting edge of biological science today, it is still in its infancy. In conjunction with research on stem cell biology and the development of potential stem cell therapies, research on approaches that prevent immune rejection of stem cells and stem cell-derived tissues and associated issues should also be actively pursued.

The possibilities are endless.....it only needs careful execution, determination and most importantly more insight into the human complexities, both in physiology as well as pathology.

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