Document heading doi: 10.21276/apjhs.2019.6.1.18 **Review Article**

Stem cells – the tiny procreators: a review article

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Abstract

Stem cells are known to be the most fascinating components of biology today and the basis for the research on these depends on the knowledge about how an organism develops from a single cell and also how healthy cells are capable of replacing the damaged cells in adult organisms. This area of science has led the scientists to investigate the possibility of cell based therapies to find treatment for many of the incurable diseases. This mode of treatment is referred to as regenerative/reparative medicine. But like many expanding fields of scientific enquiry, research on stem cells raises scientific questions about healing, ethics etc., as rapidly as it generates new discoveries.

Keywords: Stem Cells, SHED, Regeneration, Tissue engineering, Tissue reconstruction

Introduction

The loss of tooth is a redundant situation that occurs from numerous pathologies.[1]Since years, dentistry has been dealing with the replacement of missing teeth with the use of synthetic materials[2] but they fail to remodel with host bone leading to aseptic loosening or infection resulting in its failure.[1] So, the justification to this question is stem cells. Stem cell research is becoming a promising field for both tissue regeneration and implementation of regenerative medicine [3] and now have been introduced to the field of dentistry to treat various oro-facial problems, which have high impact not only on the facial appearance, but also on quality of life - especially on the ability to chew. In pediatric dentistry stem cell technology is an emerging field of study.

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Combined with tissue engineering techniques, it is possible that dental stem cells may be used to engineer a complete tooth one day.[4]

Discussion

Stem cells are the master cells of the body which meet the two basic conditions, one of self-replication and the other an ability to differentiate into at least two different type of cells. Russian histologist Alexander Maksimov in1908proposed the term stem cell for scientific use.[1]

These are three defining features of a stem cell:

- Stem cell 'self-renews': It undergoes cell division in which one daughter cell remains as a stem cell, while the other forms a particular cell type (a 'committed progenitor') by a process called 'asymmetric division'.
- Stem cell is multipotent cell, which means, it can form multiple cell types.

- Single stem cell has the capability to completely re-form a particular tissue when it is transplanted within the body.[5]

History of stem cells

The history on work done to recognize, and use stems cells for regenerative purposes dates back to 1970sand has been summarised in Table 1

Table 1: Historical aspect of Stem Cells

1970s	EC cells injected into mouse blastocysts make chimeric mice. Cultured Stem cells are explored as models of embryonic development in mice.[6]
1978	Haemotopoietic stem cell discovered in human cord blood.[7]
1981	Mouse Embryonic Stem cells are derived from the inner mass of blastocysts by scientist Martin Evans, Matthew Kaufman and Gail R. Martin. Mouse Embryonic Stem cells are grown in vitro. Embryonic Stem cells are injected into mice from teratomas.[7]
1984-88	Pluripotent, clonal cells called embryonal carcinoma (EC) cells are developed. When exposed to retinoic acid these cells differentiate into neuron-like cells and other cell types.[6]
1989	A clonal line of human embryonal carcinoma cells is derived that yields tissues from all the three primary germ layers. They have limited replicative and differentiative cells.[6]
1992	Neural stem cells cultured in vitro.[8]
1994	Human blastocysts are generated and the inner cell mass is maintained in culture. Embryonic Stem like cells form in the center and retain stem cell like morphology.[9]
1995-96	Non-human primate Embryonic Stem cells are maintained in vitro from the inner cell mass of monkeys. These cells are pluripotent and differentiate normally into all three primary germ layers.[9]
1998	Embryonic Stem cells from the inner cell mass of normal human blastocysts are cultured and maintained normally for many passages. Embryonal Growth cells also derived and grown in vitro.[7]
2000	Scientists derive human Embryonic Stem cells from the inner cell mass of blastocysts. They proliferate in vitro for a long time and form all three germ layers and teratomas when injected into immune deficient mice.[6]
2001	As human Embryonic Stem cell lines are shared and new lines are derived, more research groups are focusing attention on the differentiation of cells *in vitro.* Many methods focus on making human tissues for transplantation.[7]
2003	Dr.Songtao Shi discovers new source adult stem cells in primary teeth.[10]
2006	Scientists in England create the first ever artificial liver cells using umbical cord blood stem cells.[11]
2007	Scientists report discovery of a new type of stem cells in amniotic fluid which may be an alternative to embryonic stem cells for use in research and therapy.[11]

Types of stem cells

Two main types of stem cells are being investigated for their use in medicine research. They differ in the ability to self-renew and their degree of differentiation.[12]

- Embryonic Stem Cells
- Adult Stem Cells

Embryonic stem cells

These types of cells are derived from the embryos. Specifically, from the embryos that develop from eggs that have been fertilized *in-vitro* and can form nerve cells, muscle cells, and many other cell types.[13]

Adult stem cells

It is an undifferentiated cell that is found among differentiated cells in a tissue or organ, which can renew itself and can differentiate to yield the major specialized cell types of the particular tissue or organ with the primary roles being to maintain and repair the tissue in which they are found. The origin of adult stem cells in mature tissues is unknown unlike embryonic stem cells, which are defined by their origin (the inner cell mass of blastocyst).[14]

POSSIBLE SOURCES OF STEM CELLS

EMBRYONIC STEM CELL SOURCES[15]

- Embryos created via IVF (for infertility treatment or for research purposes)
- Embryos obtained through elective abortion
- Embryos created via somatic cell nuclear transfer or cloning (SCNT)

ADULT STEM CELL SOURCES[14]

- Bone marrow- bone marrow stromal cells

- Peripheral blood- peripheral blood stem cells
- Neurons- neuronal stem cells
- Muscles- muscle stem cells
- Liver- liver stem cells
- Pancreas- pancreatic stem cells
- Cornea and retina- corneal limbal stem cells
- Mammary gland- mammary stem cells
- Salivary glands
- Skin- dermal hair follicle stem cells
- Tendon
- Synovial membrane
- Heart
- Cartilage

DENTAL SOURCES OF ADULT STEM CELLS[1]

- Dental pulp dental pulp stem cells (DPSCs)
- Exfoliated deciduous teeth- SHED
- Dental follicle progenitor cells- DFPCs
- Stem cells from root apical papilla- SCAP
- Periodontal ligament stem cells- (PDLSCs)

Dental Pulp Stem Cells (DPSCs)

These are the highly proliferative and clonogenic cells that have been derived from enzymatically disaggregated adult human dental pulp, that form sporadic, but densely calcified nodules in vitro.[12]DPSCs can be differentiated into various types of cellsi.e.odontoblast, cardiomyocytes, osteoblast, chondrocyte, neuron cells, corneal epithelial cell, melanoma cell, adipocyte,and insulin secreting Beta cells.[16]

DPSC remain quiescent within the dental pulps, but the response is quick after an injury. DPSCs have high proliferative capacity and immediately differentiate into odontoblasts, osteoblasts and chondrocytes to produce dentin, bone and cartilage tissues respectively for this repair process.[16]

Stem Cell Handling and Cryopreservation

Permanent and deciduous teeth which are extracted can be preserved for future use with cryopreservation. The cells are cooled rapidly to subzero temperatures as low as -196˚, stopping any cellular or biochemical activity. Rapid freezing prevents ice from forming inside or around the cells and prevent dehydration.Stem cells derived from dental pulp of extracted third molars retain their ability to differentiate into multiple types of cells following thawing after cryopreservation. After two years of cryopreservation, stem cells have been able to differentiate and proliferate.[17]

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

Dr.Songtao Shi was able to isolate, grow and preserve tooth stem cell`s regenerative ability in 2003, by the use of deciduous teeth of his six-year-old daughter and he named them as stem cells from human exfoliated deciduous teeth (SHED).[10]SHED are unspecialized, immature cells in the teeth that are able to grow into specialized cell types by differentiation. These cells appear at about 6th week during the embryonic stage of human development.[18]

Taking developmental processes tissue, structure and function into consideration deciduous teeth are significantly different from permanent teeth. Therefore, it is not a surprise to find that SHED are distinct from DPSCs with respect to their higher proliferation rate, increased cell population doublings, osteoinductive capacity *in vivo*.[19]The best candidates for isolation for deciduous teeth are canines and incisors with the presence of healthy pulp that is starting to loosen.[10]

Stem Cell Storage

SHED can successfully be stored for long-term with cryopreservation and still remain viable for use. Cells harvested near end of log phase growth (approximately. 80–90% confluent) are best for cryopreservation. Liquid nitrogen vapour at a temperature of less than -150ºC is used to preserve these cells. This preserves the cells and maintains their latency and potency.[18]

Periodontal Ligament Stem Cells (PDLSCs)

Periodontal tissues arise from migrated neural crest cells during tooth development which connects the cementum to alveolar bone, and functions to support the tooth alveolar socket.[20] Melcher was first to propose the concept that stem cells reside in periodontal tissues.[21]PDLSCs in defined culture conditions differentiate into cementoblasts, collagen forming cells and adipocytes. These cells when transplanted generate a cementum/ PDL-like structure that contribute periodontal tissue repair.[22]When stimulated in an appropriate conditions, PDLSC can attain the formation of new bone, periodontal ligament and cementum.[23]

Preservation of PDLSC

Human PDLSCs can be recovered from cryopreserved PDLSCs and cryopreservation does not affect the growth capacity of these cells **(Seo et al., 2005; Vasconcelos et al.,2012)**.[24]These cells can be collected and saved for future use through preservation techniques which include freezing in liquid nitrogen. Periodontal ligament, preserved frozen in liquid nitrogen, have known to generated high proliferative PDLSC, although number of colonies was decreased in comparison with freshly isolated tissue samples.[25]

Stem Cells from Apical Papilla (SCAP)

A population of stem cells isolated from human teeth was found at the tooth root apex. These cells are called

stem cells from apical papilla (SCAP).[26]Initially these were isolated from third molars and incisors of swine by **Sonoyama *et al.*** and obtained from humans in 2008.[27]

A higher number of stem cells is seen in the dental papilla than mature dental pulp, therefore SCAPs have a greater potential for regenerating dentin than DPSCs.[28] This higher proliferative capability makes this population of cells suitable for cell-based regeneration and preferentially for forming roots.[26]

Dental Follicle Progenitor Cells (DFPCs)

Several studies have reported the isolation of progenitor/stem cells from Dental Follicle in different species, using an enzymatic digestion of the Dental Follicle to release cells, followed by a culture of the cells in a stem cell growth medium.[29]. In 2005 & 2007, **Morsczeck et al. and Kémoun et al**., respectively have identified unique undifferentiated lineage committed cells possessing mesenchymal progenitor features in the human dental follicle. The cells were referred to as Dental Follicle precursor cells (DFPCs).30DFSCsare characterized by a high proliferation rate, as well as expression of mesenchymal and neural stem cell markers.[29]

Preservation of SCAP and Dental Follicle Progenitor Cells

Cryopreservation is the most efficient approach to preserve cells for longer period. At ultra low temperature (-196 C), all the cellular metabolism ceases, this enabled biological materials could be store in inert condition for many years. However, during cryopreservation, cells often experience cryogenic injuries due to temperature fluctuation, cytotoxicity. Intracellular ice crystals formation is induced due to freezing and to combat this situation, agents known as cryoprotective agents (CPAs) are added to maintain cellular architecture.[31]

Clinical Application of Dental Stem Cells

For the treatment of many conditions stem cell based therapies are being investigated Currently, patients are being treated using stem cells for bone fractures, cancer and spinal fusion surgery.[17]DPSCs hold great clinical procedure due to their differentiation capacity and easy accessibility.[16]Stem cells found in primary teeth offer promise in craniofacial repair. Currently, patients are being treated using stem cells for bone fractutes, cancer and spinal fusion surgery.[17] Dental Stem Cells are capable of forming odontoblast-like cells and produce dentin *in vivo* and are likely to be the cell source of primary odontoblasts for the root dentin formation. Some of the potential clinical applications of dental stem cells are:

*De novo pulp regeneration:*When the entire pulptissue is lost, regeneration requires the *de novo* creation of pulp. To create functional pulp for clinical application, three issues must be considered: first, regenerated pulp tissue must be vascularised; second, newly differentiated odontoblasts should form on the existing dentinal wall of the root canal space; finally, new dentin must be produced by differentiated odontoblasts on the existing dentin.[16]

*Tooth reconstruction:*It may be possible to generate a method to biologically replace lost teeth with the help of Dental pulp stem cells. A functional biological replacement tooth must include generation of a root and periodontal ligament with nerve and blood supplies.[16]

*Neurology:*Dental pulp cells have been proposed for treatment for peripheral nerve injury. The dental pulp cells form blood vessels and myelinating tissue and contribute to the promotion of normal nerve regeneration.[16]

*Angiogenesis and Vasculogenesis:*Stem cells and endothelial progenitor cells (EPCs) can be utilized to stimulate vasculogenesis as a potential treatment for ischaemic disease. DPSCs and sub-fractions of DPSCs also serve as mode of treatment for myocardial infarction and ischaemia.[16]

Endocrinology: DPSCs have been differentiated to produce Hepatocyte like cells (HLCs) with acquired hepatocyte functions, such as glycogen storage and urea production. The use of cryopreserved tissue to generate HLCs provides a promising alternative for the treatment of liver diseases.[16]

Postnatal stem cell therapy: The process comprises of postnatal stem cells (derived from buccal mucosa) being injected into disinfected root canal systems after the apex is opened. This process has many advantages like the harvesting and delivery of autogenous stem cells by syringe, being relatively easy; and the potential of these cells to induce new pulp regeneration. However, there are several disadvantages; like the cells may have a low survival rate and they may migrate to different locations within the body. Instead, all three elements (cells, growth factors, and scaffold) must be considered, to maximize the potential for success of pulp regeneration.[10]

Pulp implantation: The pulp cells can be grown on biodegradable membrane filters to transform two dimensional into three dimensional cell cultures. The ease of growing these cells on filters in the laboratory, for evaluation of cytotoxicity of test materials, is recognized as the main advantage of this delivery system. As sheets of cells lack vascularity, only the apical portion of the canal systems will receive these cellular constructs, with coronal canal systems filled

with scaffolds capable of supporting cellular proliferation.[10]

*Osteogenic Potential :*PDLSC have lower osteogenic potential than BMSC and also dental pulp derived stem cells. *Kim et al*, reported new bone formation by PDLSC in a periimplant defect model, albeit at lower levels than BMSC. Although the potential use of PDLSC for generating graft biomaterials for bone tissue engineering in regenerative dentistry can be envisioned, as these cells are more routinely accessible, it is however necessary to delineate more refined isolated of pluripotent progenitors using genomic and proteomic marker characterization.[32]

Periodontal Regeneration: A cementum/PDL-like complex generated in surgically created periodontal defects by transplanting *in vitro* expanded human PDLSCs in a ceramic particle scaffold as demonstrated by *Seoetal. In-vitro* induction of PDLSC with dentin noncollagenous proteins increased cell differentiation along the cementoblast lineage, denoting a potential inductive role of root surface in the activation of PDLSC differentiation, which can be utilized for bioengineering applications.[32]

*Continued Root Formation:*Surgically removing the apical papilla at an early stage of the root development halted the root development despite the pulp tissue being intact. In contrast, other roots of the tooth containing apical papilla showed normal growth and development.[33]

Pulp Healing and Regeneration: Stem cells allow regeneration of pulp and root maturation to occur.[33]

Bioroot Engineering: A direct integration with bone onto surface of an implant is required for dental implants as the prerequisite for success, an unnatural relation with bone as compared with a natural tooth. SCAP and PDLSCs form a bioroot.[33]

Periodontal Regeneration: Cell transplantation have been developed to regenerate periodontium using Dental follicle stem cells (DFSCs) which could become an alternative cell source for periodontal regeneration therapy.[34]

*Repair of Craniofacial defects: S*tem cells can be used to repair craniofacial region in future and they may provide a promising alternative for reconstruction of craniofacial defects.[34]

Challenges

Stem cell research has undergone huge advancements in the past couple of years. It has proved particularly challenging for scientists to ensure the long term proliferative ability and pluripotency of embryonic stem and germ cells.Immune challenges also prove a significant barrier to the application of stem cell therapies. If the stem cells are recognized as non-self, they will be rejected and destroyed.

Conclusion

Advances in adult stem cell studies have provided a great deal of impetus for the biomedical community to convert these findings into clinical application. So here we have a populations of stem cells capable to reform bone and its marrow, cementum, dentin and perhaps even periodontal ligament, it is now possible to complete restore the hard tissues in the oral cavity using the patient‘s own cells, thereby avoiding issues of histocompatibility. Furthermore, the advancements in the techniques to genetically modify the genetic activity of stem cells during their ex vivo expansion offers the unique possibility to make a patient‘s own stem cells even better.

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