Journal of Craniomaxillofacial Research

Vol. 4, No. 4 Autumn 2017

Osteogain as a new liquid formulation for emdogain: A review of the literature

Zeinab Kadkhoda [1], Jowel Makdisi [2], Sahar Chokami Rafiei [3*]

1. Department of Periodontology, Dental Faculty of Tehran University of Medical Sciences, Tehran, Iran.

2. Department of Periodontology, Dental Faculty of Tehran University of Medical Sciences, Tehran, Iran.

3. Department of Periodontology, Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

ARTICLE INFO

Article Type:
Review Article

Received: 21 Jun 2017
Revised: 30 Jul 2017
Accepted: 30 Aug 2017

**Corresponding author:*

Sahar Chokami Rafiei

Periodontology Department, Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Tel: +98-21-88351170
Fax: +98-21-84902473
Email: Sahar_rafiei87@Yahoo.com

ABSTRACT

Objective: The purpose of this study was to review the scientific evidence regarding the efficacy of Osteogain.

Materials and Methods: A literature search for relevant english articles was performed till September, 2017 through PubMed and Google Scholar databases.

Results: After screening the titles and abstracts, eight studies were found to be relevant and included in the study while considering the specific criteria. Studies consist of six In Vitro and two Animal studies. Significantly higher amount of total adsorbed amelogenin for Osteogain has been reported by enzyme-linked immunosorbent assay (ELISA). Histologic evaluation showed higher amounts of connective tissue attachment and bone formation for Osteogain. Micro-CT analysis demonstrated that Osteogain induced significant new bone formation. Real-time polymerase chain reaction (PCR) revealed that Osteogain significantly upregulated the expression of the genes involved in osteogenesis and increased alizarin red staining.

Conclusions: Most studies indicated promising results about the use of Osteogain in periodontal regeneration. Further investigations are needed to discover the characteristics of this novel liquid enamel matrix derivative (EMD) formulation (Osteogain).

Key words: Enamel matrix derivative (EMD), Emdogain, Osteogain.

Introduction

Using Enamel matrix derivative (EMD), improves periodontal regeneration through new root cementum, periodontal ligament and alveolar bone formation [1]. The rationality for the clinical application is that enamel matrix proteins sediment on the root surface of developing teeth before cementum formation. Thus, likely have an important role in cementogenesis [2]. Currently, enamel matrix derivative in poly glycolic acid (PGA) base is produced under the name EMD but there are many concerns about the gel delivery system of the EMD. Lack of flap support and blood clot stabilization limits space maintenance and regeneration outcomes [3, 4]. In order to overcome these problems, various combinations of the biomaterials and EMD have been used till now. Some clinical studies show that these combinations provide additional benefits, while others failed to show any additional regeneration [5-7].

Recently, a liquid carrier system for enamel matrix derivative (EMD dissolved in acetic acid solution) has been

introduced called Osteogain [10,11]. Osteogain has a much more complete and stable surface coating and penetrates porous biomaterials [12,13]. So the aim of this review article was to provide scientific evidences regarding the characteristics of this liquid EMD formulation (Osteogain).

Materials and Methods

An independent electronic search of the English language literature about EMD in liquid formulations (Osteogain) was performed by two reviewers using the PubMed search engine and the Google Scholar database. The specific terms that were used for the electronic search were "Osteogain" and ["Enamel matrix derivative "or "EMD" or "Emdogain"]. The process was repeated until no further new articles could be identified. The last electronic search was performed on September, 2017. The titles and abstracts of literatures were reviewed and analyzed for the eligibility criteria (inclusion/exclusion) by reviewers. Disagreement about the included articles was resolved by discussion.

Results

The electronic search provided 7595 articles in total of which, 5964 records were identified in PubMed and 1649 records identified in Google Scholar database searching. Of the total number of articles, 424 articles were found relevant to the study; of these, 18 articles were included for full-text evaluation. After screening the titles and abstracts, eight studies were found relevant to be included in the study while considering the specific criteria. Studies consist of six In Vitro and two Animal studies, were included (Figure 1). Search results are summarized in Table1.

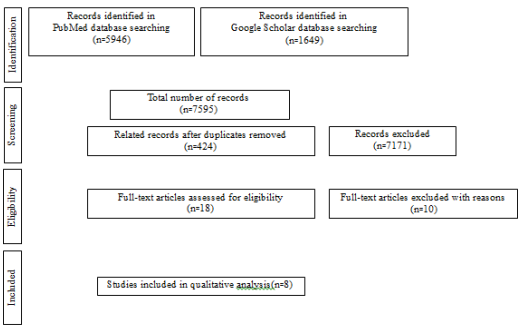

Figure 1.

-Clinical observations

Out of 8 included studies, only one animal study [14] presented data about clinical evaluation of Osteogain application in cl III furcation defects. In this study, healing period was completed without any serious complication such as material exposure, infection and suppuration.

-Histologic/ histometric observations

Six out of 8 selected studies reported histologic/ histometric evaluation of Osteogain. In one animal study by Yufeng Zhang et al, [15] the new bone formation in test defect was quantitatively assessed from sections of H&E staining and Safranin O staining. The newly formed bone was observed in bone mineral (NBM) + Osteogain group and then in NBM alone, compared to the drilled control group at all time intervals ($p<0.05$). Although no significant difference between NBM + Osteogain and NBM alone was observed at 2 weeks, statistical analysis showed that new bone formation was significantly higher in the NBM + Osteogain group at 4 and 8 weeks compared to NBM alone ($p<0.05$). This result is in agreement with an in vitro study by Richard J. Miron et al [16].

Two In Vitro studies found a significant benefit of adding Osteogain to absorbable collagen sponge (ACS) [17] and procine derived collagen membrane [18] based on alizarin red staining. In a study by Richard J Miron et al, [19] alizarin red staining was significantly higher in both osteoblasts and periodontal ligament (PDL) cells treated with either Emdogain (EMD) or Osteogain in comparison to control samples without any significant differences between EMD and Osteogain.

Yoshinori Shirakata et al [14] demonstrated that migration of junctional epithelium was more restrained in the Osteogain + ACS group than in the open flap debridement (OFD), ACS and EMD + ACS groups. Moderately thick new cellular and thin acellular cementum, with dense collagen fibers obliquely or perpendicular oriented to the root surface was observed. Highly vascularized new periodontal ligament-like tissue maintained its width up to the coronal portion.

-Radiographic observations

Only in one animal study, [15] micro-CT reconstruction was used to visualize the ability of NBM alone or NBM + Osteogain to influence new bone formation. In the unfilled control groups, little to no bone formation was observed at each time period. Defects filled with NBM material initially demonstrate a large filled area of mineralized tissue since NBM grafting particles are mineralized. Increase in bone formation was observed at 4 and 8 weeks post-implantation. A

similar trend is observed for defects filled with NBM + Osteogain when compared to NBM alone.

-Ability of different carrier to adsorb and release amelogenin over time

Among 8 included studies, in three of them, enzyme-linked immunosorbent assay (ELISA) was utilized to investigate the amount of adsorbed amelogenin when carriers were coated with Osteogain. A simple PBS rinse significantly removed over 20% more (from >90% to 70%) of the total amelogenin content from EMD in comparison to Osteogain where the total protein content remained greater than 90% of the initial concentrations. At each time period, a 20-60% significantly higher amount of total adsorbed amelogenin was found for ACS loaded with Osteogain compared to EMD. After a 10-day period, nearly 60% of the initial amelogenin protein content found in Osteogain remained present within the ACS whereas in the EMD samples, no remaining amelogenin could be quantified as values approached 0% [14]. This finding is in agreement with a study by Richard J. Miron et al [17]. In one In Vitro study, [20] a significant difference was observed between EMD-liquid and EMD-gel when the bone grafts were compared for their release of amelogenin profile over time.

The results revealed that a single rinse with phosphate-buffered saline (PBS) was able to decrease surface coating of amelogenin proteins coated by bone grafting materials with EMD-gel by over 50% in NBM, demineralized freeze-dried bone allograft (DFDBA) and calcium phosphate (CaP) study whereas had little effect on grafts coated with EMD-liquid.

Scanning Electron Microscopy (SEM) results revealed that the surface topography did not vary much from control uncoated particle surfaces following coating with EMD-liquid. When grafts were coated with EMD-gel however, a large surface coating formed over the surface of bone grafts by demonstrating regions of thicker coating, likely as a result of the PGA carrier in EMD-gel [20]. A higher number of gold-labelling was observed on NBM and DFDBA scaffolds coated with EMD-liquid when compared to EMD-gel in Transmission Electron Microscopy (TEM).

About CaP scaffolds, only a small, non-significant increase was observed. On NBM + EMD-liquid and DFDBA + EMD-liquid, most adsorption of proteins was done either within the scaffold or directly on the scaffold surface. When these scaffolds were coated with EMD-gel, this distance increased. This same trend was also observed on CaP scaffolds, although to a lesser extent [20].

-Influence of Osteogain on cell adhesion, attachment, migration and proliferation

Two studies demonstrated that the additional use of Osteogain significantly increased cell adhesion while ACS [17] or NBM [16] alone did not induce ST2 cell attachment to tissue culture plastic (TCP). While no significant differences were observed between TCP and ACS, the additional coating with Osteogain significantly decreased undifferentiated mouse cell line ST2 stromal bone marrow cell's numbers at 1, 3, and 5 days ($p < 0.05$). But in a study by NBM particles in combination with Osteogain significantly increased cell proliferation at 3 and 5 days after seeding ($P<0.05$) [17]. In one study, [19] no difference in primary human osteoblasts and PDL cell number was observed between EMD in the gel and liquid formulation and control samples at day 1, post seeding, although at 3 and 5 days post seeding, a significant increase in cell number was observed for both EMD formulations compared to control samples without any significant difference between samples treated each EMD formulations.

Barrier membranes with EMD in a liquid carrier system markedly upregulated cell attachment at 8 hours compared with barrier membranes alone. By 24 hours, cell numbers/attachment were equivalent between control and EMD coated membranes. EMD had no significant influence on cell numbers at 3 and 5 days post seeding. It was found that neither the barrier membrane alone nor its combination with EMD in a liquid carrier system had the ability to recruit progenitor cells [18]. NBM alone demonstrated no potential to induce undifferentiated mouse cell line ST2 stromal bone marrow cell's migration. The additional use of Osteogain in combination with NBM did not improve cell migration [16].

-Influence of Osteogain on cell differentiation and gene expression

As expected, several genes involved in osteogenesis were upregulated when primary human PDL cells were cultured in the presence of Osteogain [21]. Neither ACS nor Osteogain had any influence on genes encoding osteoblast differentiation markers at 3 days post seeding of the undifferentiated mouse cell line ST2 stromal bone marrow cells. However, at 14 days post seeding, ACS significantly increased Runx2 messenger

RNA (mRNA) levels up to 2- fold, and Osteogain further significantly upregulated all osteoblast differentiation markers such as runt-related transcription factor 2 (Runx2), collagen1alpha 2 (Col1 α 2), alkaline phosphatase (ALP), and bone sialoprotein (BSP) compared to TCP [17]. Osteogain + NBM significantly increased Runx2 expression at 14 days, and COL1a2 expression at 3 and 14 days after seeding as opposed to NBM alone. Furthermore, Osteogain + NBM further stimulated ALP expression at 3 days and osteocalcin (OCN) expression at 3 and 14 days after seeding [16]. Cells treated with either formulation had a slight non-significant increase in transforming growth factor-beta 1 (TGF-b1) mRNA expression in primary osteoblasts. On the other hand, TGF-b1 expression was significantly upregulated at all time periods of 8, 24 and 72 hours for PDL cells. It was then found that the gene expression of bone morphogenetic protein 7 (BMP7) was upregulated for all time periods when either osteoblasts or PDL cells were cultured with either formulation. Furthermore, both EMD formulations significantly reduced the expression of interleukin 1-beta (IL-1b) in osteoblasts and PDL cells at all time points when compared to controls. No significant differences were observed between EMD formulations at all times suggesting similar potencies on gene expression of cytokines and growth factors for both cell types [19].

It was found that both formulations had no effect on osteoblast gene expression of Runx2 however a significant upregulation was observed at time points 7 and 14 days for PDL cells cultured with either EMD formulations when compared to control samples. It was observed that both EMD formulations were able to significantly upregulate Col1 α 2 gene expression at 7 and 14 days in osteoblasts and 3, 7 and 14 days in PDL cells. Similarly, the gene expression of ALP was significantly upregulated at all time periods for both osteoblasts and PDL cells cultured in the presence of either EMD formulation.

A similar result was observed for osteocalcin mRNA expression with both EMD formulations for cells at all time points. No significant differences were observed between EMD formulations at all time points for each of the experiments [19]. The additional use of enamel matrix derivative in a liquid carrier system to barrier membranes significantly increased COL1A2 mRNA levels at 3 days post seeding, mRNA levels of ALP at 3 days and BSP expression at 14 days post seeding. No significant difference in Runx2 expression was observed at either time point [18].

Discussion

To our knowledge, no study reviewed data about Osteogain, thus this is the first one in this subject to date. Significantly higher amount of total adsorbed amelogenin for Osteogain has been reported by ELISA. Histologic evaluation showed higher amounts of connective tissue attachment and bone formation for Osteogain. Micro-CT analysis demonstrated that Osteogain induced significantly more new bone formation. Real-time PCR revealed that Osteogain significantly upregulated the expression of the genes a number of genes involved in osteogenesis and increased alizarin red staining.

Osteogain alone could never be used, because, liquid formulation prevents the material flap support and blood clot stabilization which were necessary for periodontal regeneration. Truthfully, Osteogain is designed for combination of EMD with different bone substitute materials. A bone graft placed in the bony defect might interfere with bone formation since the graft would need to be resorbed before the bone occupies the defect [22,23]. If the aim of any clinical intervention was to decrease detrimental effect of remaining particles because these remaining particles prevent periodontal regeneration, we must use EMD alone and Osteogain is not a good choice.

One animal study used Micro-CT reconstruction to evaluate the new bone formation. However micro CT was not able to accurately quantify new bone formation because of mineralized tissue contained in NBM. So another approach like histology was needed to quantify new bone formation. Two studies undifferentiated mouse cell line ST2 stromal bone marrow cell was cultured and assessed. The reason for selecting this cell was that every cell that is less differentiated, is more sensitive to differentiation to osteoblast with EMD using [22-25].

Although most studies indicated promising results about the use of Osteogain in periodontal regeneration, further clinical and preclinical studies are needed to fully elucidate the advantages of this novel liquid EMD formulation (Osteogain). Investigation in this respect is ongoing.

Author	Study Design	Study Subjects	Intervention	Variables	Outcomes
Yoshinori Shirakata et al (14)	Animal study	Chronic class III furcation defects	a:OFD + ACS b:OFD+Emdogain/ACS c:OFD+Osteogain/ACS d:OFD alone	1-ELISA 2-Histometric analysis	-Significantly higher amount of total adsorbed amelogenin for c - Both b and c resulted in higher amounts of connective tissue attachment and bone formation -c showed higher new attachment formation, cementum and new bone area.
Yufeng Zhang et al (15)	Animal study	Rat femur defect model	a:Un filled b:NBM alone c:NBM + Osteogain	1- Micro-CT analysis 2- Histometric analysis	-Significantly more new bone formation in c -More mature mineralized bone with the presence of osteocytes in c
Richard J. Miron et al (16)	In Vitro	Undifferentiated mouse cell line ST2 stromal bone marrow cells	a:NBM alone b:NBM + Osteogain	1- Cell migration, adhesion ,proliferation 2-Real-time PCR 3- Alizarin red staining	-b significantly upregulated cell adhesion -b significantly upregulated genes encoding Runx2, ALP, COL1a2 and OCN -b increased alizarin red staining vs a
Richard J. Miron et al (17)	In Vitro	Undifferentiated mouse cell line ST2 stromal bone marrow cells	a:TCP b:ACS alone c:ACS + Osteogain	1- ELISA 2- Cellular attachment and proliferation 3- Real-time PCR 4- Alizarin red staining	-ACS efficiently loaded nearly 100% of the amelogenin proteins found in Osteogain® -c significantly induced a increase in cell attachment -c resulted in increase Runx2, COL1a2, ALP, and BSP -c induced alizarin red staining
Richard J Miron et al (18)	In Vitro	Undifferentiated mouse cell line ST2 stromal bone marrow cells	a: Procine derived collagen membrane alone b:Procine derived collagen membrane + Osteogain	1- Cell recruitment, adhesion and proliferation 2- Real time PCR 3- Alizarin red staining	- A significant increase in cell adhesion in b and no significant differences for cell and proliferation -b significantly increase ALP, COL1a2 and BSP -b induced alizarin red staining vs a
Richard J Miron et al (19)	In Vitro	Undifferentiated mouse cell line ST2 stromal bone marrow cells Primary human osteoblasts and PDL cells	a:TCP b:EMD c:Osteogain	1- Cell proliferation and differentiation 2- Real-time PCR 3- Alizarin red staining	-b and c increased cell proliferation of both osteoblasts and PDL cells -b and c significantly upregulated the expression of genes encoding BMP2 and TGF-β1 as well as decreased the expression of IL-1β –c increased COL1a2 and OCN gene expression –c significantly higher alizarin red staining
Richard J. Miron et al (20)	In Vitro	-	NBM, DFDBA or CaP + EMD or Osteogain	1- ELISA 2- SEM or TEM	-EMD adsorbed less protein to the surface of grafting particles, which easily dissociated following PBS rinsing. - TEM revealed that adsorption of amelogenin proteins of EMD were significantly further from the grafting material surface -the combination of Osteogain + NBM or DFDBA adsorbed higher amounts of amelogenin than all other treatment modalities. -amelogenin proteins delivered by Osteogain were able to penetrate the porous surface structure of NBM and DFDBA
Richard J. Miron et al (21)	In Vitro	Primary human PDL cells	a:NBM alone b:NBM + Osteogain	1- Real-time PCR	-b significantly upregulated the expression of the genes including BMP2, TGF β 1, FGF, EGF and PDGF as well as some of their associated receptors -b promoted gene expression COL1a2 and ALP as well as FNT and a variety of integrin binding proteins and calcitonin receptor and annexin A5 gene expression

Table1. Main characteristics of selected studies.

Abbreviation: OFD: open flap debridement; ACS: absorbable collagen sponge; ELISA: enzyme-linked immunosorbent assay; NBM: natural bone mineral; PCR :polymerase chain reaction; Runx2: runt-related transcription factor 2; ALP: alkaline phosphatase; Col1 α 2: collagen1 alpha 2; OCN: osteocalcin; TCP: tissue culture plastic; BSP: bone sialoprotein; PDL periodontal ligament; BMP2: bone morphogenetic protein 2; transforming growth factor-beta1 (TGF-b1); interleukin 1-beta (IL-1b); DFDBA: demineralized freeze-dried bone allograft; CaP calcium phosphate; SEM :scanning electron microscopy; TEM: transmission electron microscopy; PBS: phosphate-buffered saline; FGF: fibroblast growth factor; EGF: epidermal growth factor; PDFG: platelet-derived growth factor; FNT: fibronectin.

Conclusion

Most studies indicated promising results about the use of Osteogain in periodontal regeneration. Further studies are needed to discover the characteristics of this novel EMD formulation (Osteogain).

Conflict of Interest

There is no conflict of interest to declare.

Acknowledgements

The authors confirm that they have no financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript.

References

[1] Miron RJ, Dard M and Weinreb M. Enamel matrix derivative, inflammation and soft tissue wound healing. J Periodontal Res. 2014; 50(5):555-69.

[2] Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol. 1997; 24(9):658–68.

[3] Polimeni G, Koo K.T, Qahash M, Xiropaidis A.V, Albandar J.M, Wikesjo U.M Prognostic factors for alveolar regeneration: effect of a space-providing biomaterial on guided tissue regeneration. J Clin Periodontol. 2004; 31(9): 725–29.

[4] Siciliano V.I, Andreuccetti G, Siciliano A.I, Blasi A, Sculean A, Salvi G.E. Clinical outcomes after treatment of non-contained intrabony defects with enamel matrix derivative or guided tissue regeneration: a 12-month randomized controlled clinical trial. J Periodontol. 2011; 82(1): 62–71.

[5] Aspriello SD, Ferrante L, Rubini C, Piemontese M. Comparative study of DFDBA in combination with enamel matrix derivative versus DFDBA alone for treatment of periodontal intrabony defects at 12 months post-surgery. Clin Oral Investig. 2011; 15(2):225-32.

[6] Yilmaz S, Cakar G, Yildirim B, Sculean A. Healing of two and three wall intrabony periodontal defects following treatment with an enamel matrix derivative combined with autogenous bone. J Clin Periodontol. 2010; 37(6):544-50.

[7] Miron RJ, Wei L, Bosshardt DD, Buser D, Sculean A, Zhang Y. Effects of enamel matrix proteins in combination with a bovine-derived natural bone mineral for the repair of bone defects. Clin Oral Investig. 2014; 18(2):471-78.

[8] Hoidal MJ, Grimard BA, Mills MP, Schoolfield JD, Mellonig JT, Mealey BL. Clinical evaluation of demineralized freeze-dried bone allograft with and without enamel matrix derivative for the treatment of periodontal osseous defects in humans. J Periodontol. 2008; 79(12):2273-80.

[9] Guida L, Annunziata M, Belardo S, Farina R, Scabbia A, Trombelli L. Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative in the treatment of periodontal intraosseous defects. J Periodontol. 2007;78(2):231-38.

[10] Miron RJ, Bosshardt D, Laugische O, Dard M, Gemperli AC, Buser D, et al. In Vitro Evaluation of Demineralized Freeze-Dried Bone Allograft in Combination With Enamel Matrix Derivative. J Periodontol. 2013; 84(11):1646-54.

[11] Miron RJ, Bosshardt DD, Hedbom E, Zhang Y, Haenni B,Buser D, et al. Adsorption of enamel matrix proteins to a bovine-derived bone grafting material and its regulation of cell adhesion, proliferation, and differentiation. J Periodontol. 2012; 83(7):936-47.

[12] Protivinsky J, Appleford M, Strnad J, Helebrant A, Ong JL. Effect of chemically modified titanium surfaces on protein adsorption and osteoblast precursor cell behavior. International Journal of Oral and Maxillofacial Implants. 2007; 22(4):542.

[13] Wittmer CR, Phelps JA, Saltzman WM, Van Tassel PR. Fibronectin terminated multilayer films: protein adsorption and cell attachment studies. Biomaterials. 2007; 28(5):851-60.

[14] Shirakata Y, Miron RJ, Nakamura T, Sena K, Shinohara Y, Horai N, et al. Effects of EMD-liquid (Osteogain) on periodontal healing in class III furcation defects in monkeys. J Clin Periodontol. 2017 Mar; 44(3):298-307.

[15] Zhang Y, Jing D, Buser D, Sculean A, Chandad F, Miron RJ. Bone grafting material in combination with Osteogain for bone repair: A rat histomorphometric study. Clin Oral Investig. 2016; 20(3):589–95.

[16] Miron RJ, Fujioka-Kobayashi M, Zhang Y, Caballé-Serrano J, Shirakata Y, Bosshardt DD, et al. Osteogain improves osteoblast adhesion, proliferation and differentiation on a bovine-derived natural bone mineral. Clin Oral Implants Res. 2017 Mar; 28(3):327-33.

[17] Miron RJ, Fujioka-Kobayashi M, Zhang Y, Sculean A, Pippenger B, Shirakata Y, et al. Osteogain® loaded onto an absorbable collagen sponge induces attachment and osteoblast differentiation of ST2 cells in vitro. Clin Oral Investig. 2016 Dec 1; 21(7):2265-72.

[18] Miron RJ, Fujioka-Kobayashi M, Buser D, Zhang Y, Bosshardt DD, Sculean A. Combination of Collagen Barrier Membrane with Enamel Matrix Derivative-Liquid Improves Osteoblast Adhesion and Differentiation. Int J Oral Maxillofac Implants. 2017 Jan/Feb; 32(1):196-203.

[19] Miron RJ, Chandad F, Buser D, Sculean A, Cochran DL, Zhang Y. Effect of Enamel Matrix Derivative Liquid on Osteoblast and Periodontal Ligament Cell Proliferation and Differentiation. J Periodontol. 2016 Jan; 87(1):91-9.

[20] Miron RJ, Bosshardt DD, Buser D, Zhang Y, Tugulu S, Gemperli A, et al. Comparison of the capacity of enamel matrix derivative gel and enamel matrix derivative in liq- uid formulation to adsorb to bone grafting materials. J Periodontol 2015; 86(4):578–87.

[21] Miron RJ, Shuang Y, Sculean A, Buser D, Chandad F, Zhang Y. Gene array of PDL cells exposed to Osteogain in combination with a bone grafting material. Clin Oral Investig. 2016 Nov; 20(8):2037-43.

[22] Miron RJ, Caluseru OM, Guillemette V, Zhang Y, Gemperli AC, Chandad F, et al. Influence of enamel matrix derivative on cells at different maturation stages of differentiation. PLoS One. 2013; 8(8): e71008.

[23] Miron RJ, Saulacic N, Buser D, Iizuka, T, Sculean A.Osteoblast proliferation and differentiation on a barrier membrane in combination with bmp2 and tgfbeta1. Clin Oral Investig. 2013; 17(3): 981–88.

[24] Murray G, Holden R, Roschlau W. Experimental and clinical study of new growth of bone in a cavity. Am J Surg. 1957; 93(3):385-87.

[25] Araujo MG, Lindhe J. Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. Clin Oral Implants Res. 2009;20(5):433-40.

Please cite this paper as:
Kadkhoda Z, Makdisi J, Chokami Rafiei S; Osteogain as a new liquid formulation for emdogain: A review of the literature. J Craniomaxillofac Res 2017; 4(4): 414-420