ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 3, May - 2021, Page No. : 449 - 458

Tissue Engineering: A Boon to Dentistry

[1]Dr. Maumita Bhattacharya, Senior lecturer, Dept. of Oral and Maxillofacial Pathology and Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital.

[2]Prof (Dr.) Jayanta Chattopadhyay, Dean & HOD, Dept. of Oral and Maxillofacial Pathology and Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital.

[3]Dr. Rakshith Shetty, Reader, Dept. of Oral and Maxillofacial Pathology and Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital.

[3]Dr. Subhalakshmi Sen, Senior Lecturer, Dept. of Oral and Maxillofacial Pathology and Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital.

[5]Dr. Surajit Bose, Senior Lecturer, Dept. of Oral and Maxillofacial Pathology and Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital

[6]Dr. Anchal Agarwal, Senior Lecturer, Dept. of Oral and Maxillofacial surgery, K.D. Dental College, Mathura, UP

[7]Dr. Abhishek Rathi, Reader, Dept. of Oral and Maxillofacial Surgery, Institute of Dental Studies and Technologies, Modinagar, UP

Corresponding Author: Dr. Abhishek Rathi, Reader, Dept. of Oral and Maxillofacial Surgery, Institute of Dental Studies and Technologies, Modinagar, UP

Citation of this Article: Bhattacharya M, Chattopadhyay J, Shetty R, Sen S, Bose S, Agarwal, A, Rathi A, "Tissue Engineering: A Boon to Dentistry", IJDSIR- May - 2021, Vol. – 4, Issue - 3, P. No. 449 – 458.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Tissue engineering is amongst the latest technologies having impacted the field of dentistry. Initially considered as a futuristic approach, tissue engineering is now being successfully applied in regenerative surgery. This article reviews the important determinants of tissue engineering and how they contribute to the improvement of wound healing and various surgical outcomes in the oral region. Furthermore, we shall address the clinical applications of engineering involving oral and maxillofacial pathologies and surgical procedures along with other concepts that are still in the experimental phase of development. This knowledge will aid the surgical and engineering researchers to comprehend the collaboration between these fields leading to its applications in dentistry and to ever-continuing man-made miracles in the field of human science.

Keywords: tissue engineering, dental stem cells, guided tissue regeneration, scaffold

Introduction

Mankind is advancing beyond the ability to create inanimate objects, towards the capability of replacing and regenerating living tissues. Tissue engineering is an emerging field of science aimed at developing techniques for the fabrication of new tissues to replace damaged or lost tissue organs of living organisms; and is based on the principles of cell biology and biomaterial sciences.

The current research on dental stem cells is expanding at an unprecedented rate. Henceforth, the emerging field of 'personalized medicine' has become quite popular, which refers to new medical technologies derived from aa patient's own stem cells and the use of genomic diagnostics.[1]

Langer and Vacanti defined tissue engineering as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" (Langer and Vacanti, 1993).[2]

In basis, TE attempts to mimic the function of natural tissue. Therefore, to optimize the development of functional biological substitutes, the natural circumstances of the specific tissues have to be fundamentally understood. Biological tissues essentially consist of cells, signaling systems and extracellular matrix (ECM). The cells are the core of the tissue, however, cannot function in the absence of signaling systems and/or of ECM. The signaling system consists of genes that secrete transcriptional products when differentially activated and urges cues for tissue formation and differentiation. The ECM is a meshwork-like substance within the extracellular space and supports cell attachment and promotes cell proliferation. [3,4].

Every year, a huge number of teeth are lost due to dental caries, trauma or periodontal diseases. Every dental surgeon wishes to regenerate lost teeth or their components. Tissue engineering is based on combining stem cells, growth factors and scaffolds for regenerating diseased or lost tissues. The contribution of scaffolds in tissue regeneration is indispensable as they serve as carriers to facilitate delivery of stem cells and/or growth factors at a local receptor site.

Scaffold biomaterials are classified as natural or synthetic, rigid or non rigid and degradable or non degradable. Polymers are extensively used biomaterials for constructing scaffolds and their differential characteristics are attributed to the differences in their composition, structure and arrangement of constituent macromolecules. Researches currently are directed towards tailoring the design characteristics of biomaterials such that precisely guided, coordinated, well timed interactions between the epithelial mesenchymal stem cells and scaffold matrix are encouraged.[5-7]

Fundamental elements for tissue engineering

For tissue regeneration, the integration of three elements is required, namely: the cells, scaffolds or extracellular matrix, and growth factors.

Cells

Tissue regeneration requires specialized cells capable of synthesizing the extracellular matrix specific for each tissue. Stem cells have been extensively used in regenerative medicine, since they are non-specialized and are capable of self-renewal and cell differentiation.

Stem cells may be classified according to their nature, into embryonic or adult types. Embryonic stem cells are pluripotent and apt to differentiate into cells of the three germ layers (ectoderm, mesoderm and endoderm) that consequently, may differentiate into multiple tissue cell types (Figure-1).[6]

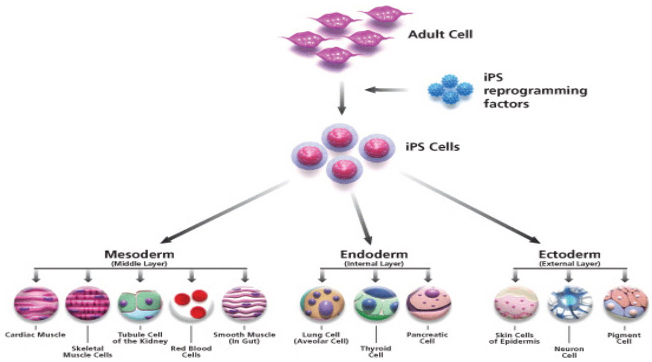

Figure 1: Cell of three germinative layer

However, in spite of this high potential differentiation, these cells raise ethical questions, such as their retrieval and possible tumorigenic potential, making it difficult to use them. Adult cells have been more frequently studied in regenerative medicine, because they are readily available, in addition to being capable of differentiating into various cell types, such as osteoblasts, chondrocytes, myoblasts, hematopoieticand neural cells, originating bone, cartilage, muscle, vascular and nerve tissue, respectively. These cells may be found in bone marrow, adipose tissue, umbilical cord and in dental tissues. With reference to dental tissues, various types of adult cells have been isolated from the periodontal ligament, papillae, primary and permanent tooth pulp. These are considered promising alternative for tissue engineering.[7]

The stem cells of dental origin, also denominated dental mesenchymal stem cells -DMSCs, perform an important function in dental repair, homeostasis, and present characteristics similar to those originated in bonemarrow. Moreover, these cells are denominated according

to their tissue of origin, such as stem cells from human exfoliated deciduous teeth – (SHED); dental pulp stem cells – (DPSCs); periodontal ligament stem cells – (PDLSCs), among others.

The stem cells found in primary tooth pulp present high potential differentiation into odontoblasts, osteoblasts, adipocytes and chondrocytes. And cells derived from pulp of permanent teeth, specifically third molar, may differentiate into odontoblasts, adipocytes, chondrocytes and myoblasts. Both primary and permanent tooth sources of stem cells maybe used for endodontic and bone regeneration. Whereas the stem cells found in periodontal ligaments differentiate into osteoblasts, cementoblasts and fibroblasts, and can beused in regenerating both periodontal ligaments and bone tissues.[8]

Scaffolds or extracellular matrix

Scaffolds are biomaterials with two-dimensional or three-dimensional architecture that provide the cells with an adequate environment, making it possible for them to migrate, proliferate and differentiate. In addition, they allow the transportation of nutrients, oxygen andcellular metabolic residues, making them a crucial element for tissue regeneration. [9]

To be considered ideal, biomaterial needs to possess some characteristics, such as biocompatibility, biodegradability, good mechanical properties, and a porous structure. Biocompatibility is one of the main characteristics since it prevent immunological, cytotoxic and inflammatory reactions by the body, which impede the regenerative performance of the biomaterial.[9]

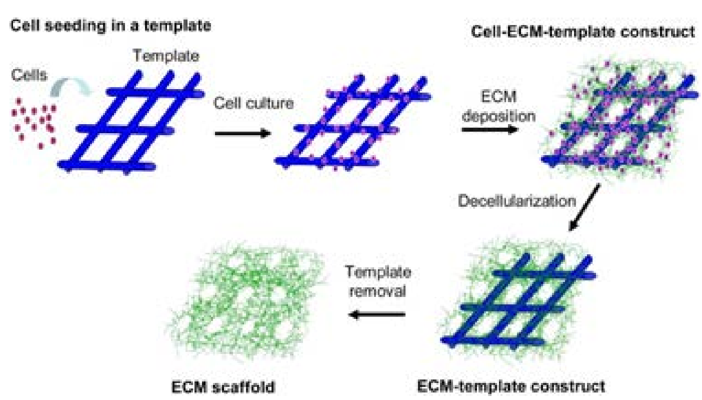

Figure 2: Extracellular matrix formation process

Moreover, the capacity for biodegradability allows the cells of the body itself to degrade the scaffold, and to make space for new tissue. So scaffolds need to have good mechanical properties and compatibility with the tissue or organ to be replaced. Also scaffolds must have pores with an adequate size and dispersion ability, since these features ensure cell penetration, diffusion of the nutrients required by the cells, vascularization of the site and removal of the residual products by taking them out of the structure. However, materials with these characters continue to be a challenge to tissue engineering.[10]

The choice of material for fabricating the scaffold is also an important factor for it to be successful. Different types of materials are used like ceramics, metals, polymers. Amongst all, polymers have gained emphasis because they are biodegradable and have great flexibility. Polymers may be of natural origin, and be composed of collagen, fibrin and hyaluronic acid; synthetic, made of polymer compounds such as Poly (lactic acid) (PLA), polyglycolic acid (PGA) andits poly copolymer (Poly(Lactide-co-Glycolide) acid) (PLGA);or hybrids (natural and synthetic).[11] However, synthetic materials have shown a higher success rate when compared with the natural types, because they provide versatility when creating the scaffold architecture, allowing control of the mechanical, physical and degradation properties. [12]

There are various techniques involved in the fabrication of scaffolds, such as lyophilizing, phase separating, foaming, rapid prototyping, and electro spinning, among others. However, the electro spinning technique has advantages compared with other techniques, because it is easy to perform, versatile, and allows better control of pore fabrication.[12]

Growth factors

Growth factors are extra cellularly secreted proteins that stimulate cell growth and bind to specific receptors in the cell membranes. Also they have the capacity to regulate cell growth, development proliferation and migration.

Different growth factors have been used in tissue engineering, among them are the bone morphogenetic proteins (BMP); Hedgehog proteins (SHH); fibroblast growth factor (FGF); interleukins; tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF).

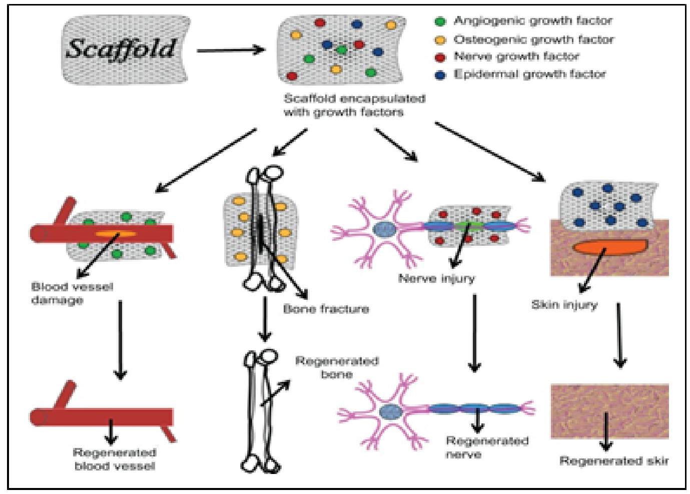

Figure 3: Growth factor effect on different parts of body.

Among these signaling molecules, the BMPs is studied and applied for dental regeneration. The BMPs can be divided into four families: the first are BMP-2 and -4; second, BPM-3 and -3B; the, BMP-5,-6, -7 and -8; and lastly, the growth/ differentiation factors- GDF-5, -6 and -7. These proteins play an important role in tooth development, and are associated with the differentiation of odontoblasts and ameloblasts, and in the development of cement and alveolar bone. In one study, using an animal model of periodontal disease, the regeneration and healing potential of BMP-6 was evaluated in periodontal defects. After the onset of periodontal disease, BMP-6 was applied to the affected regions and maintained there for 8 weeks. At the end of the study, the authors observed that the application of BPMs-6 in the areas of bone loss increased healing and the boneand ligament regeneration, demonstrating the importanceof this protein in regenerative dentistry.[13-15]

Engineering of mineralized tissues and soft tissues

Bone

Bone regeneration is perhaps the most widely investigated application of tissue engineering. Guided tissue and bone regeneration in periodontal and oral implant therapy, sinus augmentation, distraction-osteogenesis, reconstruction of oral and maxillofacial bone defects (resulting from trauma, tumour, infections, biochemical disorders, congenital defects, disease or abnormal skeletal development) are some of the clinical situations in which surgical intervention is required.[16]

Bone grafts can either be autologous, allogeneic, xenogeneic or alloplastic. Autogenous graft material from the iliac crest has long been considered as having the greatest potential for osseous regeneration. A patient's own bone, lacks immunogenicity and provides bone-forming cells, which are directly delivered at the defect site and regenerate bone or induce the surrounding cells to form bone.

The major drawbacks of autologous bone are their limited availability, need for additional surgical site and donor site morbidity. Allografts and xenografts overcome these drawbacks. However, disease transmission and immune rejection remain substantial obstacles to their implementation. Besides these, synthetic materials have also been investigated extensively as bone substitutes. While many of these materials serve as scaffolds for new bone, their treatment effect has been inconsistent across studiesand they appear to mostly produce bone repair rather than regeneration. [17]

To overcome the drawbacks of the current bone graft materials, bone tissue engineering using mesenchymal stem cells and bioactive molecules has been suggestedas a promising technique for reconstructing bone defects. Constructs for bone regeneration have been designed based on tissue-engineering principles and comprise three-dimensional scaffolds that function as a carrier for cells or bioactive molecules or both. In the cell-based constructs, living osteogenic cells are carried on scaffolds to the bony defect site to allow the development of a three-dimensional tissue structure. These seeded cells, with the potential for recruiting or differentiating into bone-forming cells, have led to successful bone formation in the management of periodontal defects, oral implant therapy, sinus augmentations and oral and maxillofacial defects. [18]

Some of the commercially available tissue engineering systems combining the signaling molecules and the scaffolds are platelet derived growth factor-BB-tricalcium phosphate (GEM21S®; OsteoHealth, Shirley, NY,USA), bone morphogenetic protein-type I collagen sponge (INFUSE®; MedtronicSonfamoreDanek, Memphis, TN, USA) and 15 amino acid sequenced type I collagen (PepGen P-15; DentsplyFriadent, Mannheim, Germany). [16]

A potential drawback of the bioactive factor-based approach is that high, supraphysiologic concentrations of these factors are needed to achieve the desired osteo-inductive result, with possible related side effects and high costs.[20]

Teeth

The goal of modern restorative dentistry is to functionally and cosmetically restore diseased/lost tooth or tooth structure. Tooth structure lost due to decay or trauma is most often replaced by restorative materials. Though these conventional restorative materials have proven to be highly effective at preserving teeth, they have a limited lifespan ultimately requiring replacement. Hence, regeneration of the lost tooth structure, as opposed to repairing/replacing, would have significant benefits.[25]

The lack of any enamel forming cells in the enamel of fully developed teeth precludes the potential for cell-based approaches for enamel regeneration. In contrast, the regeneration of dentin is feasible (if the pulp tissue is still vital and not irreversiblyinflamed) because dentin is in intimate contact with an underlying highly vascular and innervated pulpal tissue, forming a tightly-regulated "dentin-pulp complex". [26]

In the tooth replacement options, dental implants are amongst the most advanced alternative for replacing lost or missing teeth. However, factors that interfere with osseointegration can lead to failure of the implants. With advances in stem cell biology and tissue engineering, biological teeth may become an alternative for replacing missing teeth. The idea is to cultivate stem cells with odontogenic induction signals through epithelial-mesenchymal interactions, thereby programming the stem cells to adopt dental lineages and, with the help of scaffold/extracellular

matrix, to become part of a tooth. Duailibi et al. seeded cultured tooth germ cells on biodegradablescaffolds which were then implanted to bioengineer tooth tissues, while Ohazamaet al. generated tooth structures from non-dental mesenchymal cells placed in contact with embryonic oral epithelium and transplanted to an ectopic site.[27,28]

Skin and Oral Mucosa

The most successful application of tissue engineering to date is the development of skin equivalents. In dermatology, the ability to produce large amount of dermal-epidermaltissue from a small portion of the patient's skin in a short amount of time makes it possible to treat a variety of burns and chronic skin wounds. In and around the oral cavity, the need for soft tissue restoration/reconstruction is most often related to the lost gingival tissues and less frequently to the lost oral mucosa and skin in cases of disfigured tissues following severe burns, in radical resective surgery to treat invasive cancers, or in oral and maxillofacial traumatic wounds. The main goal of periodontalplastic and cosmetic oral surgery is to restore the aesthetics of the diseased or lost tissues.[29]

Many techniques have been developed over the years to restore the aesthetics of gingival and oral tissues. These include pedicle flaps, epithelialized/ nonepithelialized soft tissue autografts, or bilaminar techniques. Since they are autologous, the body does not reject these grafts. However, there are several problems associated with autologous grafts/flaps, including donor site morbidity, tissue shortage,and retention of the original characteristics of the donor tissue. Thus, clinicians have been interested in an alternative source for donor tissue. As in dermatology, tissue engineering is being explored to provide the replacement equivalent for oral tissues. Similar to oral hard tissues, there are various approaches to engineering oral soft tissues (Fig. 4).[16,30]

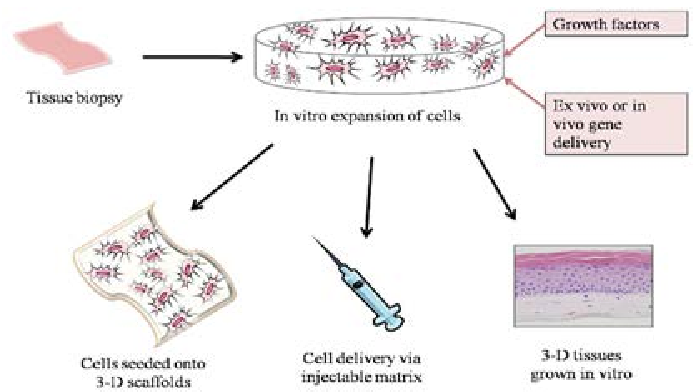

Figure 4: Tissue engineering approaches for oral soft tissues.

The desired cell types can be cultured expanded ex vivo and can be directly injected into the soft tissue defect site. They can be seeded onto porous scaffolds with or without the presence of bioactive factors and implanted into the defect area. Bilayered cell therapy is an example of a living product constructed of type1 bovine collagen and viable allogeneic human fibroblasts and keratinocytes which produce many growth factors. Another approach can be to grow three dimensional tissues ex vivo before being transplanted in vivo.[31]

The dental literature contains many reports on the applications of tissue engineeringtechniques to biopsy and grow patient's own cells on different scaffold materials, to be used as a substitute for soft tissue autografts. Tissue engineered oral equivalents havebeen explored for the management of gingival recession, inadequate keratinized gingiva, interdental papillary loss, preprosthetic surgery, and in oral and maxillofacial reconstructive surgery. Unlike skin, tissue-engineered human oral mucosa has not yet been commercialized for clinical applications. However, studies with favorable histologicaland clinical results have been carried out with tissue-engineered oral mucosa for intra- and extra-oral treatment, in the pursuit of the best predictable method of regenerating the lost soft tissues.[32]

Future Directions

Tissue engineering represents one of the most promising advances in regenerative medicine. The ability to produce new tissues and organs from the patient's own cellshas changed treatments and prognoses for numerous patients. It provides several advantages over traditional methods of treatment. It can provide unlimited source ofgraft material from a small donor site with minimal discomfort to the patient. However, the fact remains that the in vitro environment, where these tissue engineering constructsare developed, are technically different from the actual environment in the defect site in the body as they lack several systemic controls/components involved in homeostaticregulation in vivo. There are some questions open to speculation regarding what happens when these ex-vivo-developed tissue constructs are placed from the apparently stable culture environment to the more dynamic in vivo environment. Do these ex vivo expanded tissue cells adapt to the surrounding environment and remain viable throughthe crucial post-operative healing period? Or do these cells succumb to the biological and systemic influences to which they have not been subjected to in vitro? Is the new tissue formed by the implanted cells per se or by the surrounding induced or preexistingcells?[41] Further studies are necessary to maximize cell viability, optimize total cell density,optimize the bioactive molecule dose, control its delivery rate and understand the binding kinetics of the biofactors. Studies are required to design scaffolds with chemical compositions, pore sizes, and surface characteristics that allow cells tomaintain their optimum tissue-forming potential. The degradation kinetics of polymer based delivery systems need to be explored to enable control of the release profile ofgrowth factors, in order to achieve optimized concentrations of growth factors, which is a primary goal of these systems. Also, one of the most challenging issues is to understand how the various components involved in engineering tissues can beintegrated to produce predictable tissue regeneration. More research should also bedirected to the issue of how to achieve rapid vascularization of the tissue-engineeredconstructs to optimize the survival of the tissue-engineered graft. Regeneration of tissues by tissue engineering is an inevitable therapy, and continuing collaborative efforts among research scientists, engineers, funding agencies, and dental professionalsare required to pool resources to hasten its development. The unleashed potential ofregenerative therapy may benefit millions of patients each year.[42]

Conclusion

Medical research is endlessly exciting, by its very nature, continuously uncovering new facts and principles that build upon existing knowledge to modify the way we think about biological processes. In the history of science, certain discoveries have indeed transformed our thinking and created opportunities for major advancement. The isolation of pluripotent stem cells has revolutionized the field of medical science.

The aim of regenerative medicine is to stepwise recreate in-vitro all the mechanisms and processes that nature uses during initiation and morphogenesis of a given organ. Regenerative medicine has become a fashionable field and the isolation and manipulation of ESC and ASC for the creation of new functional organs will replace the missing or defective organs constitutes and enormous challenge.

The gold standard in tissue engineering for the reconstructive maxillofacial surgeon is a "made to measure" composite graft of skin, bone, cartilage, and muscle, with associated nerve and blood vessels, which is engineered from host stem cells. To achieve this, tissue engineers need improved biomimetic scaffolds, the ability to prevascularise three-dimensional tissue constructs, and to engineer the complex interface of the different facial tissues. Tissue engineering promises to provide a credible surgical option for the reconstruction of maxillofacial defects and the role of the oral and maxillofacial surgeon is to drive forward research to provide a firm evidence base for its future use.

Acknowledgement

The authors would like to thank the Department of Oral & Maxillofacial Pathology, Kusum Devi Sundarlal Dugar Jain Dental College & Hospital, Kolkata for giving the opportunity to study and write the article.

References

1. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J. In-vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein I after subcutaneous transplantation in mice. J Endodont 2008K 34:421-426.
2. Langer R, Vacanti JP. Tissue Engineering. Science 1993k260:920-926.
3. Vacanti CA. History of Tissue Engineering and A Glimpse Into Its Future. Tissue Engineering 2006;12-:1137-1142.
4. Fuchs JR, Nasseri BA, Vacanti JP. Tissue engineering: A 21st century solution to surgical reconstruction. Annual Torac Surgery 2001; 72:577-591.
5. Lanza RP, Langer RS, Vacanti J. Principles of tissue engineering. 2nd ed. San Diego, CA:Academic Press, 2000.
6. Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007;28:3587-3593.
7. BlitterswijkCAv, Thomsen P. Tissue engineering. 1st ed. Amsterdam;Boston: Elsevier/Academic Press, 2008.
8. Bianco P, Riminucci M, Gronthos S, GehronRobey PG. Bone marrow stromal stem cells: nature, biology and potential applications. Stem Cells 2001; 19:180–192.
9. Fuchs E, Segre JA. Stem cells: A new lease on life. Cell 2000; 100:143-155.
10. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: Entity or function? Cell 2001; 105:829-841.
11. Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, et al. Molecular and cellular characterization of highly purified stromal stem cells derived from human bone marrow. J Cell Sci2003; 116:1827–1835.
12. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell in vitro. BMC Cell Biol2006; 7:14.
13. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002; 81:531–535.
14. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs those from other sources: their biology and role in regenerative medicine. J Dent Res 2009; 88(9):792-806.
15. Seo B, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal

stem cells from human periodontal ligament. Lancet 2004; 364:149–155.

16. Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 2005; 84(10): 907–912.
17. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontol 2000 2006a; 40:164–172.
18. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. ProcNatlAcadSci USA 2003; 100:5807–5812.
19. Tomar GB, Srivastava RK, Gupta N, Barhanpurkar AP, Pote ST, Jhaveri HM, et al. Human gingivaderivedmesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for celltherapy in regenerative medicine. Res Commum BiochemBiophys 2010; 393(3):377-83.
20. Zhang QZ, Su WR, Shi SH, Wilder-Smith P, Xiang AP, Wong A, et al. Human gingiva-derivedmesenchymal stem cells elicit polarization of M2 macrophages and enhance cutaneous wound healing.Stem Cells 2010; 28(10):1856-68.
21. Mitrano TI, Grob MS, Carrión F, Nova-Lamperti E, Luz PA, Fierro FS, et al. Culture andcharacterization of mesenchymal stem cells from human gingival tissue. J Periodontol2010;81(6):917-925.
22. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell mediatedfunctional tooth regeneration in swine. PLoS ONE 2006; 1(1):e79.
23. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: thepotential role in pulp/dentin regeneration and bioroot engineering. J Endod2008; 34:645-651.
24. Kim BS, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissueengineering. Trends Biotechnol1998; 16:224-30.
25. Kao RT, Murakami S, Beirne OR. The use of biologic mediators and tissue engineering in dentistry.Periodontol 2000 2009; 50:127–153.
26. Bartold PM, Xiao Y, Lyngstaadas SP, Paine ML, Snead ML. Principles and applications of celldelivery systems for periodontal regeneration. Periodontol 2000 2006; 41:123–135.
27. Tabata Y. Tissue regeneration based on growth factor release. Tissue Eng2003: 9(Suppl. 1):S1–S15.
28. Boontheekul T, Mooney DJ. Protein based signaling systems in tissue engineering. Curr Op Biotechnol 2003; 14:559-565.
29. Wang AY, Leong S, Liang Y, Huang R, Chen C, Yu S. Immobilization of growth factors on collagenscaffolds mediated by polyanionic collagen mimetic peptides and its effect on endothelial cellmorphogenesis. Biomacromolecules2008; 9:2929-2936.
30. Yang S, Leong K-F, Du Z, Chua C-K. The design of scaffolds for use in tissue engineering. Part 1. Traditional factors. Tissue Eng2001; 7:679–689.
31. Habers GM, Healy KE. The effect of ligand type and density on osteoblast adhesion, proliferation, andmatrix mineralization. J Biomed Mater Res 2005; 15:855–869.
32. Park J, Lee J, Park Y, Rhee S, Lee S, Kim T, Seol Y, Lee Y, Ku Y, Rhyu I, Han S, Chung C. Enhancedbone regeneration in beagle dogs with bovine bone mineral coated with a synthetic oligopeptide. JPeriodontol2007; 78:2150–2155.
33. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. Periodontol 1999;19:40-58.

34. Sarment DP, Cooke JW, Miller SE, Jin Q, McGuire MK, Kao RT, et al. Effect of rhPDGF-BB on boneturnover during periodontal repair. J ClinPeriodontol2006; 33(2):135-40.
35. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/IIclinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB andrecombinant human insulin-like growth factor-1 in patients with periodontal disease. J Periodontol1997; 68:1186-1193.
36. Ito K, Yamada Y, Naiki T, Ueda M. Simultaneous implant placement and bone regeneration arounddental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and plateletrichplasma. Clin Oral Implants Res 2006; 17(5):579–586.
37. Wikesjo UM, Razi SS, Sigurdsson TJ, Tatakis DN, Lee MB, Ongpipattanakul B, et al. Periodontalrepair in dogs: effect of recombinant human transforming growth factor-beta1 on guided tissueregeneration. J ClinPeriodontol1998; 25:475-481.
38. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. ActaHaematol2001; 106:148-156.
39. Fowlkes JL, Thrailkill KM, Liu L, Wahl EC, Bunn RC, Cockrell GE, et al. Effects of systemic andlocal administration of recombinant human IGF-I (rhIGF-I) on de novo bone formation in an agedmouse model. J Bone Miner Res 2006; 21(9):1359-66.
40. Barboza E, Cau´ la AL, Cau´ la F, Oliveira de Souza R, Neto LG, Sorensen RG, et al. Effect ofrecombinant human bone morphogenetic protein-2 in an absorbable collagen sponge with spaceprovidingbiomaterials on the augmentation of chronic alveolar ridge defects. J Periodontol2004;75:702–708.
41. Jung RE, Glauser R, Scharer P, Hammerle CH, Sailer HF, Weber FE. Effect of rhBMP-2 on guidedbone regeneration in humans. Clin Oral Implants Res 2003; 14:556–568.
42. Kim SJ, Shun HS, Shin SW. Effect of bone block graft with rhBMP-2 on vertical bone augmentation.Int J Oral MaxillofacSurg 2010; 39(9):883-8.