Stem Cells : An Emerging Hope

Prof. (Dr.) U.S. Krishna Nayak
Dean, Senior Professor & HOD
Dept. of Orthodontics &
Dentofacial Orthopaedics,
A. B. Shetty Memorial Institute of
Dental Sciences, Derlakatte, Mangalore.

Dr. Amarnath Shenoy.
Associate Professor
Dept. of Conservative
Dentistry & Endodontics

Dr. Shruti D. Nayak
Senior Lecturer
Dept. of Oral Pathology

Yenepoya Dental College, Mangalore

Dr. Soumi Samuel
Reader
Dept. of Oral & Maxillofacial Surgery
A. B. Shetty Memorial Institute of
Dental Sciences,Derlakatte
Mangalore

Dr. Arjun Nayak,
Dental Surgeon
Nayak's Dental Speciality
Clinic, Mangalore

Abstract

Stem cells are one of the most fascinating areas of biology today. Research on stem cells is advancing knowledge about how an organism develops from a single cell and how the damaged or injured cells replace healthy cells in adult organisms. This area is leading the scientists towards cell-based therapies to treat a disease which is referred to as regenerative or reparative medicine. Dental stem cells can be used to regenerate jawbone and periodontal ligament in patients. Stem cells from umbilical cord blood are being used to treat leukaemia and blood related diseases.

Introduction

Stem cells are generally defined as clonogenic cells capable of both self renewal and multi-lineage differentiation.[1] Post natal pulp contains several niches of potential stem cells/ progenitor cells and these cells may have importance in mediating reparative dentine formation. Stem cells have a fundamental role in wound repair processes. In response to certain extracellular signals they produce multiple highly differentiated progeny even though they represent on 1% of the total cell population[2]. Post-natal stem cells have been isolated from various tissues, including bone marrow, neural tissue, skin, retina, and dental epithelium. A population of putative post-natal stem cells have also been identified in human dental pulp called dental pulp stem cells (DPSCs). Their most striking feature is their ability to regenerate a dentin pulp-like complex the composition of which is very similar to that found in natural human teeth, that is the arrangement of fibrous tissue containing blood vessels and tubules lined with odontoblasts .[1]

Stem cells have two important characteristics that make them different from other type of cells. First, they are unspecialised cells that renew themselves for long periods through cell division. The second is that under certain experimental or physiological conditions, they can be induced to become specialised cells. Such special cells can be cells that take part in heart beat and cells which produce insulin in pancreas. Two kinds of stem cells are being worked upon by the scientists. They are embryonic stem cells and adult stem cells. Stem cells isolated from human embryos are called human embryonic stem cells. The embryos used in these studies were obtained through in vitro fertilization procedures used in cases of infertility. Once their purpose was achieved these embryos were donated for research with the informed consent of the donor. [3]

Researchers have until now used artificially-inseminated oocytes which could no longer be used for originally planned implantation. ES (Embryonic Stem) cells have also been developed from oocytes donated specifically for the purpose. There are serious ethical reservations which oppose this production of embryos purely for the research purposes. EG (embryonic germ) cells can be obtained from precursor cells from oocytes and sperm cells- cells which are known as primordial germ cells. These can be isolated from several week old foetuses following artificially induced termination of pregnancy or natural termination. Somatic stem cells are obtained in adult organism or from the foetal tissue, and most importantly from organs with high reproductive capacity. Somatic cell nuclei could be transferred into denucleated oocytes and fertilised oocytes can be developed further, and ES cells can be separated from them. But these two possibilities should be kept separate.[4]

The embryo protection law is of huge importance when it comes to obtaining human ES cells and carrying out scientific projects involving them. This law identifies the beginning of an individual life as the point at which fertization is completed. That is, when the egg and sperm unite to create a new individual genome. Artificial fertilisation is also included in this law. Regarding the use of somatic stem cells no legal controls are in place. The embryo protection law and the transplantation law are not applicable. Tissue specific stem cells do not involve organs in the sense of those covered by the transplantation law. Special regulations are there for obtaining stem cells from blood. When therapeutic applications for stem cells are concerned, the pharmaceuticals law must be taken into account.[4]

Stem Cell Niches in Dental Pulp[2]

The progenitor cell or stem cells within healthy tissues maintain a quiet and inactive state and this is due to the environment in which they are found. Death of the post-mitotic odontoblasts caused due to injury or trauma stimulates a cascade of complex and yet to be understood events in which signals are released into the matrix, which then cause the stem cell population to produce a high proliferative activity and generate a terminally differentiated Odontoblastic cell. The identification of stem cell niches is best observed in situ following their activation in response to injury. Initial studies indicated that replacement odontoblasts are derived from undifferentiated mesenchymal cells in the pulp proper. More recent studies suggest that progenitor or stem cell niches reside predominantly in the perivascular regions of the pulpal cavity, from where they migrate to the site of injury.

Animal study finding suggest that progenitor cell niches reside in different locations throughout the pulpal tissue .The study was done based on notch expressions following pulp capping, since notch is purported to be an important signalling molecule which controls stem cell fate.[2]

Two different populations of stem cells can be considered; epithelial stem cells (EpSC) which can give rise to ameloblasts, and mesenchymal stem cells (MSC) odontoblasts, cementoblasts, osteoblasts and fibroblasts of the periodontal ligament.

Tooth engineering using stem cells is based on their isolation, association and culture as recombinants in vitro or ex vivo conditions to assess firstly tooth morphogenesis, and secondly cell differentiation into tooth specific cells which will give rise to enamel, dentin, alveolar bone and cementum. Adult stem cells (ASC) are quiescent, undifferentiated and slow-cycling cells which are surrounded by neighbouring cells and extracellular matrix. The micro environment housing ASC and transient amplifying cells (TAC) forms a niche. Understanding and regulation of these specialised micro environments is the key for successful ex vivo engineering of an organ with ensured functional homeostasis. In teeth, two different stem cell niches have been suggested: the cervical loop of rodent incisor for EpSC and a perivascular niche in adult dental pulp for MSC. In the dental pulp, MSCs are thought to reside in a perivascular niche, but little is known on the exact location and molecular regulation of this niche. This dental pulp perivascular niche is maintained by the Eph receptor tyrosine kinase family of guidance molecules. Other MSC populations have been isolated from human dental tissues such as the periodontal ligament and dental follicle.

Stem cells from the apical part of the papilla (SCAP)

SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.

Stem cells from the dental follicle (DFSC)

DFSC have been isolated from follicle of human third molars. These cells can be maintained in culture for at least 15 passages. STRO-1 positive DFSC can differentiate into

cementoblasts in vitro and are able to form cementum in vivo. Immortalized dental follicle cells are able to re-create a new periodontal ligament (PDL) after in vivo implantation.

Periodontal Ligament Stem Cells (PDLSC)

The PDL is a specialized tissue located between the cementum and the alveolar bone. It maintains and supports the teeth. Mesenchymal progenitor cells are involved in its continuous regeneration. PDL contains STRO-1 positive cells that maintain certain plasticity since they can adopt adipogenic, osteogenic and chondrogenic phenotypes in vitro. It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone.

Bone Marrow Derived Mesenchymal Stem Cells (BMSC)

These cells have the ability to form in vivo cementum, PDL and alveolar bone after implantation into defective periodontal tissues. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases. BMSC share numerous characteristics with DPSC and are both able to form bone-like or tooth-like structures. However, BMSC display a lower odontogenic potential than DPSC indicating that MSC from different embryonic origins are not equivalent.

Significant progress has been made with the MSC. Even then the information available for dental EpSC in humans is very limited or nil. Dental epithelial cells such as ameloblasts and ameloblasts precursors are eliminated soon after tooth eruption and this is a major problem. So the epithelial cells that could be stimulated in vivo to form enamel are not present in human adult teeth. Stem cell technology appears to be the only possibility to re-create an enamel surface.

Origin of Stem Cells[6]

Two models have been proposed to explain the persistence of stem cells throughout life. The first one suggests that a fixed number of primitive cells are laid down during embryogenesis to supply the body's needs throughout its lifetime. Stem cells are recruited into proliferation, differentiation and development as required. The stem cell pool declines as stem cells differentiate to contribute mature cells to the tissue according to this theory. The second model also says that a small population of embryonic cells arises during embryonic development, but these cells have self reproducing capacity and produce daughter cells which retain the same developmental and proliferative potential of original parent cells. So the recruitment does not necessarily lead to a reduction as in first model. Evidence strongly supports the second model.

Haemopoietic stem cells are formed during embryogenesis and colonise the bone marrow. Here they contribute to neonatal and adult haemopoiesis. Stem cells associate with the complex range of cells and extracellular matrix of bone marrow stroma. Research has shown that different kinds of blood cells are all derived from a common stem cell pool in the marrow. Thus Haemopoietic system is multipotent. The rate of stem cell self renewal must be balanced against differentiation and mature cell formation and loss. The ability of self renewal which helps them to persist is common to all stem cells.

Stem Cells in Disease[6]

Dysregulation of stem cells and their more differentiated progeny is thought to contribute to several clinical conditions such as Myelodysplastic syndrome, Secondary polycythaemia, Polyposis coli, Warts, Lentigo maligna and some small cell lymphomas. Several genes that promote cellular division have been identified. Mutations or inappropriate expressions of these genes are thought to be able to stimulate cells to divide. The genes which are suspected in promoting malignant events are called transforming oncogenes. Anti oncogenes or tumour suppressor genes is the term used to describe a set of genes that restrict cell-division. These tumour suppressor genes are lost or mutated in several malignancies. Polyposis to gastrointestinal carcinoma is thought to be due to a combination of both transforming oncogenes and tumour suppressor gene.

Adult stem cells share at least two characteristics. They make identical copies of themselves for long periods of time; this ability is known as long-term-self-renewal. They can also give rise to mature cell types that have characteristic morphologies (shapes) and specialized functions. Before they achieve their fully differentiated state the stem cells generate an intermediate cell type or types, and this cell is called precursor cell or progenitor cell. These precursor cells in foetal or adult tissues are partially differentiated cells that divide and give rise to differentiated cells. Adult stem cells are rare and their primary functions are homeostasis and, within its limitations, to replace diseased or dead cells. As an example it can be cited that only 1 in 10,000- 15,000 cells in the bone marrow is a hematopoietic (blood forming) stem cell (HSC). Adult stem cells are dispersed in tissues throughout the mature animal and behave differently depending on their local environment. In the bone marrow HSCs are being constantly generated and they differentiate into mature types of blood cells.

Unlike embryonic cells, which are defined by their origin, adult stem cells share no such means of characterization. The origin of adult stem cells in any mature tissue is not known. Most of the information about adult cells comes from study done in mice. Bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of skin and digestive system, cornea, retina, liver and pancreas are the adult tissues containing stem cells. To be called as an adult stem cell, the cell should be capable of self renewal for the life time of the organism. In a complex organism as a human being it is nearly impossible to design an experiment to track the fate of adult stem cells in vivo over an individual's entire life time. Clonogenic property of the adult stem cells is also difficult to demonstrate in vivo. In practice scientists show that a stem cell is clonogenic in vitro or that a purified population of candidate stem cells can repopulate the tissue.

Adult stem cell should also be capable of giving rise to fully differentiated cells that have mature phenotypes, are fully integrated into the tissue, and are capable of specialised functions that are appropriate for the tissue. Researchers rely on two characteristics to prove their claim of having identified adult stem cells. Appropriate cell morphology and identification of surface markers in the differentiated cell types that identify them as belonging to the tissue are those two characteristics. Some studies demonstrate that the differentiated cells derived from adult stem cells are truly functional. Few studies show that the cells are integrated into the differentiated tissue in vivo and that they interact properly with neighbouring cells.[3]

Dental Pulp Stem Cells & Ageing[2]

Pulp cell populations when analysed histomorphometrically, indicate that there is an age related reduction. This includes sub-Odontoblastic cells which may be a potential progenitor cell niche.[7] It may be that the size of the pool of stem/ progenitor cells is relatively small and only a relatively low number of cells are able to participate in specific dentinogenic regenerative responses. It is speculated that biomimetic materials or bio fillings may be effective in stimulating natural repair and influence the effectiveness and lifespan of the restorations, thus maintaining the vitality of the compromised pulp. For the success of such treatment, the stage of dental development may be of importance.[2]

One of the most promising recent developments is that a study showed stem cells from teeth can create islet-like cells which produce insulin in a glucose responsive manner, suggesting a potential therapy for type 1diabetes.[8]

Adult Human Pulp Stem Cells[9]

Dentin is a mineralised tissue which has a great similarity to bone. Limited dentinal repair occurs throughout life in postnatal organisms. DPSCs have been found to form sporadic but densely calcified nodules in vitro. Experiments in immunocompromised mice showed that when DPSCs were transplanted with hydroxyapatite/ tricalcium phosphate a dentin like structure was generated by them with collagen fibres running perpendicular to the mineralising surface as found in vivo. It also contained

dentin enriched protein and dentin sialophosphoprotein. The newly formed dentin was lined with human odontoblasts like cells and an interstitial tissue reminiscent of pulp. In contrast to BMSCs, DPSCs did not support the establishment of a hematopoietic marrow or adipocytes, elements that are absent in dental pulp tissue in vivo.

Studies have shown that transplanted DPSCs can not only commit to the Odontoblastic lineage but also reside in the pulp-like connective tissue as fibroblast like cells, and it is possible that these fibroblast-like cells belong to a population of a more primitive reserve cells responsible for the dentin formation in the secondary transplantation. An antibody specific to DSP protein has also been found in the study.[1]

In a study stem cells from human exfoliated deciduous teeth were identified to be a population of highly proliferative, clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts.[10]

Stem Cells or Progenitor Cells in Dentine Regeneration [2]

It is still unclear how the inflammatory processes affect stem/progenitor cells or the molecular signalling processes responsible for their differentiation. The ability of stem cells to migrate to areas of injury from other niches in unaffected areas of the pulp, in order to replace or damage lost cells remains still an ongoing research. It is now established that the growth factors sequestered within the dentin matrix, influence and direct the processes of reactionary and reparative dentinogenesis. Angiogenic growth factors are present among the cocktail of growth factors found within the mature dentine matrix. Their release might be the key to the local up- regulation of angiogenesis at the injury site and thus indirectly influence the pool progenitor cells for regeneration.

Osteogenesis and dentinogenesis [11]

Osteogenesis and dentinogenesis begin at 2-4 weeks post transplantation, and eventually lead to the regeneration of a bone/marrow organ structure and a dentin/pulp-like complex in BMSSC and DPSC transplants, respectively. After 16 weeks more mineralised tissue is observed which proves that BMSSCs and DPSCs have continuous potential of forming mineralised tissue after organ-like structure are formed. So both BMSSCs and DPSCs are not only able to differentiate into osteoblasts/odontoblasts in vivo early in the transplantation process, but also are capable of inducing host cells to participate in tissue regeneration by formation of a hematopoietic marrow and a pulp like complex.[11]

Comparisons Between Human Embryonic Stem Cells & Embryonic Germ Cells [3]

The ES cells derived from human blastocysts and from human EG cells are similar in many aspects. In both cases the cells replicate for an extended period of time, show no chromosomal abnormalities and generate both XX and XY cultures and express a set of markers regarded as characteristic of pluripotent cells. Under proper conditions they both differentiate into endoderm, mesoderm and ectoderm.

However they differ in tissue sources from which they are derived and they also vary with respect to their growth characteristics in vitro and their behaviour in vivo. If injected into immunocompromised mice colonies human ES cells will generate teratomas containing differentiated cell types, while human EG cells will not.

Potential Uses of Human Embryonic Stem Cells [3]

Various uses have been proposed but their major use is in their potential use in transplant therapy. This means that they have an important role in replacing or restoring tissue that has been damaged by injury or disease. Human embryonic stem cells have been used for therapeutic transplants. Diseases like Parkinson's disease, diabetes, traumatic spinal cord injury, Purkinje cell degeneration, heart failure, and Osteogenesis imperfecta may be treated using these cells. But in treating these diseases it should be seen that human ES cells should be directed to differentiate into specific cell types prior to transplant.

One of the advantages of using ES cells as compared to adult stem cells is that ES cells have an unlimited ability to proliferate in vitro. They are more likely to be able to generate a broad range of cell types through directed differentiation. One potential disadvantage of the use of human ES cells for transplant therapy is the propensity of the undifferentiated ES cells to induce the formation of tumours (teratomas). Suicide genes can be inserted into transplanted ES derived cells. These suicide genes trigger the death of the cells should they become tumerogenic.

Human ES derived cells would be advantageous for transplantation purposes if they do not trigger immune rejection. The potential immunological rejection of human ES derived cells might be avoided by genetically engineering the ES cells or by using nuclear transfer technology to generate cells that are genetically identical to the person who receives the transplant.

By studying the human ES cells in vitro, it may be possible to identify the genetic, molecular, and cellular events that lead to problems such as spontaneous abortion caused due to congenital birth defects and placental abnormalities. Human ES cells could also be used to test candidate therapeutic drugs. These cells could also be employed to screen potential toxins. With the help of human ES cells it may be possible to develop a new method for genetic engineering.[3]

Conclusion

Predicting the future of stem cell applications is impossible, particularly given this early stage of stem cell biology. It may be still very early to say which stem cells, or which methods for manipulating the cells will best meet the needs of basic research and clinical applications. Conducting more research is the only answer to this. Dental EpSC can be isolated from post-natal teeth but exhibit complex problems that strongly limit their clinical application in humans. Other sources are thus required. Ideally these sources should be easily accessible, available from adult individuals and the derived cells must have potential for enamel matrix production. The use of non dental EpSC will only be possible with the transfer of genes, creating an odontogenic potential to non-dental epithelia prior to any association with mesenchymal cells. This is certainly one of the most exciting goals of the next decade in tooth engineering.

References

1. S.Gronthos, J. Brahim,W. Li, L W Fisher et al. Stem cell properties of human dental pulp. J Dent Res 2002 81 (8): 531-35
2. Alastair J. Sloan, Rachel J. Waddington. Dental stem cells: what, where, how? Int J of Paed Dent 2009; 19: 61-70.
3. Stem cells .Internet search. http:// www.stemcellmx.com. Last visited in May 2012.
4. Wolf rum R. Stem cells: ethical and legal challenges. Max plank research. 2001; 64-67.
5. Bluteau G, Luder H U, De Bari, Mitsiadis T A, Stem cells for tooth engineering, Eur cell and mat; 2008:16: 1-9.
6. Clark B R, Dexter T M. stem cells in normal growth and disease. Basic molecular and cell biology. 48-69
7. Murray P E, Stanley HR, Mathews JB, Sloan AJ, Smith AJ, ageing human odontometric analysis. Oral surg oral med oral Pathol oral radiol 2002a; 93: 474-82.
8. Dental stem cell research. Emerging field, advancing research leads to exciting potential for dental stem cell applications. Internet search. www.store-a-tooth.com. Last visited in May.
9. Paul H. Krebsbach, Pamela GR, Dental and skeletal stem cells: potential cellular therapeutics for craniofacial regeneration. J den edu; 66:6:766-73
10. Masako M, Stan G, Mingrui Z, Bai L et al.SHED: stem cells from human exfoliated deciduous teeth. 2003; 10:5807-12.
11. Batouli S, M. Miura, J Brahim, T W Tsutsui et al. comparison of stem cell mediated Osteogenesis and dentinogenesis. J Dent Res 2003; 82:12:976-8