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Effect of concentrated growth factors on function and Wnt3a expression of human periodontal ligament cells *in vitro*

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Abstract

Concentrated growth factors (CGFs), the new generation of platelet concentrate products, appear to exhibit superior potential for tissue regeneration. However, there are only a few studies supporting this. This study was designed to investigate the effect of CGFs on proliferation and alkaline phosphatase (ALP) activity of human periodontal ligament cells (hPDLCs) *in vitro*. Furthermore, as bone homeostasis is fundamentally controlled by Wnt-mediated signals, we also investigated Wnt3a expression of hPDLCs after treatment of CGFs. hPDLCs and CGFs were obtained from the same volunteer. CGFs or combination of recombined human TGF-β1 (rhTGF-β1) and PDGF-AB (rhPDGF-AB) were added to hPDLCs in different concentrations. The rate of proliferation was analyzed by an MTT assay. ALP activity was assessed using p-NPP assay. Quantitative RT-PCR was used to evaluate the gene expression of Wnt3a. In a range of concentrations, CGFs significantly promoted the proliferation of hPDLCs in a dose-dependent manner. ALP activity was also enhanced by CGFs in a dose-dependent and time-dependent manner. The stimulatory effect of CGFs was much greater than rhTGF-β1 and rhPDGF-AB combination. Quantitative RT-PCR results showed that Wnt3a mRNA expression was increased at 24 h in hPDLCs treated by CGFs. CGFs can enhance hPDLCs proliferation and ALP activity and may have great potential in clinical and biotechnological applications.

Keywords

Alkaline phosphatase activity, concentrated growth factors, cell proliferation, periodontal ligament cells, Wnt3a

History

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Introduction

Periodontitis is an inflammatory disease that leads to the loss of tooth-supporting tissues. Conventional periodontal treatment is generally unable to promote regeneration of the damaged periodontal structures [1,2]. One reason is that conventional treatment methods lack of active induction to cells. Furthermore, residual healthy periodontal ligament cells at the periodontal lesion are very few due to the inflammatory damage [3,4].

Polypeptide growth factors have shown important roles in the growth and differentiation of cells involved in periodontal wound healing [5,6]. Recombinant form of growth factors may have positive effects in experimental studies but is not practical to apply clinically because of complexity of application and their high costs [7,8]. Platelets, a major resource of autogenous growth factors, among the first cells to reach a wound site and in response to tissue damage, provide hemostasis and the secretion of those growth factors that play prominent role in bone healing [9].

Concentrated growth factors (CGFs) are new generation of platelet concentrate products [10]. CGFs are produced by centrifuging blood samples at alternating and controlled speeds using a special centrifuge (Medifuge, Silfradentsrl, Italy). CGFs generation is characterized by three phases: (1) a superior phase represented by the serum (blood plasma without fibrinogen and coagulation factors); (2) an interim phase represented by a very large and dense polymerized fibrin block with aggregated platelets and CGFs; and (3) a dense, viscous lower red portion consisting of coagulated red blood cells. Different centrifugation speeds permit the isolation of a much larger and denser fibrin matrix richer in growth factors than typically found in platelet-rich plasma or platelet-rich fibrin. And the combination of fibrins and cytokines within CGFs becomes a powerful bio-scaffold with an integrated reservoir of growth factors for tissue regeneration. Rodella et al. evaluated the morphological ultrastructure of CGFs and assayed the presence of TGF-β1 and VEGF. They observed a fibrin network constituted by thin and thick fibrillar elements, and multiple platelet cell elements were observed forming a cell aggregate trapped among the fibrin network [11]. Their study demonstrated the presence of transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) in CGF and red blood cell layers, suggesting that an improved CGFs isolation procedure could optimize the amount of growth factors in the CGF layer. Furthermore, their results showed a high number of CD34-positive cells in CGFs – CD34 having been demonstrated to play an important role in vascular maintenance, neovascularization, and angiogenesis.

In theory, CGFs appear to exhibit superior potential for tissue regeneration in clinical and biotechnological applications. However, there are only a few studies supporting this. In 2014, Yu et al. investigated the proliferation and differentiation of beagle periodontal ligament stem cells (PDLSCs) co-cultured with CGFs [12]. Their result showed that CGFs significantly promoted the proliferation of beagle PDLSCs and exhibited a dose-dependent effect on the activation and differentiation of the stem cells.

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During regeneration of adult tissues, the Wnt family plays essential roles by regulating cellular processes including proliferation, differentiation, and apoptosis [13,14]. Wnt genes encode secreted glycoproteins. And Wnt proteins transduce multiple signaling cascades including the canonical Wnt/β-catenin pathway, the Wnt/Ca^{2+} pathway, the Wnt/polarity pathway, and the pathway that regulates spindle orientation and asymmetric cell division [15].The canonical Wnt/β-catenin signaling pathway has been found to play a critical role in skeletal development and osteogenesis [16,17].

Therefore, the purpose of this study was to investigate the effect of CGFs on proliferation and differentiation of human periodontal ligament cells (hPDLCs) *in vitro*. Furthermore, as bone homeostasis is fundamentally controlled by Wnt-mediated signals, we also investigated Wnt3a expression of hPDLCs after treatment of CGFs.

Materials and methods

Cell isolation and cell culture

hPDLCs were obtained from extracted healthy teeth removed for orthodontic reasons from young healthy volunteers. All volunteers were informed of the nature of this study and signed an informed consent prior to their participation. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 2000, and the study protocol was reviewed and approved by the university ethical board (Peking University, School and Hospital of Stomatology). The periodontal ligament tissues attached to the middle one-third of the roots were removed by a surgical scalpel and then minced, placed in 35-mm culture dishes in Dulbecco's modified minimum essential medium (DMEM; Gibco BRL, Grant Island, NY, USA) supplemented with 20% (v/v) fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 U/mL penicillin G, and 100 μg/mL streptomycin and were overlaid with sterile cover slips. Cultures were maintained at 37°C in an incubator with an atmosphere consisting of 95% air, 5% CO_2, and 100% relative humidity. Cells were passaged until confluence was reached. Then cells were subcultured in fresh DMEM containing 10% FBS under the standard incubation conditions. Cells of the third passage were used for the experiment.

CGFs preparation

CGFs were obtained from the same volunteer for hPDLCs. Venous blood was drawn in sterile Vacuette tubes (Greiner Bio-One, GmbH, Kremsmunster, Austria) without anticoagulant solutions. These tubes were then immediately centrifuged (Medifuge, Silfradentsrl, Sofia, Italy) using a program with the following characteristics: 30-s acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 4 min at 2700 rpm, 3 min at 3000 rpm, and 36-s deceleration and stop. At the end of the process, three blood fractions were created (Figure 1): (1) a superior phase represented by the serum (blood plasma without fibrinogen and coagulation factors, platelet poor plasma [PPP]); (2) an interim phase represented by a very large and dense polymerized fibrin block containing the CGFs, white blood cells, and stem cells; and (3) the lower red blood cell layer.

CGFs' gel layer was collected and pressed onto membranous film to maintain the slow release of growth factors in it.

Treatment of hPDLCs

There were five groups used in the present study: negative control group (standard cell culture without CGFs), 1 CGF (standard cell culture + CGFs from 1 mL fresh whole blood), 3 CGFs (standard cell culture + CGFs from 3 mL fresh whole blood), 5 CGFs (standard cell culture + CGFs from 5 mL fresh whole blood), and positive control group (standard cell culture + 40 ng/mL rhPDGF-AB 100 ng/mL rhTGF-β1). The standard cell culture was composed of DMEM containing 10% FBS, 100 U/mL penicillin G and 100 μg/mL streptomycin.

The hPDLCs were seeded in 6-well culture plates (Corning Life Sciences, Acton, MA, USA) at a density of 5×10^6 cells/well and were incubated for 24 h in 2 mL of DMEM containing 10% FBS to allow cells to attach and resume exponential growth. After 24 h, the baseline of the experiment, the medium was removed and replaced with 2 mL of DMEM containing 10% FBS. Then, CGFs or growth factors were added and experimental checking points were at 24 h, 72 h, and 7 days following inoculation.

Cell proliferation

Cell proliferation analysis was performed using the MTT assay according to the cell proliferation kit protocol (Sigma-Aldrich, St. Louis, MO, USA) at different time points. In the MTT test, tetrazolium salts were transformed by active enzymes of the cells into intracellular formazan deposits; the amount of color produced was directly proportional to the number of viable cells. Absorbance was determined at 490 nm with a microplate reader (Bio-Rad Model 550, Hercules, CA, USA).

Detection of alkaline phosphatase activity

At different time points, alkaline phosphatase (ALP) activity in the hPDLC cell layer was measured by *p*-nitrophenyl phosphate (*p*-NPP) substrate reactions (Sigma-Aldrich, St. Louis, MO, USA). After the medium was removed, cells were washed twice

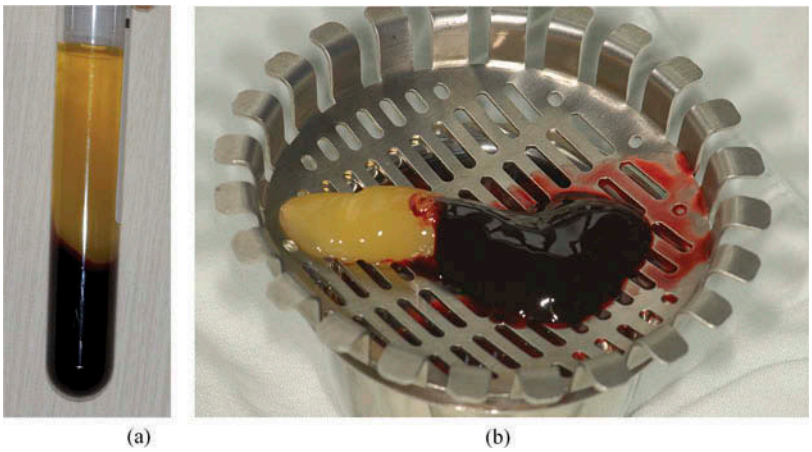

Figure 1. CGFs. (a) Three blood fractions were obtained through centrifuge process: (1) a superior phase represented by the serum; (2) an interim phase represented by a very large and dense polymerized fibrin block containing the CGFs, white blood cells, and stem cells; and (3) the lower red blood cell layer. (b) CGFs gel and red blood cells layer separated from platelet poor plasma.

with PBS and solubilized by the addition of 100 μL/well of 0.2% Triton X-100 and agitation on a plate-shaker for 20 min. The lysates were then collected and centrifuged at 13 000 × *g* for 5 min at 4°C, and the supernatants were used for the determination of ALP activity. The reaction mixture contained cell extract and the substrate (0.1 M diethanolamine buffer pH 10.5, 0.5 mM $MgCl_2$, 12 mM *p*-NPP) in a final volume of 0.5 mL. The mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 mL of 0.5 M NaOH. The *p*-nitrophenol formed was spectrophotometrically measured at 405 nm using a microplate reader (Bio-Rad Model 550).

Reverse transcription and real-time quantitative polymerase chain reaction (PCR) analyses

hPDLCs were treated by three kinds of culture: negative control (standard cell culture without CGFs), 3CGFs (standard cell culture + CGFs from 3 mL fresh whole blood), and positive control (standard cell culture + 40 ng/mL rhPDGF-AB 100 ng/mL rhTGF-β1). The standard cell culture was the same as above.

The hPDLCs were seeded in 6-well culture plates (Corning Life Sciences, Acton, MA, USA) at a density of 5×10^6 cells/well and were incubated for 24 h in 2 mL of DMEM containing 10% FBS to allow cells to attach and resume exponential growth. After 24 h, the baseline of the experiment, the medium was removed and replaced with 2 mL of DMEM containing 10% FBS. Then, CGFs or growth factors were added and experimental checking points were at 24 h, 72 h, and 7 days following inoculation.

QuantitativeRT-PCR was carried out. Total RNA was isolated from cultured hPDLCs using TRIzol reagent (Invitrogen, Carlsbad, California, USA) and transcribed to cDNA using a reverse transcription kit (M-MLV, Promega, USA) to synthesize cDNA according to the manufacturer's instructions. Specific primer for human Wnt3a cDNA was generated by selecting specific nucleotide sequences corresponding to the following oligonucleotides (Table I). Amplification was performed and the response procedure was consisted of 95°C for 10 min, 95°C for 15 s, and 60°C for 60 s for 40 cycles.

We performed three independent studies and confirmed similar results.

Statistical analysis

All reported values are the means of triplicate samples, and tests were repeated twice. Data were analyzed using SPSS version 10.0 (Chicago, IL, USA). Statistical analysis of the data was performed by one-way analysis of variance followed by the Tukey *post hoc* test for comparisons. For all tests, statistical significances were accepted for *P* values lower than 0.05.

Results

Effect of CGFs on hPDLCs proliferation

The effects of CGFs on hPDLCs growth are shown in Figure 2. CGFs treatment induced a strong time-dependent increase of hPDLCs proliferation. A significant difference between CGFs-treated cells and negative control cells became apparent at 24 h and was further increased at 72 h and 7 days ($P < 0.01$). The cell proliferation increased from 1CGFs to 3CGFs group accordingly ($P < 0.001$). However, when CGFs increased to 5CGFs, the proliferation of hPDLCs decreased ($P < 0.05$). The effect of CGFs on cell proliferation was shown in Figure 3.

There was a statistically significant increase in hPDLCs proliferation when the sample contained 3CGFs compared to the rhPDGF-AB and rhTGF-β1 combination at three time points ($P < 0.005$). At 72 h and 7 days, the addition of 1CGFs was more stimulatory compared to rhPDGF-AB and rhTGF-β1 combination stimulated hPDLCs proliferation ($P < 0.01$). 5CGFs were less stimulatory than rhPDGF-AB and rhTGF-β1 combination at 24 h and 7 days ($P < 0.01$).

Table I. Gene-specific primers for RT-PCR analysis.

Genes	Sequence	Prob size (bp)
Wnt3a	F: 5′-CATCTTTGGCCCTGTTCTGGA-3′ R: 5′-GCGTGTCACTGCGAAAGCTACT-3′	90
β-actin	F: 5′-CCTGGCACCCAGCACAAT-3′ R: 5′-CCGATCCACACGGAGTACTTG-3′	68

Effect of CGFs on hPDLCs ALP activity

Compared with negative control and rhPDGF-AB and rhTGF-β1 combination, all of the CGFs group significantly promoted the hPDLCs ALP activity at three time points ($P < 0.01$) (Figure 4). The promoting effect was dose dependent and time dependent ($P < 0.001$). The maximum effect was obtained at 5CGFs.

Effect of CGFs on mRNA Expression of Wnts in hPDLCs

Wnt3a, a member of canonical Wnt signaling pathway was chosen in this experiment. Expression of Wnt3a was examined by quantitative RT-PCR. As shown in Figure 5, at 24 h, the gene expression of Wnt3a was significantly increased in 3CGFs group compared with cells treated by negative or positive control group ($P < 0.01$). At 72 h and 7 days, the gene expression of Wnt3a in all groups decreased dramatically ($P < 0.001$).

Discussion

CGFs are produced by centrifuging venous blood using a special centrifugation procedure. The alternated and controlled speed centrifugation permits the isolation of a larger and denser fibrin matrix compared to PRF. The fibrin block is obtained as comprising three-dimensional polymer networks with interwoven fibers, all collected in a single phase in the form of gel. A large number of platelets are caught in the fibrin network, which make the release of growth factors more slowly. In theory, CGFs could become a powerful bio-scaffold with an integrated reservoir of growth factors for tissue regeneration.

PDLCs are major cells in periodontium. In the present study, CGFs were able to significantly stimulate hPDLCs proliferation in a dose-dependent manner when CGFs concentration increased from 1CGFs to 3CGFs. And ALP activity was also promoted by CGFs in a dose-dependent and time-dependent manner. Quantitative RT-PCR results showed that Wnt3a mRNA expression was increased with time in hPDLCs treated by 3CGFs.

CGFs are mitogenic for a variety of cell types. Yu et al. evaluated proliferation and osteogenic differentiation of beagle PDLSCs co-cultured with CGFs [12]. Their results showed that CGFs significantly promoted the proliferation of PDLSCs and exhibited a dose-dependent effect on the activation and differentiation of the stem cells. Qin et al. found that CGFs increased Schwann cell proliferation and neurotrophic factor secretion and promoted functional nerve recovery *in vivo* [18].

Rodella et al. demonstrated the presence of TGF-β1 and VEGF in CGFs and red blood cell layers, suggesting that an improved CGFs isolation procedure could optimize the amount of growth factors in the CGFs layer [11]. Our unpublished data also showed that the levels of TGF-β1, PDGF-BB, IGF-1, and VEGF in CGFs

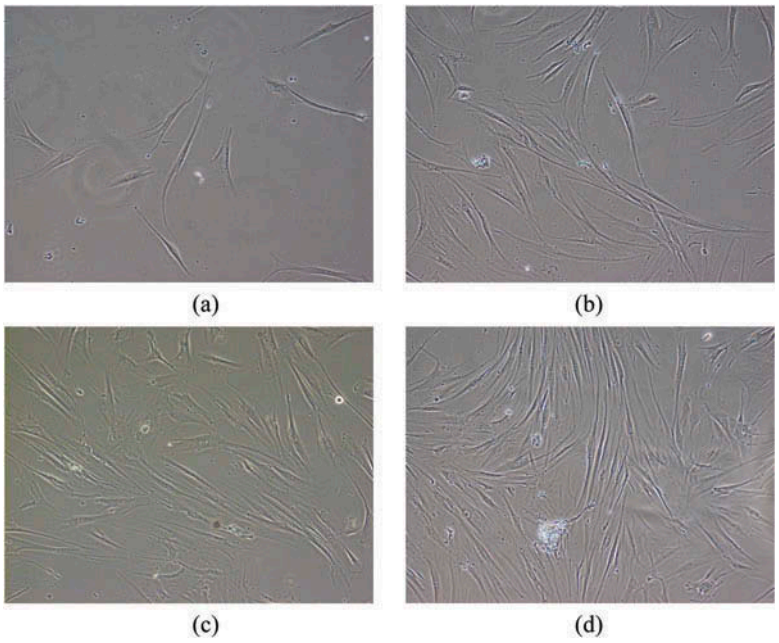

Figure 2. The effects of CGFs on hPDLCs growth at different time points. (a) baseline; (b) 24 h; (c) 72 h; and (d) 7 days.

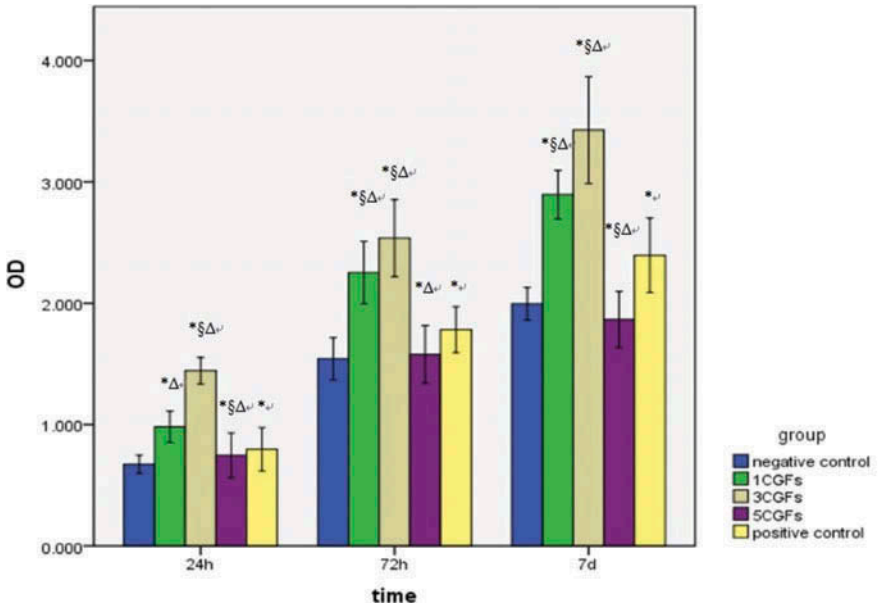

Figure 3. The effect of CGFs on hPDLCs proliferation at different time points. The result represents the means ± SD of six replicates. Negative control: standard cell culture without CGFs; positive control: standard cell culture + 40 ng/mL, rhPDGF-AB 100 ng/mL, rhTGF-β1; *compared with negative control, $P < 0.01$; §compared with positive control, $P < 0.01$; Δcompared with other CGFs groups at different time points, $P < 0.001$.

were significantly higher than those in PPP ($P = 0.000$). CGFs activity in promoting cell proliferation may be due to its biologic constituents – abundant growth factors that are involved in tissue regeneration. Polypeptide growth factors have been shown to play an important role in the growth and differentiation of cells involved in periodontal wound healing [5,6]. Unlike PRP, CGFs do not dissolve rapidly following application. Instead, the strong fibrin gel in the matrix addition is slowly remodeled in a similar manner to a natural blood clot. Thus, CGFs prolong the duration of growth factor activity, which is conducive for growth factor synergy, and enhance cell proliferation and osteogenic differentiation.

Yu et al. observed PDLSCs proliferation increased following CGFs treatment in a dose-dependent manner when the concentration of CGFs increased from 1CGFs to 3CGFs [12]. In the present study, when the concentration of CGFs increased from 3CGFs to 5CGFs, the accelerating role on cell proliferation decreased. 3CGFs seemed to be the optimal concentration of CGFs on hPDLCs proliferation. Previous studies have demonstrated that the high concentrations of PRP may also exhibit an inhibitory effect on the cultured cells [19]. This was similar in the present study.

Besides the negative control, we used rhPDGF-AB and rhTGF-β1 combination as positive controls. 1CGFs was significantly stronger compared to the standard cell culture and the rhPDGF-AB and rhTGF-β1 combination at 72 h and 7 days ($P < 0.01$). And 3CGFs showed significantly more stimulatory compared to rhPDGF-AB and rhTGF-β1 combination at all time points ($P < 0.005$). The mechanism responsible may be explained as follows: There are other growth factors in CGFs that may play the role. The efficiency of natural growth factors derived from blood in CGFs may be greater than recombined growth factor *in vitro*. Another

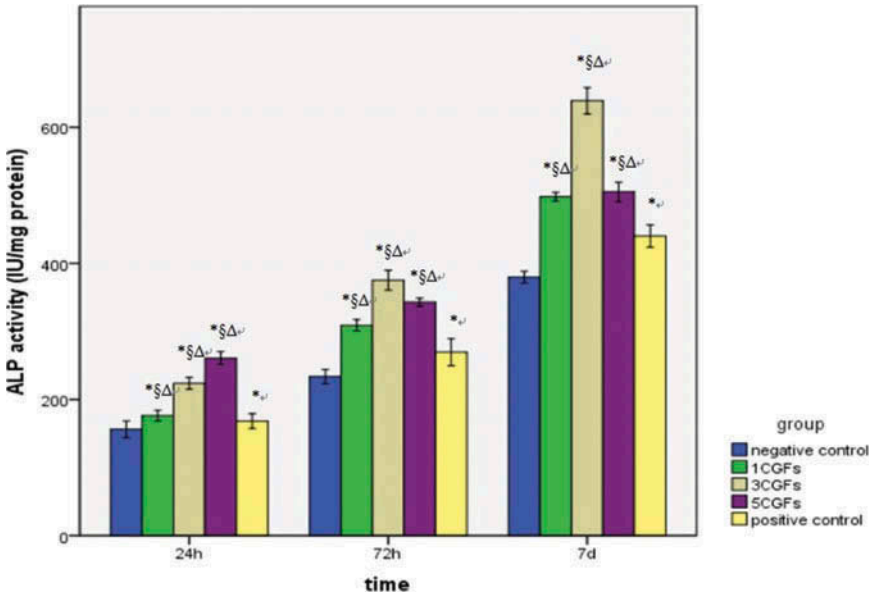

Figure 4. The effect of CGFs on ALP activity of hPDLCs at different time points. The result represents the means ± SD of six replicates. Negative control: standard cell culture without CGFs; positive control: standard cell culture + 40 ng/mL, rhPDGF-AB 100 ng/mL, rhTGF-β1; *compared with negative control, $P < 0.001$; §compared with positive control, $P < 0.01$; $^{\Delta}$compared with other CGFs groups at different time points, $P < 0.001$.

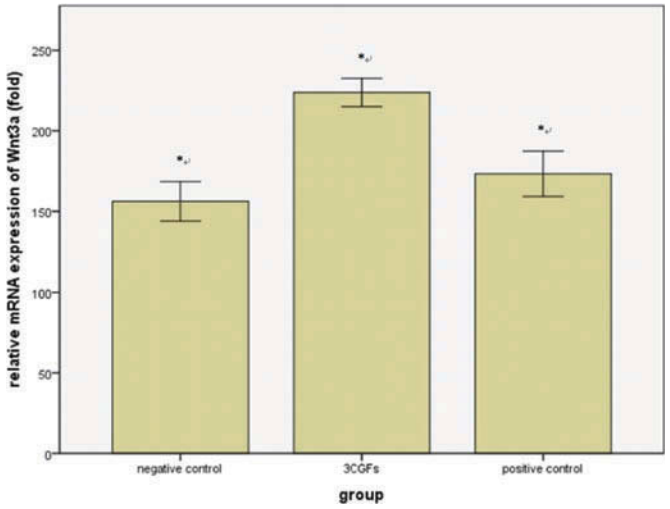

Figure 5. The effect of CGFs on gene expression of Wnt3a of hPDLCs at 24 h. The result represents the means ± SD of six replicates. Negative control: standard cell culture without CGFs; positive control: standard cell culture + 40 ng/mL, rhPDGF-AB 100 ng/mL, rhTGF-β1; *compared with other groups, $P < 0.01$.

possible reason may be synergism among various growth factors [20,21].

Aside from proliferation, the present study also examined osteogenic differentiation of hPDLCs following treatment by CGFs, by assessing ALP activity, a representative marker of bone forming. The results showed that CGFs significantly enhanced the hPDLCs ALP activity in a dose-dependent and time-dependent manner. And all of the CGFs concentrations significantly promoted ALP activity compared with rhPDGF-AB and rhTGF-β1 combination. Previous studies indicated that high concentrations of platelets may lead to an increase in osteoblast differentiation at the cost of cell proliferation [22]. This may explain the different roles of 5CGFs on PDLCs proliferation and ALP activity in the present study.

We chose 3CGFs as the optimal concentration according to the previous work and explored the possible intracellular pathways that affected the function of CGFs in hPDLCs. Expression of Wnt3a by hPDLCs was examined by real-time RT-PCR. Gene encoding Wnt3a was identified. The results showed that gene expression of Wnt3a was significantly increased at 24 h in 3CGFs group.

When contacting with hPDLCs, CGFs release various growth factors. These factors combine with their specific receptors on cell membrane immediately and thus result in the activation of intracellular signal transduction pathways, which causes final changes in cell functions. Previous studies have reported that TGF-β1 can induce the expression of Wnts in some cells or tissues and found the interaction between them [23]. Yang et al. found crosstalk between BMP-2 and Wnt/β-catenin signaling pathway in human keratinocytes [24]. This may partly explain the activation of Wnts expression in hPDLCs treated by CGFs.

Another reason may be that Wnt/β-catenin signaling participates in the proliferation and osteogenic differentiation of hPDLCs treated by CGFs in early phase. Liu et al. found that canonical Wnt signaling modulated osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions [25]. Heo et al. revealed that Wnt/β-catenin signaling enhanced osteoblastogenic differentiation of human periodontal ligament fibroblasts [26].

Conclusion

In conclusion, within the limitation of the present study, CGFs significantly promote the proliferation and ALP activity of hPDLCs, and the effects seem more stimulatory compared to rhPDGF-AB and rhTGF-β1 combination. Furthermore, we first report Wnt3a expression by hPDLCs after treated by CGFs. This indicates the participation of Wnt/β-catenin signaling in CGFs-inducing cell proliferation and differentiation in early phase. The present study provides evidence and basis for applications of CGFs in clinical and biotechnological area. Future studies are required to investigate the mechanism of CGFs, and we need more *in vivo* studies to identify the effect of CGFs in periodontal regeneration.

Acknowledgments

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Declaration of interest statement

The authors declare that there are no conflicts of interest in this study. This work was supported by Ministry of Science and Technology of China under contract International Science & Technology Cooperation Program foundation Nr.1019.

References

1. Heitz-Mayfield LJ, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. Periodontol 2000 2013;62 (1):218–231.
2. Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? Periodontol 2000 2009;51:208–219.
3. Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. J Clin Periodontol 2008;35(8 Suppl):117–135.
4. Srivastava S, Tandon P, Gupta KK, Srivastava A, Kumar V, Shrivastava T. A comparative clinico-radiographic study of guided tissue regeneration with bioresorbable membrane and a composite synthetic bone graft for the treatment of periodontal osseous defects. J Indian SocPeriodontol 2015;19(4):416–423.
5. Darby IB, Morris KH. A systematic review of the use of growth factors in human periodontal regeneration. J Periodontol 2012;21(3):416–422.
6. Maeda H, Fujii S, Tomokiyo A, Wada N, Akamine A. Periodontal tissue engineering: defining the triad. Int J Oral Maxillofac Implants. 2013;28(6):e461–471.
7. Stavropoulos A, Wikesjö UM. Growth and differentiation factors for periodontal regeneration: a review on factors with clinical testing. J Periodontal Res 2012;47(5):545–553.
8. Cochran DL, Oh TJ, Mills MP, Clem DS, McClain PK, Schallhorn RA, McGuire MK, Scheyer ET, Giannobile WV, Reddy MS, et al. Randomized clinical trial evaluating rh-FGF-2/β-TCP in periodontal defects. J Dent Res 2016 Feb 23. pii: 0022034516632497.
9. deObarrio JJ, Arauz-Dutari JI, Chamberlain TM, Croston A. The use of autologous growth factors in periodontal surgical therapy: platelet gel biotechnology–case reports. Int J Periodontics Restorative Dent 2000;20(5):486–497.
10. Corigliano M, Sacco L, Baldoni E. CGF- unapropostaterapeutica per la medicinarigenerativa. Odontoiatria – no1 anno XXIX - Maggio 2010;1:69–81.
11. Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Scarì G, Sacco L, Batani T, Rezzani R. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Technol 2011;74(8):772–777.
12. Yu B, Wang Z. Effect of concentrated growth factors on beagle periodontal ligament stem cells in vitro. Mol Med Rep 2014;9(1):235–242.
13. Tian A, Benchabane H, Wang Z, Ahmed Y. Regulation of stem cell proliferation and cell fate specification by wingless/Wnt signaling gradients enriched at adult intestinal compartment boundaries. PLoS Genet 2016;4;12(2):e1005822.
14. Nemoto E, Sakisaka Y, Tsuchiya M, Tamura M, Nakamura T, Kanaya S, Shimonishi M, Shimauchi H. Wnt3a signaling induces murine dental follicle cells to differentiate into cementoblastic/osteoblastic cells via an osterix-dependent pathway. J Periodontal Res 2015. doi: 10.1111/jre.12294.
15. Sassi N, Laadhar L, Allouche M, Zandieh-Doulabi B, Hamdoun M, Klein-Nulend J, Makni S, Sellami S. The roles of canonical and non-canonical Wnt signaling in human de-differentiated articular chondrocytes. Biotech Histochem 2014;89(1):53–65.
16. Mao L, Liu J, Zhao J, Chang J, Xia L, Jiang L, Wang X, Lin K, Fang B. Effect of micro-nano-hybrid structured hydroxyapatite bioceramics on osteogenic and cementogenic differentiation of human periodontal ligament stem cell via Wnt signaling pathway. Int J Nanomedicine 2015;10:7031–7044.
17. Ahmadzadeh A, Norozi F, Shahrabi S, Shahjahani M, Saki N. Wnt/β-catenin signaling in bone marrow niche. Cell Tissue Res 2016;363 (2):321–335.
18. Qin J, Wang L, Sun Y, Sun X, Wen C, Shahmoradi M, Zhou Y. Concentrated growth factor increases Schwann cell proliferation and neurotrophic factor secretion and promotes functional nerve recovery in vivo. Int J Mol Med 2016;37 (2):493–500.
19. Giusti I, D'Ascenzo S, Mancò A, Di Stefano G, Di Francesco M, Rughetti A, Dal Mas A, Properzi G, Calvisi V, Dolo V. Platelet concentration in platelet-rich plasma affects tenocyte behavior in vitro. Biomed Res Int 2014;2014:630870. doi: 10.1155/2014/630870.
20. Açil Y, Ghoniem AA, Wiltfang J, Gierloff M. Optimizing the osteogenic differentiation of human mesenchymal stromal cells by the synergistic action of growth factors. J Craniomaxillofac Surg 2014;42(8):2002–2009.
21. Cui X, Breitenkamp K, Lotz M, D'Lima D. Synergistic action of fibroblast growth factor-2 and transforming growth factor-beta1 enhances bioprinted human neocartilage formation. BiotechnolBioeng 2012;109(9):2357–2368.
22. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res 2006;17(2):212–219.
23. DiRenzo DM, Chaudhary MA, Shi X, Franco SR, Zent J, Wang K, Guo LW, Kent KC. A crosstalk between TGF-β/Smad3 and Wnt/β-catenin pathways promotes vascular smooth muscle cell proliferation. Cell Signal 2016;28(5):498–505.
24. Yang L, Yamasaki K, Shirakata Y, Dai X, Tokumaru S, Yahata Y, Tohyama M, Hanakawa Y, Sayama K, Hashimoto K. Bone morphogenetic protein-2 modulates Wnt and frizzled expression and enhances the canonical pathway of Wnt signaling in normal keratinocytes. J Dermatol Sci 2006;42(2):111–119.
25. Liu W, Konermann A, Guo T, Jäger A, Zhang L, Jin Y. Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 2014;1840(3):1125–1134.
26. Heo JS, Lee SY, Lee JC. Wnt/β-catenin signaling enhances osteoblastogenic differentiation from human periodontal ligament fibroblasts. Mol Cells 2010;30(5):449–454.