Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 03, pp.67164-67169, March, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STEM CELLS - THEN, NOW AND FUTURE!!

Dr. Fathimath Nishana, K., Dr. Manjushree Kadam, Dr. Thasneem, A.A., Dr. Faima Banu and Dr. Fathimath Nihala, K.

A.J. Institute of Dental Sciences, Kuntikana, Mangalore -575004, India

ARTICLE INFO

Article History:
Received 17th December, 2017
Received in revised form
22nd January, 2018
Accepted 04th February, 2018
Published online 30th March, 2018

Key words:
Stem cells,
Periodontal Ligament Stem Cells,
Tissue Engineering.

ABSTRACT

Currently regeneration of tooth and periodontal damage still remains a great challenge. Stem cell-based tissue engineering raised novel therapeutic strategies for tooth and periodontal repair. Stem cells for tooth and periodontal regeneration include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and stem cells from human exfoliated deciduous teeth (SHEDs), dental follicle stem cells (DFSCs), dental epithelial stem cells (DESCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)**.** To date, substantial advances have been made in stem cell-based tooth and periodontal regeneration. Translational investigations have been performed such as dental stem cell banking and clinical trials. In this review, we present strategies for potential applications of stem cells in periodontal therapy.

Citation: Dr. Fathimath Nishana, K., Dr. Manjushree Kadam, Dr. Thasneem, A.A., Dr. Faima Banu and Dr. Fathimath Nihala, K., 2018. "Stem cells - then, now and future!!", *International Journal of Current Research*, 10, (03), 67164-67169.

INTRODUCTION

Periodontitis is a disease of the periodontium characterized by irreversible loss of connective tissue attachment and supporting alveolar bone. These changes often lead to an aesthetically and functionally compromised dentition (Lin *et al.,* 2008). The purpose of periodontal therapy is to provide a dentition that functions in health and comfort for the life of the patient. Periodontal therapy involves two primary components. First elimination of the periodontal infection, by eliminating the pathogenic periodontal micro flora, which induces substantial favorable clinical changes in the periodontium. However, the anatomic defect resulting from active periodontitis still persists and is represented clinically by loss of clinical attachment, increased probing depths, and radiographic bone loss. The substantial efforts made to alter the defect, represent the second component of periodontal therapy. The primary approaches to correct these defects include new attachment, resective, and regenerative procedures **(**Steven *et al.,* 1996). Regeneration is defined as a reproduction or reconstitution of a lost or injured part. It is, therefore, the biologic process by which the architecture and function of lost tissues are completely restored. Periodontal regeneration is regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum (AAP 1992).

******Corresponding author:* **Dr. Fathimath Nishana,**
A.J. Institute of Dental Sciences, Kuntikana, Mangalore -575004, India.

Recently, the isolation of adult stem cells from human periodontal ligament has presented new opportunities for tissue engineering. In order to use successfully, a thorough understanding of stem cell and their role in regenerating periodontal tissue is required. A ''stem cell'' refers to a clonogenic, undifferentiated cell that is capable of self-renewal and multi-lineage differentiation. In other words, a stem cell is capable of propagating and generating additional stem cells, while some of its progeny can differentiate and commit to maturation along multiple lineages giving rise to a range of specialized cell types (Bongso *et al.,* 2005). Depending on intrinsic signals modulated by extrinsic factors in the stem cell niche, these cells may either undergo prolonged self-renewal or differentiation. The use of stem cells with these technologies may constitute novel strategies for regenerative periodontal therapy.

THE HISTORY OF STEM CELLS (Bongso *et al.*, 2004)

- **1878-** The first attempts were made to fertilize mammalian eggs outside the body**.**
- **1959 -** First animals made by in-vitro fertilization (IVF).
- **1968 -** The first human egg is fertilized in vitro.
- **1978** - The first IVF baby is born in England.
- **1984-88**- Pluripotent, clonal cells called embryonic carcinoma (EC) cells are developed. When exposed to retinoic acid these cells differentiate into neuron-like cells and other cell types.

- **1989**- A clonal line of human embryonic carcinoma cells is derived that yields tissues from all three primary germ layers. They have limited replicative and differentiative capacity.
- 1994- Human blastocysts are generated and the inner cell mass is maintained in culture. ES like cells form in the center and retain stem cell like morphology.
- **2003** - Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth.
- **2004-05** - Korean researcher KwangWoo-Suk claims to have created several human embryonic stem cell lines from unfertilized human oocytes. The lines were later shown to be fabricated.
- **January 2007** - Scientists at Wake Forest University led by Dr Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid. This may potentially provide an alternative to embryonic stem cells for use in research and therapy.
- **January 2008** - Human embryonic stem cell lines were generated without destruction of the embryo.
- **January 2008** - Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts.

WHAT ARE STEM CELLS?

Stem cells are cells that display the following properties

- are unspecialized
- capable of dividing and renewing themselves for long periods
- can give rise to specialized cell types

(Brivanlou *et al.,* 2003)

Stem cells are unspecialized

One of the fundamental properties of a stem cell is that it does not have any tissue specific structure that allow it to perform specialized functions. A stem cell cannot work with its neighbors to pump blood through the blood stream.

Stem cells are capable of dividing and renewing themselves for long periods

Stem cells can replicate themselves. When cells replicate themselves many times over, it is called **Proliferation.** Stem cells that proliferate for many months in the lab can yield millions of cells.

Stem cells can give rise to specialized cells

When unspecialized stem cells give rise to specialized cells, the process is called Differentiation**.** There are signals inside and outside cells that trigger stem cell differentiation. A cell gene controls the internal signals, which are interspersed along the 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. Stem cell is a broad term used to describe a wide variety of cells from varying sources. Stem cells can be broadly divided into two categories – embryonic and adult. Embryonic stem cells are totipotent cells, capable of differentiating into virtually any cell type, as well as being propagated indefinitely in an undifferentiated state (Evans and Kaufman, 1981). Due to regulatory issues associated with the use of embryonic stem cells, and the difficulty in controlling their growth and differentiation, recent attention has been focused on stem cells derived from adult tissues. Indeed, from a practical standpoint, adult stem cells are more appropriate for periodontal tissue engineering purposes. Although it is accepted that adult stem cells have a more restricted differentiation potential compared with the totipotent properties of embryonic stem cells, these cells still fulfill the basic characteristics of stem cells – abilities to self-renew, generate large numbers of progeny and differentiate into multiple mature cell types (Fortier, 2005).

EFFECTIVENESS OF THE STEM CELLS

A stem cells *Potency* its capacity or efficiency specifies to differentiate into different cell types and accordingly the cells can be divided into several categories of efficiency

- Totipotent stem cells: These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent. Totipotent stem cells can differentiate into embryonic and extra embryonic cell types. Such cells can construct a complete, viable, organism.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.
- Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.
- Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells) (Motwani *et al.,* 2016).

CLASSIFICATION OF STEM CELLS

Stem cells can be classified into four broad types based on their origin. (Bongso et al 2005).

They are

1. Stem cells from embryos
2. Stem cells from the fetus
3. Stem cells from the umbilical cord and
4. Stem cells from the adult:

- Hematopoietic stem cells (bone marrow and peripheral blood)
- Mesenchymal stem cells (bone marrow stroma)
- Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs)
- Periodontal Ligament Stem Cells (PDLSCs)
- Dental follicle stem cells
- Dental epithelial stem cells
- Cementoblast - like stem cells

STEM CELLS IN HUMAN PERIODONTAL LIGAMENT

The presence of multiple cell types (fibroblasts, cementoblasts and osteoblasts) within the postnatal periodontal ligament has led researchers to speculate that these cells may share common

ancestors. The possibility that progenitor cells might exist in the postnatal periodontal ligament has been recognized for some time but until recently had never been formally proven (Coura *et al.,* 2008). These cells are believed to provide a renewable cell source for normal tissue homeostasis and periodontal wound healing. The first reported isolation and identification of mesenchymal stem cells in human periodontal ligament was in 2004. Since then, there has been considerable activity trying to understand the function of these cell populations and their interactions with each other with a view to laying the fundamental groundwork for clinical applications in regenerative periodontics. A number of studies have now been carried out to confirm the presence of MSC-like cells in the periodontal ligament. These have not been limited to human but also include mouse, rat and sheep. All of these studies have confirmed that periodontal ligament stem are cells multipotent in nature and while the initial studies indicated this to include an ability to differentiate into osteoblast, cementoblast (Wenjun *et al.,* 2015).

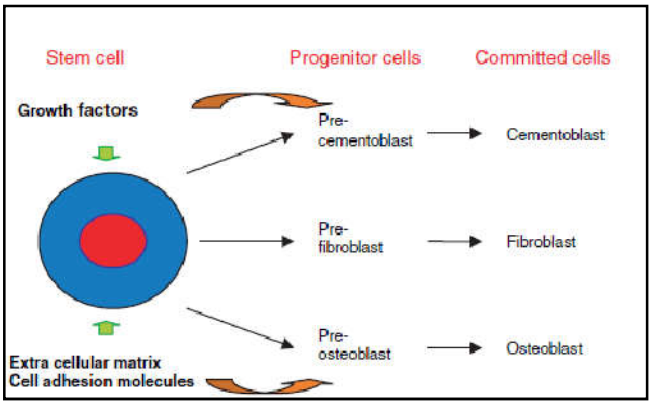

Fig.1. Progenitor cells of periodontal ligament

POTENTIAL APPLICATIONS OF STEM CELLS IN PERIODONTAL THERAPY

Identification of stem cells in postnatal dental tissues has presented exciting possibilities for the application of tissue engineering as well as gene and cell-based therapies in reconstructive dentistry. The use of stem cells with these technologies may constitute novel strategies for regenerative periodontal therapy.

Tissue engineering

Tissue engineering is a specialized field of science based on principles of cell biology, developmental biology and biomaterials science to fabricate new tissues to replace lost or damaged tissues.

Successful tissue engineering requires an appropriate extracellular matrix or carrier construct which contains regulatory signals and responsive progenitor cells. A potential tissue engineering approach to periodontal regeneration involves incorporation of progenitor cells and instructive messages in a prefabricated three-dimensional construct, which is subsequently implanted into the defect site.

This strategy eliminates some of the limitations associated with conventional regenerative procedures because of direct placement of growth factors and progenitor cells into the defect site overcomes the normal lag phase of progenitor cell recruitment to the site (Langer et al.,1993).

Requirements for successful tissue engineering

Biomechanical features

- Space maintenance
- Barrier or exclusionary features

Biological functions

- Biocompatibility
- Incorporation of cells
- Incorporation of instructive messages

Space maintenance within the defect site

It has been recognized that bone will grow into an adjacent tissue space provided that space can be maintained and soft tissue in growth prevented. Such natural phenomena should be used to advantage when considering tissue engineering and placement of bioengineered matrices for regeneration. The engineered material should be of sufficient form to allow placement into a defect and prevent subsequent collapse of the repositioned tissues into the defect site.

Barrier or exclusionary functions

The engineered tissues should act as a barrier to the in growth of unwanted tissues. To achieve this, design features will have to be incorporated whereby the external surface may be exclusionary yet the internal scaffold conducive to new tissue in growth.

Biocompatibility and design features

The scaffold material should be either biocompatible with the tissues to be regenerated or biodegradable, allowing for gradual replacement with regeneration.

Table 1. Tissue engineering approaches in periodontics

Technique	Advantages	Complications
Cell injection	Easy delivery Injected stem or precursor cells can induce the formations of extracellular matrices and blood vessels	Low cell survival Cells may not differentiate
Cultured tissues	Easy to grow in the laboratory Increased stability compared with cell injection	Tend to be very small in size without vasculature Very fragile
Porous scaffolds	Supports cell organization and promotes vascularization	Delay between implantation and vascularization
Three-dimensional printing	Multiple cell types can be precisely positioned	Inconsistent results
Inject able scaffolds Simple delivery	Can mediate regeneration by providing biomedical cues	Inconsistent results

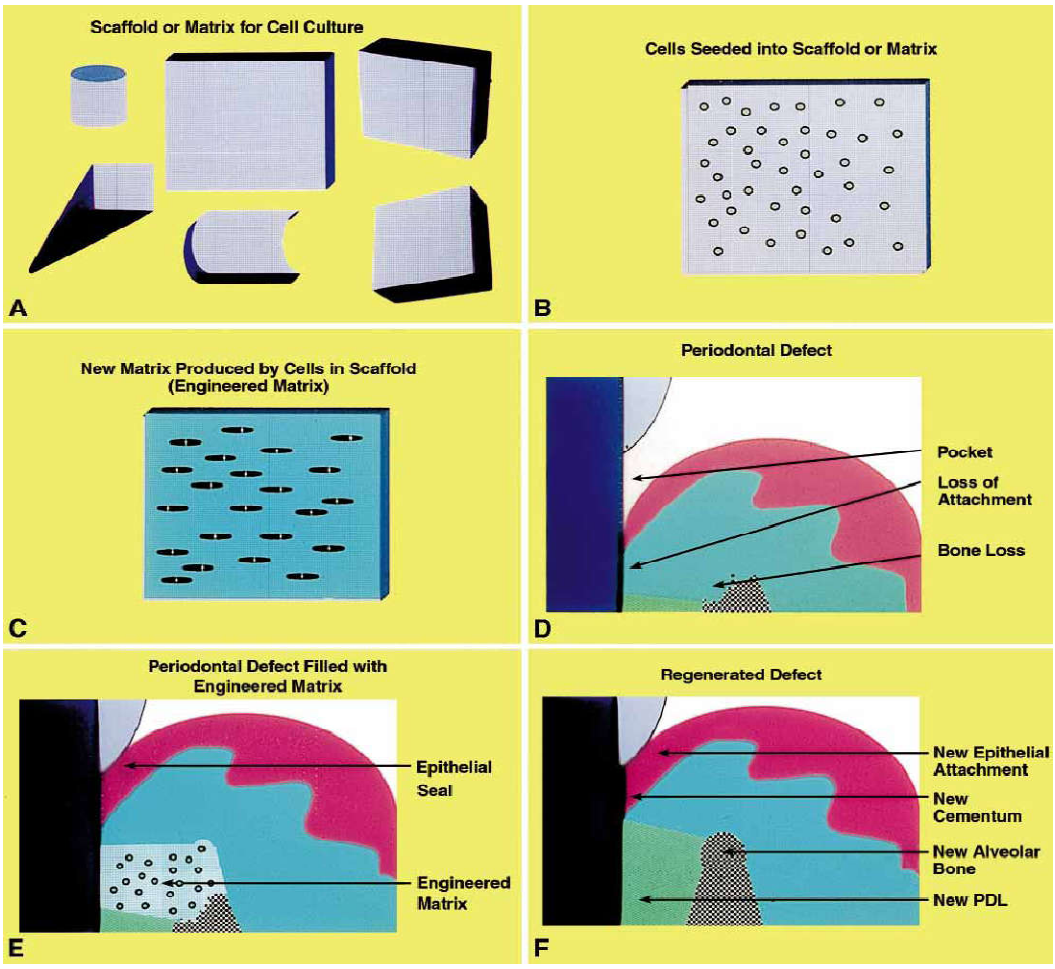

Fig. 2. Tissue engineering approach to periodontal regeneration

Synthetic scaffold can be prepared which can be cut and molded into, desired shapes and forms. B. Cells from the periodontal with a tissue (either as primary cell cultures or even genetically manipulated for the expression of specific matrix component can be seeded cells are cultured in vitro C. The seed cell iscultured for a specified time to allow synthesis of an appropriate matrix suitable for plantation. D. Periodontal defect. E. The ''engineered'' matrix is implanted into the periodontal defect site which adequate epithelial seal, will lead to a regenerative response.F. Fully regenerated periodontal defect resulting from implanted engineered tissue PDL (periodontal ligament) (Bartold *et al.,* 2006)

Tissue design features need to include considerations of pore size for both cell attachment and incorporation in vitro as well as subsequent tissue maturation during in situ regeneration. Scaffold material include calcium phosphate, hydroxyapatite, extracellular matrix components (collagens, hyaluronan and fibronectin) polyglycolic acid and other synthetic bioresorbable materials. In the case of the periodontium, a biodegradable material that would degrade slowly over time and be replaced with a soft connective tissue compatible with periodontal ligament, together with new alveolar bone and root surface cementum with appropriately orientated and inserted collagen fibers, would be desirable.

Incorporation of cells with appropriate phenotype for ongoing periodontal regeneration

Cells with a ''periodontal regenerative phenotype'' it should be possible to culture and subsequently incorporate these cells into a suitable biodegradable scaffold for immediate introduction into a periodontal defect.

Incorporation and bioavailability of instructive Messages

The synthetic matrix should be bioresorbable but constructed from a material with a suitable affinity for the adsorption of appropriate growth/differentiation factors as well as integrins, cell receptors and other instructive molecules normally found in regenerating tissues. Agents that promote cell adhesion, permit proliferation of progenitor cells and can be easily incorporated into a scaffold or matrix for suitable slow release with adequate delivery kinetics will become the major focus of interest.

CURRENT PROBLEMS

It is easy to be engulfed by a tide of enthusiasm for these innovative techniques that promise to revolutionize surgical practice, but it is important to recognize the enormity of some of the problems that, as yet, have not been satisfactorily addressed

- **Immunogenicity**: Unless individuals pre-donate cells to generate a unique bank of 'spare-part organs', the problems of the host immune response to allogeneic or xenogeneic tissues must be overcome. Current immunosuppressive regimen is costly and are associated with significant deleterious effects.
- **Preservation**: Assuming that the problems of immunogenicity can be overcome with 'wrappers' or other techniques, a method of preserving these engineered artificial tissues for 'off-the-shelf' use must be identified. Ever since the discovery of cryoprotective

agents like glycerol and its successful preservation of human gametes and embryos, science has tried to freeze ever more complex tissues.

- **Vascularization and Organogenesis**: A fundamental limitation to the size of any implantable living construct is its blood supply. Complex tissues can receive nutrients and oxygen (and eliminate waste) by diffusion over only a few micrometres. Thus, any attempts at fabricating complex tissue must address the problems of angiogenesis. Incorporation of angiogenetic factors such as vascular endothelial growth factor is one potential solution, encouraging accelerated ingrowth of vessels from the host.
- **Ethics**: Many of the tissue engineered projects outlined above utilize cells and tissue from allogenic and xenogenic sources, both of which generate intense ethical debate.

FUTURE CHALLENGES

Biological challenges

Despite biological evidence showing that regeneration can occur in humans, complete and predictable regeneration still remains an elusive clinical goal, especially in advanced periodontal defects. Basic discoveries on periodontal stem cells have emerged from cell culture and animal models which does not always translate to the human situation (Yokohama-Tamaki, 2006)**.** Further research is needed to elucidate the cellular and molecular events involved in restoring lost periodontal tissues before a reliable biologically - based therapy can be developed.

Technical challenges

Biologically, the matrix scaffold should have good biocompatibility for the cellular and molecular components normally found in regenerating tissues. The optimal mechanism of propagation and incorporation of human PDLSCs cells into a carrier scaffold still needs further refinement. Further studies are needed to understand the conditions that induce lineage-specific differentiation and efficacy of in vitro expanded stem cells derived from regenerating periodontal defects. Refinement of current techniques to facilitate laboratory handling of these cells and to maximize their regenerative potential represents a long-term endeavor if these cells are to be used in clinical periodontics (Zhao *et al.,* 2006).

Clinical challenges

There are a number of clinical barriers in MSC-based clinical therapy that must be understood and overcome immune rejection, tumour growth and efficacy of cell transplantation (Choumerianou *et al.,* 2008**).** The challenge relating to genomic stability and the risk of tumorigenesis following stem cell transplantation are major safety considerations because reliable methods to eliminate undifferentiated embryonic stem cells from culture are yet to be established (Bongso *et al.,* 2008), and current studies lack long-term follow-up to draw firm conclusions (Yu *et al.,* 2006)

CONCLUSION

Regeneration of tissues destroyed by periodontitis has long been an altruistic goal of periodontal therapy. Periodontal regeneration requires consideration of many features that parallel periodontal development, including the appropriate progenitor cells, signaling molecules and matrix scaffold in an orderly temporal and spatial sequence. Some of the main issues are identification of the optimal precursor cell types, establishment of growth and differentiation conditions that meet safety and good manufacturing practice standards, and manipulation of the surrounding environment to allow transplanted cells to survive and function. To summarize, the promise of stem cell therapies is an exciting one, but significant technical hurdles remain that will only be overcome through years of intensive research.

REFERENCES

American Academy of Periodontology. Glossary of Periodontal Terms, 3rd edition. 1992. Chicago: *American Academy of Periodontology,*

Bartold, PM., McCulloch, CAG., Narayanan, AS. and Pitaru, S**.** 2000. Tissue engineering: a new paradigm for periodontal regeneration base on molecular and cell biology. *Periodontol.,* 2006;24:253-69.

Bongso, A. and Lee, EH. 2005. Stem cells: their definition, classification and sources. In Stem Cells: from bench to bedside (pp. 1-13).www.worldscibooks.com

Bongso, A. and Richards, M. 2004. History and perspective of stem cell research. *Best Pract Res Clin Obstet Gynaecol.,* 18(6):827-42.

Brivanlou, AH., Gage, FH., Jaenisch, R., Jessell, T., Melton, D. and Rossant, J. 2003. Stem cells. Setting standards for human embryonic stem cells. *Science*, 300: 913–16.

Choumerianou, DM., Dimitriou, H. and Kalmanti, M. 2008. Stem cells: promises versus limitations. *Tissue Eng Part B Rev.,* 14: 53–60.

Evans, MJ. and Kaufman, MH. 1981. Establishment in culture of pluripotent cells from mouse embryos. *Nature,* 292:154-6.

Fortier, LA. 2005. Stem Cells: Classification, controversies and clinical applications. *Vet Surg.,* 34:415-23.

Garrett, S. 1996. Periodontal regeneration around natural teeth. *Ann Periodontol.,* 1(1):621-66.

Harold CSlavkin, P. and Mark Bartold. 2000. Challenges and potential in tissue engineering. *Periodontol.,* .2006;41:9-15.

Langer, R. and Vacanti, JP. May 14, 1993.". Tissue Engineering." *Science,* 260(5110):920-6.

Lin, NH., Gronthos, S. and Bartold, PM**.** 2008. Stem cells and periodontology. *Aust Dent J.,* 53:108-21.

Lin, NH., Gronthos, S. and Mark Bartold, P. 2000. Stem cells and future periodontal regeneration. *Periodontology,* 2009 ;51(1):239-51.

Motwani, BK., Singh, M. and Kaur, G. 2016. Stem cells: a new paradigm in dentistry. *J App Dent Med Sci.,* 2(1):139-45.

Newman, MG., Takei, HH. and Carranza, F. Textbook of *clinical periodontology.,* 11th edition.

Yokohama-Tamaki, T., Ohshima, H., Fujiwara, N., Takada, Y., Ichimori, Y., Wakisaka, S., Ohuchi, H. and Harada, H. 2006. Cessation of Fgf10 signaling, resulting in a defective dental epithelial stem cell compartment, leads to the transition from crown to root formation. *Development,* Apr 1;133(7):1359-66.

Yu, J. and Thomson, JA. 2006. Embryonic stem cells. In: National Institutes of Health (NIH) (ed). Regenerative medicine 2006. Bethesda: *Department of Health and Human Services*, 1–12.

Zhao, M., Jin, Q., Berry, JE., Nociti, FH Jr, 2004. Giannobile WV, Somerman MJ. Cementoblast delivery for periodontaltissue engineering. *J Periodontol.*, 75: 154–161

Zhu, W. and Liang, M. 2015. Periodontal ligament stem cells: current status, concerns, and future prospects. *Stem cells international.*

****