ECRONICON OPEN ACCESS

DENTAL SCIENCE
Research Article

The Osteoinductive Properties And Influence of "Emdogain" (Straumann) on Colony-Forming Properties of Human Osteogenous Progenitor Bone Marrow Cells *Ex vivo*

Biloklytska G F[1], Panchenko L M[2] and Braun I E[1]*

[1]*Department of conservative dentistry, National medical academy of postgraduate education named after P L Shupyk, Ukraine*

[2]*Institute of traumatology and orthopedics of National Academy of Medical Sciences of Ukraine, Ukraine*

***Corresponding Author:** Braun Iuliia, Department of conservative dentistry, National Medical Academy of Post-Graduate Education named after P L Shupyk, Dental Scientific Medical Center, Kyiv, Ukraine.

Received: December 23, 2014; **Published:** December 31, 2014

Abstract

Generalized periodontitis (GP) is widespread disease among working category of patients that is very actual for Ukraine where periodontology still is not recognized specialty. Modern data demonstrate presence of connection and influence of GP on development of chronic diseases that is very important point for general health care of citizens, especially of Ukraine. According to Biloklytska G., an effective treatment of GP for citizens of Ukraine is among priorities, where statistics of disease prevalence shows very high percent of patient`s involvement. Growing tendency among our patients revealed fact of great want of patients to leave natural tooth on place instead of extraction. This fact enforces practicing dentists to search for more favor and biology based treatment techniques of GP. Especially it is important during usage of additional materials and medications for periodontal regeneration. This means that all used modern materials should fulfill all the demands, especially when we deal with regenerative periodontology, when the periodontal regeneration highly expected. Usage of enamel matrix proteins in periodontology is still very perspective promoting periodontal regeneration, influencing on different parts of periodontal wound regeneration. Also the regeneration of bone tissue as component of periodontal complex can be enhanced due to biological mechanisms support. In the review the preliminary data about details of occurrence of osteoinductive properties of "Emdogain" (Straumann) and the influence on colony forming properties of osteongenous bone marrow cells will be showed. The preliminary results will be showed and discussed.

Keywords: *Generalized periodontitis; Periodontal regeneration; Enamel matrix proteins; Emdogain; amelogenin; Osteogenous progenitor human bone marrow cells; Colony forming activity; Osteinduction*

Abbreviations: GP: Generalized Periodontitis; PD: Periodontal Disease; PDL: Periodontal Ligament; EMPs: Enamel Matrix Proteins; APP: Acute Phase Proteins; HERS: Hertwig`s Epithelial Root Sheath; ALP: Alkaline Phosphatase; OC: Osteocalcin; TGF-β: Transforming Growth Factor β; CTGF: Connective Tissue Growth Factor; VEGF: Vascular Endothelial Growth Factor; HMVECs: Human Microvascular Endothelial Cells; BMP: Bone Morphogenetic Protein; BSP: Bone Sialoprotein; OPG: Osteoprotegerin; CFFU: Colony-Forming Fibroblast Units

Introduction

Periodontal disease (PD) involves the chronic and progressive destruction of the tooth supporting tissues, mainly the periodontal ligament (PDL), root cement and alveolar bone, whose regeneration is most complicated and under present scientific interest [1-5].

This fact can be explained by all collected data about complex structure and functional details of these data [1-8]. However tooth loss as the most widespread consequence that means exact data about all regenerative mechanisms are absent [3-5,9,10]. A major discovery was the finding that periodontal tissue regeneration could be achieved by application of acidic extract of enamel matrix proteins (EMPs), and later- by the FDA approved medical device termed Emdogain [3-5,11-14]. It is well also known that PD cause chronic and progressive destruction of the tooth supporting tissues, which attaches the tooth to the alveolar bone of the jaw, leading to progressive bone resorption process [3-5,13,15]. But the complete periodontal regeneration is still under plural investigations, profound investigations are not able to supply understanding of all cell-to-cell regenerative mechanisms [1,2,5,7,14-18]. Also regeneration of root cement is among main scientific interest, having different cement types, cells whose function and genesis are still unknown [1,2,5,7,14,18].

And regeneration of the periodontium is a major goal in the treatment of teeth affected by periodontitis [1,2,5,7,14,17,19]. It was demonstrated, in the periodontal regeneration model, migration of cells from the adjacent unwounded PDL into the wounded area [16], bone marrow stromal cells also have the potential to regenerate the periodontium [20]. It was demonstrated that amelogenin stimulates cascade of events due to remodeling and regeneration of periodontal tissues [3,4,7,14]. Another data showed the expression of amelogenin mRNA isoform-M180 in macrophages, suggesting the endogenous expression of amelogenin in osteoclasts [21]. Also authors suggested that during alveolar bone regeneration after induced inflammation, amelogenin might act by increasing bone formation over resorption process. It may function in the fine balance between bone formation by osteoblasts and bone resorption by the osteoclasts, possibly through the RANKL/RANK mediated pathway [22,23]. Amelogenin expression is highest in bone cells adjacent to PDL and in the periosteal cells, as compared to bone cells distal to the PDL and the periosteum [3,4]. This can appear because the bone adjacent to the PDL absorbs all the mastication forces and bone turnover, remodeling are much higher in this area, as compared to bone remote to the PDL region [3-5]. Periosteum is the main site of bone growth and remodeling, and it contains mainly progenitor cells [5]. The findings from animal study on dogs suggest that in the normal uninjured animal, amelogenin expression is increased at sites of high bone activity and remodeling [3]. A similar phenomenon was seen in the regenerated alveolar dog bone; amelogenin was highly expressed in endosteal cells, osteoblasts, and cells surrounding blood vessels in the regenerated area, and to a much lesser extent in bone cells distal to the regenerated area [4]. Authors detected amelogenin expression both in osteoclasts and in osteoblasts lining the alveolar bone trabeculae [3,4].

Pericytes whose are involved in regulating of angiogenesis, differentiate along several lineages including osteogenic and chondrogenic phenotypes. The finding that amelogenin might promote angiogenesis in the regenerating tissues explained in *in vitro* and *in vivo* findings demonstrating chemotaxis of endothelial cells and induction of blood vessel formation by EMPs [24,25]. Chronic periodontal disease is similar to systemic inflammation characterized by the mediators such as acute phase proteins, inflammatory cytokines as IL-6 and coagulation factors as a fibrinogen [19,17]. PD could be the case of the generalized acute phase response [9,10]. Among others also changes in the level of numerous serum proteins occur these proteins are therefore called acute phase proteins (APP) [9]. Several biological activities of APPs were described e.g., enhancement of nonspecific response complement activation, involvement in blood clotting and fibrinolytic activities defence against iron lost [9,10]. Some APPs participate in limiting the inflammatory reaction and in clearance of its products, the facilitate phagocytosis; participate in repair processes by influencing fibroblast activity and reorganization of new developed connective tissue healing wound. They may also affect activity of the immune cells [8-10,16]. Oxidative stress also is postulated to be increased in patients with periodontitis and oral mucosa diseases. It is suggested that oxidative cell injury caused by reactive oxygen species contributes to the development of inflammatory changes of oral mucosa [9]. Knowing the fact that Hertwig`s epithelial root sheath (HERS) cells have synthetic activity, they also secrete molecules into extracellular milieu [1,2]. While some studies showed EMP expression on the developing root surface [1,2,7,14], others have claimed that HERS` cells produce cementum proteins [1,2]. Based on findings of the occasional expression of EMPs along the forming root, the idea developed that EMPs plat pivotal role in the differentiation of progenitor cells into cementoblasts that specifically produce acellular extrinsic fibre cementum [7,14].

Systemic review and meta-analysis concluded that periodontal therapy does not result in a significant reduction of serum acute phase proteins levels [26], as well as alfa 1-antichymotrypsin [6], and alfa 1-acid glycoprotein [6]. Emdogain is an acidic extract of extracellular enamel matrix, and induces a heterogeneous mixture of mainly hydrophobic polypeptides encoded by several genes [7,14,27]. It was not clear however, which of the EMPs induce the regeneration, and what molecular mechanisms are involved [27]. It was not clear however, which of the EMPs induce the regeneration, and what molecular mechanisms are involved [27]. For decades amelogenin was thought to be exclusively an enamel (epithelial origin) protein. However, in more recent years, amelogenin has also been detected in dentin matrix [7,14,28], odontoblasts [29], in remnants of Hertwig`s root sheath and in PDL cells [30,31], in long bone cells some of which are multi-potent stem cells [3], osteocytes, osteoblasts, osteoclasts, cartilage chondrocytes, in growth plate cells [4]. The relatively large number of amelogenin alternatively spliced mRNA translated polypeptides and the fact that amelogenin is expressed in different tissues (calcifying and soft tissues) and of different embryonic origin, possibly reflect different functions of amelogenin [4]. Application of amelogenin induces local neovascularization to support the developing tissues, and possibly might change the fine balance between osteoclast and osteoblast activity, favoring regeneration over the natural process of degeneration after induction of chronic periodontitis in experiment. This degeneration process is characterized by connective tissue and epithelial proliferation into injured area, an event thought to prevent proper regeneration of the periodontal tissues [4]. The bone matrix is known to contain BMPs, highly potent growth/differentiation factors that induce the differentiation of progenitor cells into osteoblasts. However, the dentin matrix appears to have a higher osteoinductive capacity [1,2,32,33]. *In vitro* and *in vivo* studies showed the chondrogenic and osteogenic activities of recombinant amelogenin polypeptides [32,33]. These findings are in line with those obtained with enamel matrix and amelogenin [34,35]. However another authors showed that EMD failed to enchance proliferation of stromal osteoblastic cells obtained from the bone marrow of young adult male rats [12]. In contrast, treatment with EMD of human osteoblastic cells (SaM-1) from one patient [36] and of rat femoral bone marrow stroma [37], mouse preosteoblast cell line [38], significantly stimulated cell proliferation. However, when alveolar bone cells were used, no effect of EMD on cell proliferation was observed, but the bone cells showed the greatest attachment response to EMD [39]. In contrast, it was showed that growth of human mandibular osteoblasts from one patient was significantly increased by EMD [40]. In heterogeneous cell population from rat bone marrow, EMD had no significant effect on cell proliferation, alkaline phosphatase (ALP) activity, and mRNA expression of type I collagen, osteocalcin (OC) [18]. Using an organoid culture system with human primary osteoblasts it was observed a significant increase in cell proliferation [41]. It was observed that EMD promotes motility of different osteoblastic cell lines better than the control groups, whereas the proliferation rates depended on the cell type [42]. In another study where a chondrogenic cell line, EMD markedly increased cell proliferation [43]. It was showed that EMD stimulated proliferation of human bone marrow stromal cells in a dose-dependent manner [11]. EMD treatment of human osteoblastic cells (Saos-2) increased cell proliferation [44]. Using human bone marrow stromal cells, it was showed that EMD down regulated type I collagen synthesis and ALP activity, whereas the decrease in OC synthesis was not statistically significant. *In vitro* mineralization was reduced in EMD-treat cells [11]. Also authors showed that EMD up-regulated the release of TGF-β1 from primary rat osteoblasts into the culture medium [45]. Also when human osteoblastic cells (Saos-2) were treated with EMD, a dose-dependent increase in the expression of connective tissue growth factor (CTGF), a mediator of TGF-β1 was observed [44]. EMD-induced CTGF expression and in vitro mineralization were significantly reduced in the presence of TGF-β inhibitor and treatment with anti-CTGF antibody, respectively. Using the bone wound-healing model in rat femurs [46] and in rat parietal bone [47], authors noted a significantly higher bone volume fraction of newly formed bone volume trabeculae 7 days after injury in the EMD group compared with the control [48]. Also authors suggested that effects of EMD also depend on the local osseous environment. It was showed that exposure of different cell types like: PDL cells, gingival fibroblasts, osteosarcoma cell line on EMD, resulted in enhanced wound-fill rates [48,49]. The early healing stages showed more effect on PDL than of gingival fibroblasts. The effects of EMD on microvascular endothelial cells (HMVECs) were investigated and it was suggested that low concentrations resulted in significant stimulation of HMVEC proliferation, and HMVEC chemotaxis when PDL cells were present [50]. All doses tested increased angiogenesis. HMVECs in combination with EMD stimulated a 750% increase in PDL cell migration compared with controls.

Also it was determined at almost 400% increase in VEGF concentration by ALP-positive PDL cells and a significant increase in TGF-β production in both ALP-positive and ALP-negative PDL cells in EMD-stimulated conditioned media [50]. Also authors demonstrated that in human PDL cells EMD down-regulates the expression of genes involved in early inflammatory events of wound healing, whereas genes encoding growth and repair-promoting molecules were up-regulated [51]. The increased expression of BMP-2, BMP-7, BSP, cementum attachment protein-23 (CP-23) was noted and two putative cementum markers [52]. EMD significantly stimulated total protein synthesis by human PDL fibroblasts [49]. EMD exposure to human PDL fibroblasts resulted in significantly enhanced osteocalcin (OC) and osteoprotegerin (OPG) levels [8].

Even if it was showed existence of not significant statistical difference in between groups where EMD were applied or not [9,10], better tendency for healing in EMD treated part of periodontium [13], details of osteoinductive properties of this material can be very useful for practical usage. Also regeneration of bone and its enhancement are important for periodontal regeneration, especially the knowledge about human bone marrow cells behavior under application of EMP can be important for practical day by day usage in different clinical cases.

Aim

To investigate presence of osteoinductive properties of "Emdogain" (Straumann) and evaluate the influence on osteogenous progenitor cells- colony-forming fibroblast units (CFFU) of human bone-marrow *ex vivo.*

Materials and methods

Cloning of CFFU of human bone marrow was provided according to methodic of Fridenshtein and Lalikina [53] in modification of Astachova [54]. For investigation the cancellous iliac bone was taken from healthy patients out of inflammatory and degenerative-dystrophic lesions during orthopedic surgical operation.

The cancellous bone for investigation was take in the conditions of operating room, put into container with feeding solution "199". Further processing of the collected material was provided in laboratory box under sterile conditions. The cell cloning procedure was taken under standard conditions during 14 days without changing of feeding solution in Ru-containers (culture containers) under temperature of 37°C in gaseous blend containing 5% of CO_2 in atmosphere oxygen with usage of lethally irradiated rabbit bone marrow cells in role of feeder.

For investigation of direct action of "Emdogain" (Straumann), "Pre-Gel" (Straumann) *ex vivo* 4 series experiments of cloning CFFU of human bone marrow were provided. In 1st experimental group into culture container during explantation of bone marrow cells "Pref-Gel" (Straumann) was added. In 2nd experimental group "Pref-Gel" (Straumann) and "Emdogain" (Straumann) were added into culture container during explantation of bone marrow cells. In 3rd experimental group only "Emdogain" (Straumann) was added into culture container. In 4th experimental group, as a control group, the cloning of CFFU was provided without adding of any additional material.

The regeneration potential of bone tissue was assessed according to value of cloning effectiveness of CFFU of human bone marrow among 10^5 nuclear containing cells.

The cloning effectiveness was assessed according formula:

Where K - means amount of colonies that grew up in cultural container x 10^5; N - means amount of cells that were explanted into cultural container.

The statistics was provided according computer program analysis "Statistica". The middle values presented as M ± m, where M - the middle meaning of value, m - standard deviation of middle value meaning.

$$ECFFU = \frac{K}{N} \times 10^5$$

The 4 experimental series of CFFU cloning of human bone-marrow were provided: 1 group - with adding of etching gel "Pref-Gel"; 2 group - with adding of "Pref-Gel" with "Emdogain"; 3 - with adding of "Emdogain" only and 4 - without adding of any preparations (control). The action was evaluated according to cloning effectiveness of CFFU of human bone-marrow among 10^5 nucleus containing cells. 9 experimental and 6 control colonies were cultivated.

Results and Discussion

In 1st and 2nd experimental groups the growth of stromal fibroblasts wasn't detected. The effectiveness of CFFU cloning = 0. In 3rd group in the mean 145 colonies of CFFU grew up with cloning effectiveness of 15,10 ± 0,95 among 10^5 nucleus-containing cells. In control group in the mean 120 colonies of CFFU grew up. The cloning effectiveness was 12,48 ± 1,24 among 10^5 nucleus-containing cells. Ad oculus the colonies from control and investigated groups weren't differ from each other. "Emdogain" (Straumann) on 20,8% in comparison with control group, enhanced amount of CFFU colonies in bone-marrow, increasing specific gravity of multilayer colonies, giving evidence about its osteoinductive properties.

Conclusion

The presented investigation showed the direct action of "Emdogain" (Straumann) with additional components on osteogenous progenitor CFFU of human bone marrow of iliac bone ex vivo, the certain details of action were determined. The obtained data can be useful for regenerative periodontal surgical procedures. Adding of "Pref-gel" (Straumann) for etching procedure into culture of stromal fibroblasts of human bone marrow completely depress the colonies growth. "Pref-Gel" and combination of "Pref-gel" with "Emdogain" completely depresses proliferation and differentiation of CFFU in human bone marrow *ex vivo.* The cloning activity was absent. This means that EDTA for etching stage application, which is chemically active component of "Pref -Gel" (Straumann) influences negatively on vital cells of bone tissue that must be taken into consideration during practical usage. During application providing periodontal surgical operations having exposed bone in area of bone pockets, it may influence on vital cells leading to their death in area of operating periodontal wound.

Knowing the fact that according proper instruction guidelines it is recommended to etch the root surface previously to "Emdogain" application, it is need able to notice the importance, which can be questionable, of combined usage of etching procedure with EDTA and afterwards EMD application.

This situation can be explained with difficulties to prevent entering of etching gel-like EDTA into deep bone pocket with exposed bone after preparation procedures including influence of exposition timing. Also this detail is important while the bone pockets are containing and deep where in most of cases its need able to fill all pocket in aim to prevent root surface against blood coverage. From the other hand the obligatory usage of etching of root surface due to EDTA application is under contradiction and it is important to notice that additional protective measures for bone preservation against EDTA penetration should be provided otherwise.

In contrast "Emdogain" (Straumann) alone enhances growth of CFFU colonies in bone marrow due to increased specific gravity of multilayer colonies, giving evidence about existence of osteoinductive properties. Even if the grew colonies from CFFU were not differ from each other in different investigated groups, "Emdogain" (Straumann) strongly enhanced their formation in 20,8%, that can be assessed as strongly enhanced.

Profound knowledge about details of influence of EMD on bone tissue may provide developing of more precise and accurate periodontal surgical procedures with less invasion and tissue preservation. This may provide additional EMD usage in dental practice dealing with bone regenerative procedures, periodontal wound healing enhancement.

Bibliography

1. Bosshardt DD. "Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels". *Journal of Clinical Periodontology* 35.Suppl. 8 (2008): 87-105.
2. Bosshardt DD and A Nanci. "Hertwig`s root sheath, enamel matrix proteins, and initiation of cementogenesis in porcine teeth". *Journal of Clinical Periodontology* 31.3 (2004): 184-192.
3. Haze A., *et al.* "Amelogenin expression in long bone and cartilage cells and bone marrow progenitor cells". *The Anatomical Record* 290.5 (2007): 455-460.
4. Haze A., *et al.* "Regeneration of bone and periodontal ligament induced by recombinant amelogenin after periodontitis". *Journal of Cellular and Molecular Medicine* 13.6 (2009): 1110-1124.
5. Hagi TT., *et al.* "Regenerative periodontal therapy". *Quintessence International* 45.3 (2014): 185-192.
6. Glurich I., *et al.* "Systemic inflammation in cardiovascular and periodontal disease comparative study". *Clinical and Diagnostic Laboratory Immunology* 9.2 (2002): 425-432.
7. Hammastrom L., *et al.* "Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins". *Journal of Clinical Periodontology* 24.9 Pt 2 (1997): 669-677.
8. Lossdorfer S., *et al.* "Enamel matrix derivative promotes human periodontal ligament cell differentiation and osteoprotegerin production *in vitro*". *Journal of Dental Research* 86.10 (2007): 980-985.
9. Ide M., *et al.* "Effect of treatment of chronic periodontitis on evels of serum of acute-phase inflammatory and vascular responses". *Journal of Clinical Periodontology* 30.4 (2003): 334-340.
10. Kurhanska-Flisykowska A., *et al.* "Effectiveness of Emdogain in the periodontal treatment". *Przeglad Lekarski* 69.10 (2012): 1046-1048.
11. Guida L., *et al. "In vitro* biologic response of human bone marrow stromal cells to enamel matrix derivative". *Journal of Periodontology* 78.11 (2007): 2190-2196.
12. Gurpinar A., *et al.* "Effect of enamel matrix derivate on mouse fibroblasts and marrow stromal osteoblasts". *Journal of Biomaterials Applications* 18.1 (2003): 25-33.
13. Hagenaars S., *et al.* "Soft-tissue wound healing following periodontal surgery and Emdogain applicaton". *Journal of Clinical Periodontology* 31.10 (2004): 850-856.
14. Hammastrom L. "Enamel matrix, cementum development and regeneration". *Journal of Clinical Periodontology* 24.9 Pt 2 (1997): 658-668.
15. Miron RJ., *et al.* "*In vitro* evaluation of demineralized freeze-dried bone allograft in combination with enamel matrix derivative". *Journal of Periodontology* 84.11 (2013): 1646-1654.
16. King GN and FJ Hughes. "Bone morphogenetic protein-2 stimulates cell recruitment and cementognesis during early wound healing". *Journal of Clinical Periodontology* 28.5 (2001): 465-475.17.
17. Loos BG. "Systemic markers of inflammation in periodontits". *Journal of Periodontology* 76.11 Suppl (2005): 2106-2015.
18. Van den Dolder J., *et al.* "The effect of Emdogain on the growth and differentiation of rat bone marrow cells". *Journal of Periodontal Research* 41.5 (2006): 471-476.
19. Loos BG., *et al.* "Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients". *Journal of Periodontology* 71.10 (2000): 1528-1534.
20. Kawaguchi H., *et al.* "Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal sem cells". *Journal of Periodontology* 75.9 (2004): 1281-1217.
21. Deutsch D., *et al.* "Amelogenin, a major structural protein in mineralizing enamel, is also expressed in soft tissues: brain and cells of the hematopoietic system". *European Journal of Oral Sciences* 114.Suppl 1 (2006): 183-189.
22. Hatakeyama J., *et al.* "Amelogenin-mediated regulation of osteoclastogenesis, and periodontal cell proliferation and migration". *Journal of Dental Research* 85.2 (2006): 144-149.

23. Hatakeyama J., *et al.* "The receptor activator of nuclear factor-kappaB ligand-mediated osteoclastogenic pattway is elevated in amelogenin-null mice". *The Journal of Biological Chemistry* 278.37 (2003): 35743-35748.
24. Collett GD and AE Canfield. "Angiogenesis and pericytes in the initiation of ectopic calcification". *Circulation Research* 96.9 (2005): 930-938.
25. Yuan K., *et al.* "Enamel matrix derivate exhibits angiogenic effect *in vitro* and in murine model". *Journal of Clinical Periodontology* 30.8 (2003): 732-738.
26. Ionnidou E., *et al.* "Elevated serum interleukin (IL-6) in soloi-organ transplant recipients is positively associated with tissue destruction and IL-6 gene expression in the periodontium". *Journal of Periodontology* 77.11 (2006): 1871-1878.
27. Saito K., *et al.* "Amelogenin binds to both heparan sulfate and bone morphogenetic protein 2 and pharmacologically suppresses the effect of noggin". *Bone* 43.2 (2008): 371-376.
28. Nebgen DR., *et al.* "Identification of the chondrogenic-inducing activity from bovine dentin (bCIA) as a low-molecular-mass amelogenin polypeptide". *Journal of Dental Research* 78.9 (1999): 1484-1494.
29. Papagerakis P., *et al.* "Expression of amelogenin in odontoblasts". *Bone* 32.3 (2003): 228-240.
30. Fong CD and L Hammastrom L. "Expression of amelin and amelogenin in epithelial root sheath remnants of fully formed rat molars". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 90.2 (2000): 218-223.
31. Janones DS., *et al.* "Immunocytochemical examination of the presence of amelogenin during the root development of rat molars". *Archives of Oral Biology* 50.5 (2005): 527-532.
32. Veis A. "Amelogenin gene splice products: potential signaling molecules". *Cellular and Molecular Life Sciences* 60.1 (2003): 38-55.
33. Veis A., e*t al.* "Specific amelogenin gene splice in culture products have signaling effects on cells in culture and in implants *invivo"*. *The Journal of Biological Chemistry* 275.52 (2000): 41263-41272.
34. Urist MR. "Bone histogenesis and morphogenesis in implants of demineralized enamel and dentin". *Journal of Oral Surgery* 29.2 (1971): 88-102.
35. Wang W. "Ectopic bone induction by human fetal enamel proteins". *Zhonghua koukiang yixue zazhi* 28.6 (1993): 362-364. (Article in Chinese)
36. Mizutani S., *et al.* "Involvement of FGF-2 in the action of Emdogain on normal human osteoblastic activity". *Oral Diseases* 9.4 (2003): 210-217.
37. Keila S., *et al.* "*In vitro* effects of enamel matrix proteins on rat bone marrow cells and gingival fibroblasts". *Journal of Dental research* 83.2 (2004): 134-138.
38. He J., *et al.* "Emdogain promotes osteoblast proliferation and differentiation and stimulates osteoprotegerin expression". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* 97.2 (2004): 239-245.
39. Rincon JC., *et al.* "Enchanced proliferation, attachment and osteopontin expression by porcine periodontal cells exposed to Emdogain". *Archives of Oral Biology* 50.12 (2005): 1047-1054.
40. Galli C., *et al.* "Osteopretegerin and receptor activator of nuclear factor-kappa B ligand modulation by enamel matrix derivate in human alveolar osteoblasts". *Journal of Periodontology* 77.7 (2006): 1223-1228.
41. Pischon N., *et al.* "Effects of an enamel matrix derivate on human osteoblasts and PDl cells growth in organoid cultures". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* 102.4 (2006): 551-557.
42. Klein MO., *et al.* "*In vitro* assessment of motility and proliferation of human osteogenic cells on different isolated extracellular matrix components compared with enamel matrix derivate by continuous single-cell observation". *Clinical Oral Implants Research* 18.1 (2007): 40-45.
43. Narukawa M., *et al.* "Enamel matrix derivative stimulates chondrogenic differentiation of ATDC5 cells". *Journal of Periodontal Research* 42.2 (2007): 131-137.
44. Heng NH., *et al.* "Enamel matrix derivative induces connective growth factor expression in human osteoblastic cells". *Journal of Periodontology* 78.12 (2007): 2369-2379.

45. Dacy JA., *et al.* "Effects of phosphated titanium and enamel matrix derivatives on osteoblast behavior *in vitro*". *International Journal of Oral and Maxillofacial Implants* 22.5 (2007): 701-709.
46. Kawana F., *et al.* "Porcine enamel matrix derivative enhances trabecular bone regeneration during wound healing of injured rat femur". *The Anantomical Record* 264.4 (2001): 438-446.
47. Sawae Y., *et al.* "Effects of enamel matrix derivative on mineralized tissue formation during bone wound healing in rat parietal bone defects". *Journal of Electron Microscopy* 51.6 (2002): 413-423.
48. Hoang AM., *et al.* "Amelogenin is a cell adhesion protein". *Journal of Dental Research* 81.7 (2002): 497-500.
49. Rodrigues TL., *et al.* "Effects of enamel matrix derivative and transforming growth factor-β1 on human periodontal ligament fibroblasts". *Journal of Clinical Periodontology* 34.6 (2007): 514-522.
50. Schlueter SR., *et al.* "*In vitro* effects of enamel matrix derivative on microvascular cells". *Journal of Periodontology* 78.1 (2007): 141-151.
51. Parkar MH., *et al.* "Gene expression profiles of periodontal ligament cells treated with enamel matrix proteins *in vitro*:analysis using cDNA arrays". *Journal of Periodontology* 75.11 (2004): 1539-1546.
52. Kemoun P., et al. "Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivative (EMD) *in vitro*". *Cell and Tissue Research* 329.2 (2007): 283-294.
53. Friedenstein A Ya and KS Lalikina. "Induction of bone tissue and osteugenous progenitor cells". Moscow: Meditstein, 1973.
54. Astachova VS. "Comparative characteristics of ksenofiders during cloning of human bone marrow stromal fibroblasts". *Bulletin of experiment, biology and medicine,* 1982.

Volume 1 Issue 1 December 2014