Comparison of the effects of different bone allografts with and without growth factors on proliferation, osteogenic differentiation and mineralization of MG-63 osteoblast-like cells

Abstract:

Background and aim: Predictable regeneration of alveolar bone defects has always been an important therapeutic challenge in implant dentistry. Allografts including FDBA and DFDBA are some substitutes being widely used and reported as having osteoinductive activities with some degrees of controversy. The aim of this study is to determine the effect of growth factors on osteoinductive activities of different bone materials.

Materials and Methods: MG-63 cells were exposed to 60 mg amounts of four different commercially available freeze-dried bone allografts with or without 5 ng/mL of two growth factors (singular or in combination). After 24 and 72 hours of incubation, the effect of water-soluble allograft released materials and soluble growth factors on cell viability and proliferation was assessed using methyl thiazol tetrazolium (MTT) assay. Cell differentiation and mineralization was respectively assessed by real-time quantitative reverse transcription PCR (qRT-PCR) and alizarin red staining after 72 hours of exposure.

Results: The effect of different GFs on cell/allograft containing plates was affected by the allograft type. Early proliferative and late osseoinductive effects of GFs were more consistent in TGF-β rather than PDGF. PDGF only showed limited osseoinductivity in terms of accelerating BSP and OC genes.

Conclusion: based on the results of this study, TGF-β can have additional osseoinductive effect on allografts/cells combination and its application may be beneficial in *in vitro* and clinical regenerative studies.

Key words: Bone allograft, Growth factor, Osteoblast differentiation, Mineralization

Introduction:

Alveolar bone loss is a common consequence of periodontal disease progression which can finally lead to tooth mobility and then, an inevitable tooth loss. Additionally, it can complicate further implant therapy due to some residual bone defects (1). Since the prevalence of generalized periodontitis has been reported as high as 5-15%, the predictable and complete regeneration of alveolar defects has been an important therapeutic challenge in implant dentistry (2). Using various procedures like guided tissue regeneration (GTR), guided bone regeneration (GBR), and using enamel-matrix proteins are some examples to overcome this challenge (3). In 1923, bone grafts were first used by Hedegus for the regeneration of defects caused by periodontal lesions (4), and later in 1965 by O'Leary and Nabers (5).

Among all four types of bone graft materials, allografts seem to overcome some complications related to using autografts, like patient's more pain and discomfort (6), more cost and the limitation of intraoral donor sites (7). Demineralized Freeze-Dried Bone Allograft (DFDBA) has been successfully used in the treatment of periodontal, peri-implant, and furcation defects for three decades (8). The exposure of osteoinductive factors like bone

morphogenic protein (BMP) by *in vitro* HCL acid demineralization in DFDBA, has improved its osteoinductive potential via accelerating osteoblastic/chonroblastic differentiation from precursor cells (9-11). It has been said that the osteoinductivity of these graft materials can largely vary depending on the manufacturers' preparation protocol, donor's properties, and the particle size; therefore, it appears that many of current commercial product may have a limited osteoinductive activity, if not totally lack it (12-15).

In our previous *in vitro* study (16) on the osteoinductivity of different DFDBAs (Osseo^{+}, Alloss, Cenobone), we concluded that all three were osteoinductive and had the ability to promote osteogenic differentiation of SaOs-2 cells. On the other hand, it has also been shown that the quantity of BMPs in almost all different commercial DFDBAs is less than which can be really osteoinductive and so it is necessary to add complementary BMP to them (17). Platelet-derived growth factor-BB (PDGF-BB), a potent chemotactic and mitotic factor on mesenchymal cells, can evidently promote healing. Also, it has shown a potent stimulatory effect on extracellular matrix (ECM) synthesis (18). The effect of rhPDGF-BB on granulation tissue development in diabetic ulcers has also been well confirmed (19). Transforming growth factor-β (TGF-β) stimulates chemotaxis and survival of osteoblasts (20), ectopic bone formation, and when delivered by Matrigel matrix and implanted in class II and III furcation defects of mandibular molars, induced periodontal tissue regeneration in a primate model (21). So, it is likely that we can benefit from these two growth factors (PDGF-BB and TGF-β) to enhance bone regeneration in defect sites selected for further implant placement.

Considering the lack of enough knowledge and consistency about the real benefits of adding growth factors (GFs) to current bone allografts, we designed this experimental *in vitro* study primarily aimed at evaluating osseoinductivity of two commercial FDBAs (CenoNone^{TM}, NonDemin^{TM}) and two DFDBAs (CenoBone^{TM} and OsteoDemin^{TM}) with or without growth factors (PDGF-BB and TGF-β) via assessing cell viability/proliferation (quantitative MTT assay), cell differentiation (osteogenic gene expression via quantitative real-time PCR) and cell mineralization (qualitative Alizarin red staining) in human osteoblast cell line (MG-63).

Materials and Methods:

-Cell culture and treatment

MG-63 (osteosarcoma) human osteoblast-like cells were provide from Pasture Institute of Iran (NCBI code: C555) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, UK) supplemented with 1% antibiotic (penicillin and streptomycin), and 10% fetal bovine serum (37°C, 5% CO_2 and 95% humidity). Cells at logarithmic growth phase, with the density of 35,000 cells per well of 6-well culture plates (SPL, Korea). After 24 hours of incubation, precisely weighed 60 mg amounts of four sterile bone allograft groups (Table 1) were directly added to 2 mL culture medium/well (3 wells for each sample; n=3). The Cells treated with culture medium only (without allografts or growth factors) was considered as the positive control for cell viability and proliferation (no cytotoxicity) and negative control for osteoblastic differentiation and mineralization. Then, 5 ng/mL of recombinant human TGF-β1 growth factor (PEPROTECH, England) and recombinant human PDGF-BB growth factor

(PEPROTECH, England) were added (singular and or combined) to control cells and allograft treated cells.

-Cell viability and proliferation quantitative analysis using MTT assay

In order to evaluate and compare the effects of understudied bone allografts and growth factors (in singular or in combination with each other) on cell viability/proliferation the MTT (Methyl Thiazol Tetrazolium) assay was performed. At 24 and 72 hours post-treatment, the medium in each well was removed and replaced with the medium containing 10% MTT dye (5 mg/mL stock solution) (Sigma-Aldrich, Germany). After three hours of incubation at 37°C, the MTT medium was completely removed and replaced with the same volume of dimethyl sulfoxide solvent to dissolve purple Formazan crystals; 100µL of the colored solution from each group was added to each well of a 96-well plate (six repetitions) and the optical density (OD) was read at 570 and 620nm wavelength by the Elisa Reader (Anthos 2020, Salzburg, Austria).

- Cell differentiation quantitative analysis using Real-time PCR

In order to evaluate and compare the effects of understudy bone allografts and growth factors (in singular or in combination with each other) on cell differentiation, the expression of three osteoblast differentiation marker genes (Alkaline phosphatase, ALP - Bone Sialo Protein, BSP – Osteocalcin, OC) genes, were analyzed using quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR). At 72 hours post-treatment, the total RNA was extracted from treated and non-treated (control) cells using RNeasy Mini Plus Kit (Qiagen, USA). Then, complementary DNAs (cDNas), were synthesized using a Quanti Tect Reverse Transcription kit (Qiagen, USA), according to the manufacturer's instructions. Amplification of specific products was performed with 20 µL reaction mixtures contained 0.5 µl of forward and reverse primers (250 nM final concentration, 60°C annealing temperature) (Bioneer, South Korea( )Table 2), 2 µL of cDNA, 10 µL of Syber® Green PCR Master Mix (Qiagen, Hilden, Germany), and 7 µL of DNase-free water using Real-Time PCR detection system (Roche, Germany). Three repetitions were made for each group per each test (mean± SD). After normalizing the mean Ct values, the changes in expression of ALP, OC and BSP genes were evaluated in comparison with the expression of internal control gene (Ribosomal protein L13a, RPL) using Delta Delta Ct formula.

-Cell mineralization qualitative analysis using Alizarin-red staining

In order to evaluate and compare the effects of understudy bone allografts and growth factors (in singular or in combination with each other) on cell mineralization, the calcified nodule production were analyzed with Alizarin Red staining. At 72 hours post-treatment, the MG-63 treated and non-treated (control) cells were washed with phosphate buffered saline (PBS) solution and then fixed with ice-cold 70% ethanol (1 hour incubation at room temperature). After several rinsing with deionized water, 2% Alizarin Red solution (Sigma-Aldrich, Germany) was added (30 minutes incubation at room temperature). After several rinsing with

deionized water, the cells were evaluated under an inverted light microscope (10X magnification) for detection of orange-red calcified nodules.

-Statistical analysis

The results were analyzed with GraphPad Prism (V.6.01 - USA) using one-way ANOVA and Tukey's post hoc test. Pairwise comparisons were made using t-test and the relevant graph was plotted. Stars (*) on the columns indicate statistically significant difference with the control group (P value<0.05).

Results:

- Effect of bone allografts/growth factors on cell viability and proliferation

The percentage of viability and proliferation of non-treated control cells (exposure to culture medium only, without bone allografts or growth factors) is considered 100%.

As seen in figure 1-A, 24 hours after treatment, the combination of TGF-ß and PDGF-BB growth factors has significantly higher effect on viability and proliferation (33% increase vs control) than each growth factor in singular (the same effect, 15% significant difference vs control). The Combination of CenoBone™ PDFDBA (partially demineralized) and PDGF-BB has increased cell viability compared to control group (12%), TGF-ß only treated and allograft only treated group (22%). In contrast, the combination of TGF-ß and PDGF-BB growth factors with this allograft significantly reduced cell viability (38% reduction compared to control).

A significant increase (36%) in cell viability and proliferation is seen in OsteoDemin™DFDBA (fully demineralized) only treated cells and also in combination with TGF- ß treated cells was seen. In contrast, the combination of this allograft with PDGF-BB and with both TGF-ß/PDGF, significantly reduced cell viability (18% reduction compared to control).

No significant difference in cell viability and proliferation was seen in four state of CenoBone™ FDBA (mineralized) treated cells (allograft only, allograft in combination of FGF-ß, allograft in combination of PDGF-BB, allograft in combination of both TGF-ß and PDGF-BB), compared to non-treated control group, 24 hours after treatment.

According to the results, NonDemin™ FDBA (mineralized) allograft only (38%), in combination with TGF-ß only (46%), in combination with PDGF-BB only (64%) and in combination with both TGF-ß and PDGF-BB (30%) increases the viability and proliferation, 24 hours after treatment. In this allograft group, the positive effect of allograft/PDGF-BB growth factor was significantly higher.

As seen in figure 1-B, 72 hours after treatment, the growth factors (in singular or in combination) slightly (7-10%) reduced cell viability (non-cytotoxic) of non-allograft treated cells. This reduction in cell viability and proliferation was seen in CenoBone™ PDFDBA only treated and CenoBone™ PDFDBA in combination with TGF-ß treated cells (30%). Viability and proliferation reduction was slighter in PDGF-BB only and PDGF-BB in combination with TGF-ß treated cells (20%). Also 14% significant increase in cell viability was seen after 72 hours treatment with both growth factors, in compare to 24 hours post-treatment (time-dependent increase).

No significant difference in cell viability and proliferation was seen in four state of OsteoDemin™ DFDBA treated cells (allograft only, allograft in combination of FGF-ß, allograft in combination of PDGF-BB, allograft in combination of both TGF-ß and PDGF-BB), compared to non-treated control group, 72 hours after treatment. Also, 15% significant increase in cell viability was seen in 72-hours treated cells with PDGF-BB (in singular and in combination with TGF-ß), in compare to 24 hours post-treatment (time-dependent increase).
Significant decrease in cell viability (52% reduction compared to non-treated control, cytotoxic) observed in CenoBone™ FDBA treated cells. This reduction was slighter (40%) in allograft treated cells in presence of growth factors. Also, time-dependent reduction (72-hours vs 24-hours) was seen in these groups.
According to the results, NonDemin™ FDBA allograft only (11%, non-cytotoxic) and in combination with both growth factors (40%, cytotoxic), reduced cell viability. The viability and proliferation in TGf-ß only and PDGF-BB only treated cells was similar to controls.

- Effect of bone allografts/growth factors on expression of osteogenic marker genes

Figure 3-A shows the effect of understudy bone allografts and growth factors (in singular or in combination, 72 hours exposure) on the expression of ALP gene (early marker of osteoblast differentiation) in comparison with its expression in the negative (culture medium, without differentiation potential) control groups. The expression of ALP gene, as the early marker of osteoblastic differentiation, increases at the beginning of differentiation and is gradually down regulated by completion of differentiation (time-dependent decrease in gene expression). When pre-osteoblasts transform to fully differentiated osteoblasts (at the beginning of matrix mineralization), expression of BSP (as mid-late marker) and OC (as late marker of osteoblastic differentiation, late stage of maturation) increases.

As seen in Figure 3-A, if the expression rate in control group is considered to be 1, the significant highest expression of ALP occurred in presence of Cenobone™ FDBA treated cells: 23 times the control group in allograft only treated, 16 times in allograft/RGF-ß, 22 times in allograft/PDGF-BB and 17 times in allograft in combination with both TGF-ß and PDGF-BB. In CenoBone™PDFDBA only and in combination with PDGF-BB treated cells, 3 times increase in ALP expression was seen, compared to control. This increase was higher in allograft treatment in combination with TGF-ß only or TGF-ß/PDGF-BB (~3.5 time's vs control). Also, same expression pattern of expression but with lower rate was seen in OsteoDemin™DFDBA treated groups.

Figure 3-B shows the effect of allografts/growth factors on BSP gene expression (mid-late marker of osteoblastic differentiation) compared to its expression in the negative control group. The highest level of expression (6-7 times the control group) was seen in NonDemin™PDFDBA, OsteoDemin™DFDBA and NonDemin™FDBA treated groups especially in combination with TGF-ß growth factor (in singular or in combination with PDGF-BB). No significant differences in expression of BSP was seen among CenoBone™ FDBA treated groups (~2 times the control group).

Figure 3-C shows the effect of allografts/growth factors on expression of OC gene (late marker of osteoblastic differentiation) compared to its expression in the negative control group. The highest level of expression (3 times the control group) was seen in NonDemin™FDBA treated groups especially in combination with TGF-ß growth factor (in singular or in combination with PDGF-BB). Two times increase in expression of OC was seen in CenoBone™FDBA only treated cells and also in allograft in combination with both growth factors. Increase in OC expression (1.5 times) Cenobone™DFDBA treated cells in combination with TGF-ß or TGF-ß/PDGF-BB. No significant differences was seen in CenoBone™ PDFDBA treated groups. No significant differences in expression of OC was seen in CenoBone™ PDFDBA treated groups compared to control.

- Effect of bone allografts/growth factors on mineralization of MG-63 cells

Figure 3 shows the qualitative (microscopic. 10X magnification) results of Alizarin Red staining of cells exposed to 60 mg of understudy bone allografts with or without growth factors (in singular or in combination), compared to control group (non-treated/undifferentiated cells) 72 hours after treatment. Red calcified nodules (indicative of completion of differentiation and calcification) was seen in all treated groups (in contrast to non-treated control). The highest frequency was seen in presence of NonDemin™ FDBA group (allograft only and in combination with growth factors). Nodules are not very clear in other groups; arrows in Figure 3 point to the nodules.

Discussion:

Regarding the current use of many commercially available allografts in dental market and their recent application with recombinant growth factors especially in advanced periodontal regenerative therapies, this experimental *in vitro* study was aimed at determining whether the studied allografts show osteoinductive activities or not; and also, how this could be influenced by adding different growth factors. Since none of the studied allografts (without GFs) significantly reduced cell proliferation in 24 hours comparing to the control group, it can be concluded that they may have not early osteoinductive activity. In contrast, at 72 hours, two experimental groups harboring Cenobone FDBA and Cenobone DFDBA showed a significant reduction in cell viability; probably suggesting of inducing late osteoblastic differentiation by these two allograft groups; because proliferation and differentiation are two dynamic and reversed-direction phases in human osteoblast-like cell cycle (22, 23). This finding is exactly consistent with the results of AlP and BSP gene expression at the same time point, in which these two groups of allografts, even without any GF, had a significantly higher AlP gene expression compared to the control group (P value<0.05). In our previous *in vitro* study in 2012 (16), we found the same results for Cenobone DFDBA which showed a concurrent reduction of cell proliferation and an increase in osseodifferentiaton of SaOS-2 cells at 48 hours.

The primary aim of this study was to clarify the osteoinductive effects of two important GFs, PDGF and TGF-β, either individual or together, on MG-63 cells cultured in plates containing four different allografts. As it was previously reported, different trials of GFs on MG-63/allografts culture plates lead to somehow diverse results in terms of cell viability and

osseodifferentiaton compared to their control group; but in general, the pattern of additional osteoinductive effects of GFs, especially in terms of gene expression, was more similar within demineralized (including PDFDBA and DFDBA) or non-demineralized (including Cenobone and NonDemin FDBAs) experimental allograft groups compared to their control plates containing only allografts. So, it can be concluded that the osteoinductive potential of GFs may be to some degree influenced by the used allograft type. In a recent study by the same authors in 2014 (under publication), we encountered a relevant conclusion; the type of allograft is to be more influential factor than concentration or particle size on the osteoinductive property in MG-63 cell line.

According to different studies, FDBAs and DFDBAs vary in terms of their particle size and particle heterogeneity, their mineralized composition, BMP content, and the manufacturing protocol which all can influence their osteoconductive as well as osteoinductive features (24-28). Although FDBA may have the same BMP content in its organic matrix, it does not have osteoinductive capability same as DFDBA (29). Evidence suggests that maximum osteoinduction is observed when there is 2% residual calcium remaining in DFDBA, and it is believed that this small percentage of calcium acts as a nidus for hydroxyapatite crystal formation (30). FDBA may provide a better scaffold than DFDBA for space maintenance and may also be more osteoconductive (31). Moreover, in that relevant study by the same authors in 2014, assessing the *in vitro* effects of mineralized and demineralized bone allografts on viability, proliferation and differentiation of MG-63 cells, the superior efficacy of the mineralized group was attributed to their close calcium/phosphate to that in normal bone (1.67), which was earlier introduced by Greenspan (2012) as a determinant of allograft osteogenic activity (32). So, perhaps these inherent differences between two allograft groups may be the reason of those intergroup different findings. Thus, due to the important osteoconductive and osteoinductive role of allografts, it will be a logic measure to select between more similar allografts when assessing the specific and meticulous additional osteoinductive effects of GFs or other signaling molecules in future studies. So, in order to avoid confusion and reach a relevant conclusion, we did report and analyze our results in 4 groups according to the allograft type.

In the present study, the effect of TGF-β or PDGF alone on MG-63 cell cultures was reported as a significant acceleration of cell viability/proliferation in the first 24 hours of exposure. Also, when they were combined together (TGF-β/PDGF), they showed a double positive effect on MTT results, suggestive of a real synergism. As time passed, typically they caused a decrease in cell viability rate, demonstrating the initiation of osteoblastic differentiation as early as 72 hours post-exposure. TGF-β has proved to have the ability of inducing osteoblastic differentiation and also playing a crucial role in BMP-mediated osteogenesis in various in vitro (33) and clinical studies (34). Also, PDGF-BB is believed to have a strong mitogenic effect on osteoprogenitor cells in concentrations as low as 10-20 mg/ml (35).

The interesting point is the different response of MG-63 cells to GFs in terms of viability or gene expression when they are mixed with allografts; it means that they do not necessarily show the expected typical behavior of late (72 h) GF-mediated osteoblastic differentiation in all allograft groups, perhaps suggesting the combined interaction of GFs and allografts on cells. For example, TGF- β could not accelerate early (24h) proliferation of cells in plates having PDFDBA

Cenobone and FDBA Cenobone. Similarly, this GF could not decrease late (72h) proliferation in DFDBA OsteoDemin and FDBA NonDemin culture plates. Even more interesting, TGF-β increased the 72h MTT rate in FDBA Cenobone cultures compared to allograft-lacking controls. In an *in vitro* study by Mott (2002), it was concluded that PDGF/ TGF-β –enriched DFDBA could result in significant proliferation and osteoblastic activity of murine pre-osteoblasts in 7 days compared to the control group (36). Also, the favorable and beneficial effect of either PDGF-BB or TGF-β in combination with different bone substitutes has been proved in various regenerative clinical studies (34, 37, 38). This controversy between our results and those of other studies can be attributed to the particular conditions of this study in terms of cell types, duration of the experiment, concentration of GFs, or different bone substitute materials and so on, which requires further scrutiny and more studies.

Going deep into the results of adding TGF-β to cell-allograft cultures, we can easily observe a significant early (24h) proliferative effect on OsteoDemin DFDBA and NonDemin FDBA and also, a late (72h) osteoinductive effect on Cenobone PDFDBA and Cenobone FDBA. The question of whether the inter-manufacturers' differences such as special cutting and sizing process of particles can easily determine the pattern of how different allografts would affect cell proliferation under the influence of TGF-β, is to be answered (39).

As it is generally expected, adding GFs to cell-allograft cultures will should in an increase in osseodifferentiaton genes expression; it is well proved by our findings except for applying GFs only on MG-63/Cenobone FDBA cultures where 72h AlP and OC gene expression showed a significant reduction compared to control cultures without GF. Again, it raises a question about the potential influence of the allograft type on MG63-GFs interaction which has to be further dealt with in a well-designed trial in future. In the other hand, the most relevant, conducive and desirable osteoinductive benefits of adding both GFs were observed in the PDFDBA (Cenobone) group compared to all other allografts which could be attributed to the partial demineralization procedure executed while manufacturing and can be the title of further studies. This was consistent with the results gained in early (24h) and late (72h) MTT rates which showed a greater cell proliferation in both time points which may be a good evidence of osseodifferentiaton.

Another noteworthy finding of the present study was the pattern of increasing AlP, OC, BSP gene expression which was generally more consistent in specially two demineralized allografts; inversely, the AlP gene was not expressed in cells cultured in FDBA (NonDemin) with or without GFs. Even, its expression was significantly decreased in FDBA (Cenobone) group under the influence of PDGF, TGF-β, or PDGF/TGF-β compared to their control plates lacking GF. Furthermore as it was previously noted, OC gene expression was also decreased in FDBA (Cenobone) group cultured with either PDGF or TGF-β. The reason why these two GFs caused a reduction in AlP and OC gene expression in addition to the absence of significant increase in BSP gene expression on only FDBA (Cenobone) group, is yet unknown.

Another notable finding about gene expression profile of pre-osteoblastic MG-63 cells under the influence of GFs, was observed in only NonDemin FDBA plates where the AlP gene was not expressed in 72 hours of the experiment despite triplication. Comparing this with results from qualitative Alizarin-red staining test may seem somehow controversial; because cells in NonDemin FDBA plates exhibited the most frequent calcified nodule formation and the highest expression of OC and BSP among all allograft groups. As it is highly clear, AlP is an early

marker of osteoblastic differentiation which its highest expression coincides with the deceleration of cell proliferation, indicating the initiation of calcified nodule formation ultimately resulting in peripheral mineralization. Perhaps this mismatch can be explicated by an inherent biologic property of MG-63 cells, namely their inconsistent mineralization profile (40). Moreover, other reports showed that MG-63 cells have low ALP enzyme activity and did not mineralize (41, 42).

Another interesting finding relates to the interaction of two types of GFs (PDGF and TGF-β) on osseodifferentiaton of MG63 cells in only the FDBA (Cenobone) group: applying either of the two GFs conduced to a significant reduction in 72h OC gene expression; while, simultaneous application of them had reversely resulted in a significant increase. As this finding was not encountered in any other experimental group, it can be attributed to a specific unknown interaction of TGFβ-PDGF with MG63 cell in expressing OC gene. Unfortunately there are not relevant studies about the manner of osseodifferentiaton gene expression in MG-63 cell under the influence of GFs; but interestingly there is an old study by Hung and colleagues (1997) which unveils a cross-talk of PDGF-BB and TGF-β in exerting their mitogenic effect on this cell line. In this study, it was shown that TGF-β1 inhibits PDGF mitogenicity in MG-63 cells by selectively suppressing two out of three PDGF receptor signaling pathways (43). So, the presence of such an unknown and complex interaction in the expression of AlP, BSP, and OC genes by MG-63 cells could be probable as well.

By comparing osteoinductive effects of either two GFs, PDGF or TGF-β, it can be well concluded that in general TGF-β has more noticeable effects in increasing AlP, OC, and BSP gene expression in on almost all allograft groups. Meanwhile, the osteoinductive effect of PDGF alone was observed only in 72h BSP and OC gene expression of cells in only PDFDBA (Cenobone) and FDBA Cenobone group, respectively. Even, PDGF could not enhance the expression of BSP and OC genes as mid-late and late osteoblastic markers. Unlike our results, Mezawa and colleagues (2009) reported BSP mRNA increase by PDGF-BB (5 ng/mL) in SaOs-2 cells at 12 hours (44). Maybe this can be due to the concentration used in this study (5 ng/mL), the type of target cells (MG63) with their definite stopped position in cell cycle position; as the responsiveness to PDGF of human bone cells varies with the stages of differentiation cycle . Perhaps far more different results will be gained when using different concentrations. In this study, we recruited MG63 cells which are human osteosarcoma-derived osteoblast-like cells. Using other human pre-osteoblastic cells like U2OS, CAL72, TE85 may help unmasking the precise real effects of these two GFs and their putative potent osteoinductivity.

Considering the detailed results, we can observe that in this study, the GF-mediated up/down regulation of three genes involved in osseodifferentiaton does not follow the same pattern even within a given allograft group influenced by an individual GF. For example, while TGF-β caused a significant 72h up regulation of AlP and BSP genes, it was not able to increase OC gene expression in PDFDBA Cenobone group. There are many such examples which require more studies in future to answer.

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Conflict of Interests

The authors report no conflicts of interest related to this study.

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