Review Article

Stem cells: A potential regenerative future in dentistry

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In recent years, the field of dentistry has embossed its presence by taking major leaps in research and further bringing it into practice. The most valuable ongoing research in regenerative dentistry is the study on stem cells. It was instituted that stem cells grow rapidly and have the potential to form specialized dentin, bone, and neuronal cells. These neuronal cells can be used for dental therapies and can provide better treatment options for patients. The stem cells based therapies could help in new advances in treating damaged teeth, inducing bone regeneration and treating neural injury as well.

Key words: Dentistry, genetics, periodontal regeneration, stem cells

Introduction

Judging from the explosion of articles not only in scientific journals, but also in the mass media and on the internet, one could say the term "*stem cells*" has become linked to the word "cure."[1]

In the face of extraordinary advances in the prevention, diagnosis, and treatment of human diseases, devastating illnesses such as heart disease, diabetes, cancer, and diseases of the nervous system, such as Parkinson's disease and Alzheimer's disease, continues to deprive people of health, independence, and well-being. Research in human developmental biology has led to the discovery of human stem cells.

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.100749

Stem cells are primitive cells found in all multi-cellular organisms that are characterized by self-renewal and the capacity to differentiate into any mature cell type. These stem cells have the awesome potential for regeneration and may be used to replace or repair damaged cells, and have the potential to drastically change the treatment of conditions like cancer, Alzheimer's and Parkinson's disease and even paralysis.

There are 2 main types of stem cells – embryonic stem cells and adult stem cells – which are classified according to their origin and differentiation potential.[2]

Mesenchymal stem cells (MSCs), a type of the adult stem cells that can be harvested from bone marrow and other sources such as liver, umbilical cord, placenta, adipose tissue, synovial membrane, amniotic fluid and even teeth,[3] have increasingly played a central role in regenerative medicine. Their attractiveness is found in their multipotency to differentiate and develop into various types of tissues such as adipose, cartilage, and bone,[4] as well as their promising use in patient-specific gene therapy.[3]

Stem cells are defined as having the capacity for extensive self-renewal and for originating at least one type of highly differentiated descendant.[5]

Research in the stem cell field grew out of findings by Canadian scientists Ernest A. McCulloch and James E. Till in the 1960.[6,7]

Stem cells are cells that have the following capabilities: First, they are able to continuously produce daughter cells having the same characteristics as themselves (self-renewal); secondly, they can generate daughter cells that have different, more restricted properties, and finally, they can re-populate a host *in vivo* (differentiation).[8]

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Sources of Stem Cells

There are many potential sources for stem cells.

1. *Embryonic stem (ES) cells* are derived from the inner cell mass of a blastocyst from a 4 or 5 days old embryo.
2. *Embryonic germ (EG) cells* are collected from fetal tissue at a somewhat later stage of development (from a region called the gonadal ridge).
3. *Adult stem cells* that are derived from mature tissues and are found in adult tissues. They act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.[9]

The development of mouse ES cells in 1981 provided the paradigm and much of the technology, for the development of human ES cells, but the concept of a pluripotent embryonic cell is far older than that.[10] As human ES cells can produce most, if not all, the differentiated cell types in a human body, they may provide unlimited cell resources for cell therapy. However, these require intensive studies for better understanding human ES cell properties before they could be used in the clinical applications. In 1992, Brigid Hogan[10] and her colleagues reported the direct derivation of EG cells from mouse primordial germ cells. These EG cells have a developmental capacity, very similar to that of ES cells, though they differ in their expression of some imprinted genes. The mouse ES cell provides a benchmark for definition of the generic requirements for ES cells. Its key features are these: It is derived from a pluripotent cell population; it is stably diploid and karyotypically normal *in vitro*; it can be propagated indefinitely in the primitive embryonic state; it can differentiate spontaneously into multiple cell types representative of all 3 embryonic germ layers, both in teratomas after grafting or *in vitro* under appropriate conditions; and it can give rise to any cell type in the body, including germ cells, when allowed to colonize a host blastocyst.[10]

Generic Criteria for Pluripotent Embryonic stem or Embryonic germ cells

1. Originate from a pluripotent cell population
2. Maintain normal karyotype
3. Immortal and can be propagated indefinitely in the embryonic state
4. Clonally-derived cultures capable of spontaneous differentiation into extra-embryonic tissue and somatic cells representative of all 3 embryonic germ layers in teratomas or *in vitro*.[10]

Thus far, only mouse EG or ES cells meet these generic criteria. Primate ES cells meet the first 3 criteria's, but not the last.

Growth of Stem Cells in the Laboratory

Growing cells in the laboratory is known as cell culture. Human embryonic stem cells are isolated by transferring the inner cell mass into a plastic laboratory culture dish that contains a nutrient broth known as culture medium. The cells divide and spread over the surface of the dish and form clonogenic adherent cell clusters with a fibroblastic morphology (colony-forming units-fibroblast [CFU-F]) *in vitro*. The inner surface of the culture dish is typically coated with mouse embryonic skin cells (feeder layer). The reason for having the mouse cells in the bottom of the culture dish is to give the inner cell mass cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium.[8] Recently; scientists have begun to devise ways of growing embryonic stem cells without the mouse feeder cells because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells by adding a single cytokine, leukemia inhibitory factor (LIF) into the culture medium could sustain mouse ES cell self-renewal in the absence of feeders.[11,12] Periodontal ligament stem cells (PDLSCs) were identified as a specific MSC population derived from periodontal ligament with expression of array of osteogenic markers like alkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), and osteocalcin; mesenchymal stem cell marker STRO-1; and tendon marker scleraxis. Also, human ES cells can be maintained on stromal cells in the presence of basic fibroblast growth factor (b-FGF).

Characteristics of Mesenchymal Stem Cells

MSCs are described as multipotent because of their ability, even as clonally isolated cells, to exhibit the potential for differentiation into a variety of different

cells/tissue lineages. MSCs have the ability to differentiate along specific mesenchymal lineages and when induced to do so, to remain in a quiescent undifferentiated state until provided the signal to divide asymmetrically, and finally, to undergo many more replicative cycles than normal, fully-differentiated cells.[13]

Properties of Stem Cells

All primate pluripotent stem cells grow in more rounded clumps with indistinct cell borders express alkaline phosphatase activity. In humans, there are 4 different isozymes of alkaline phosphatase. EC cells express the tissue non-specific form and a form of the enzyme that can be detected by antibodies that react with the germ cell or placental form.[8] The pluripotent cells require a mouse embryonic fibroblast feeder-cell layer for support. In the case of mouse ES and EG cells, this requirement can be replaced by LIF or by related members of this cytokine family, but pluripotent human EC cells, rhesus monkey ES cells, and human ES cells will not respond to LIF in such a fashion.[10]

Periodontal Ligament Stem Cells

The concept that stem cells may reside in the periodontal tissues was first proposed almost 20 years ago by Melcher. Since periodontal regeneration is essentially a re-enhancement of the development process including morphogenesis, cytodiferentiation, extracellular matrix production and mineralization, such processes support the concept that some mesenchymal stem cells remain within the periodontal ligament and are responsible for tissue homeostasis. These mesenchymal stem cells serve as a source of renewable progenitor cells generating cementoblasts, osteoblasts, and fibroblasts throughout adult life. The periodontal ligament stem cell cultures exhibit approximately 30% higher rates of proliferation compared to the growth of cultured bone marrow stromal stem cells. It appears that these cells maintain this capacity of higher growth potential beyond 100 population doublings before *in vitro* senescence is noted.[14]

The putative stem cell marker, STRO-1, used to isolate and purify bone marrow stromal stem cells, is also expressed by human periodontal ligament stem cells and dental pulp stem cells.[14]

Clinical Application of Stem Cells

Besides in other fields of medicine, mesenchymal stem cells play an important role in the field of dentistry as it could help in regeneration of vital structures like bone, cementum, periodontal ligament fibers, and dental pulp.

The regeneration of bone is a key issue at the forefront of current tissue engineering applications, owing to the ease of use and accessibility of osteoprogenitor cells. The use of natural and synthetic biomaterials as carriers for MSC delivery has shown increasing promise for orthopedic therapeutic applications, especially bone formation. Recent advances in the field of biomaterials have led to a transition from non-porous, biologically inert materials to more porous, osteoconductive biomaterials, and, in particular, the use of cell-matrix composites. A number of delivery vehicles have been successfully used in cell-matrix composites *in vivo*, such as porous ceramics of hydroxyapatite and β-tricalcium phosphate loaded with autologous MSCs.[15]

Several craniofacial structures—such as the mandibular condyle, calvarial bone, cranial suture, and subcutaneous adipose tissue—have been engineered from mesenchymal stem cells, growth factor, and/or gene therapy approaches. Adult mesenchymal stem cells have advantages over embryonic stem cells for tissue engineering of the mandibular condyle, because adult mesenchymal stem cells (MSCs) can be obtained from the same individual and readily induced to differentiate into both chondrogenic and osteogenic cells.[16,17] Biomimetic scaffolds are frequently needed to enable cell growth and differentiation to occur in an environment that has been previously unfamiliar to either biologists or engineers.

MSCs have the potential for the regeneration of mammalian dental tissues. Bio-engineered teeth can be derived from cultured tooth bud cells. Deciduous teeth contain a population of more immature multipotent stem cells, which are capable of forming dentin-like structures but not a complete dentin-pulp complex. The developed bio-engineered tooth had a well-defined pulp chambers, odontoblasts, pre-dentin, and dentin. It also contained

a morphologically correct enamel organ consisting of stellate reticulum, stratum intermedium, ameloblasts, and dental enamel. In addition, putative Hertwig's root sheath epithelia were also present.[14,18,19] Dental pulp stem cells were capable of generating a reparative dentin-like structure directly on the surface of human dentin.[20]

It has been demonstrated that human PDL contains a population of multipotent post-natal stem cells that can be expanded *ex vivo*, providing a unique reservoir of stem cells.

The progenitor cells of the PDL appear to be morphologically heterogeneous. This confirms observations, both in osteogenic tissue and wounded PDL, that progenitors can synthesize DNA at various stages of their differentiation.

The goal of gene-enhanced periodontal regeneration is to reclaim the lost regenerative capacity within the PDL space. While gene enhanced tissue engineering can be used in conjunction with stem cells, this technique has the greatest potential if it can be adapted for use with easily harvestable fully mature cells (e.g. gingival fibroblasts, periodontal ligament fibroblasts). These cells are then genetically-enhanced to express growth factors that are involved in the initial formation of both dental and periodontal attachment tissues. In short, this approach is intended to mimic the normal biological process that occurs as these tissues are formed early in development.[21,22]

Aging of Mesenchymal Stem Cell

A number of changes occurred in physiological, functional, and molecular parameters of stem cells during long-term cultures. These changes included:

- Typical Hayflick Phenomenon of cellular aging
- Gradual decreasing proliferation potential
- Telomere shortening
- Impairment of functions

The proliferative potential of MSC decreases faster after 120 days of *in vitro* expansion.[23]

Challenges

Vast unequal resources, differential standards of public health, and uneven opportunities for health care within and between countries comprises a major hurdle in achieving substantial success in the field of genetics. The World Health Organization has reminded member states that "justice demands equitable access to genetic services" and has also stated that "Genetic services for the prevention, diagnosis, and treatment of disease should be available to all, regardless of the cost factor, and should be provided first to those whose needs are the greatest."

Along with these, funding for the purpose of research should be taken into account. The federal government is the only realistic source for such an infusion of funds. Without the stimulus of public funding, new treatments could be substantially delayed.[9]

Conclusion

Electron microscope radio-autography was used in an attempt to identify any relationship between the location and degree of differentiation of progenitor cells in the periodontal ligament (PDL). Ligament fibroblasts were classified on the basis of their nuclear/cytoplasmic ratio, and their distance to the closest blood vessel measured. It was determined that an undifferentiated paravascular progenitor cell population exists, and that the PDL also contains progenitor cells showing a range of cytodifferentiation. This demonstrated that postnatal stem cells can be retrieved from solid-frozen human periodontal ligament.

The pursuit and production of knowledge through scientific research is an undertaking that offers enormous intellectual rewards for researchers while also performing an important social function. The work of scientists is, and should be, conditioned and directed by consideration of broader human values. This means that the development of public policy, especially where highly controversial matters are involved, must take all interested sectors of the public into account. It is only through broad-based participation that the values of all stakeholders in the research enterprise can be carefully considered and weighed.

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Cite this article as: Narang S, Sehgal N. Stem cells: A potential regenerative future in dentistry. Indian J Hum Genet 2012;18:150-4.

Source of Support: Nil,**Conflict of Interest:** None declared.