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Comparative evaluation of the therapeutic potential of two platelet concentrates related to regenerative endodontics

Sakka Dimitra[1*], Digka Anna[2], Karakota Maria[3], Gounari Eleni[4], Koutsiouki Theodora[5], Skoura Lemonia[6], Koliakos Georgios[7], Lyroudia Kleoniki[8]

[1]Postgraduate student, Department of Endodontology, Dental School of Aristotle University of Thessaloniki, Thessaloniki, Greece, ORCHID ID: 0000-0001-8362-6970

[2]DDS, PhD, Department of Endodontology, Dental School of Aristotle University of Thessaloniki, Thessaloniki, Greece, ORCHID ID: 0000-0002-3167-7554

[3]Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[4]Biologist, PhD student, Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[5]Department of Biochemistry, AHEPA University Hospital, Thessaloniki, Greece, Thessaloniki, Greece

[6]Department of Microbiology, AHEPA University Hospital, Thessaloniki, Greece, Thessaloniki, Greece

[7]Professor, Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[8]Professor, Department of Endodontology, Dental School, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract:

The aim of this study was the comparison of the concentration of two major growth factors, platelet derived growth factor BB (PDGF-BB) and vascular endothelial growth factor (VEGF), in two platelet concentrates (PC), concentrated growth factor (CGF) and platelet rich plasma (PRP), as well as the evaluation of the age effect in their concentrations. PRP is a well-studied platelet concentrate with very high congregations of active blood components crucial for Regenerative Endodontic Procedures (REP). CGF has been found to promote the in vitro proliferation, migration and differentiation of stem cells of apical papilla, but it has not been used clinically, yet. Twenty healthy individuals participated in this study, in two age groups, 20-30 and 40-50 years old. 10 ml of venous blood was drawn from each participant, 5ml of which was stored with anticoagulant agent in order to prepare the PRP and the rest 5ml were immediately centrifuged to prepare the CGF. All specimens were stored at sterile Eppendorf tubes at -80°C. PDGF-BB and VEGF levels were identified using ELISA kits and the results were used for statistical analysis, by using SPSS Statistics. PDGF-BB levels were higher in PRP than in CGF of the same donor, though VEGF show no significant difference. According to this study PRP contains higher concentration of PDGF-BB compared to CGF, but there is no significant difference between the levels of VEGF. Growth factor levels do not seem to have important differences between the two age groups.

Keywords: Platelet concentrates, regenerative endodontics, platelet-rich-plasma, concentrated growth factor, pulp regeneration

Corresponding author: Sakka Dimitra

Postgraduate student, Department of Endodontology, Dental School of Aristotle University of Thessaloniki, Thessaloniki, Greece, ORCHID ID: 0000-0001-8362-6970. Email: dimitra92sakka@gmail.com

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Introduction

Permanent immature teeth diagnosed with necrotic pulp remain a challenge in clinical endodontics. The classical techniques of mineral trioxide aggregate (MTA) apical plug and long-term calcium hydroxide apexification have been successfully used for years, leaving, nevertheless, these teeth susceptible to root fractures, due to thin dentinal walls and short root length.[1] Recently, regenerative endodontic procedures (REP), based on tissue engineering, have been used for the treatment of these teeth, showing promising results.

Stem cells, scaffold and growth factors are the "holy triad" of tissue engineering.[2]

Lately, platelet concentrates, such as platelet-rich-plasma (PRP) and platelet-rich-fibrin (PRF), have been used as scaffolds, with or without blood clot. The rationale is that they contain higher amounts of platelets than the blood clot and therefore higher concentrations of growth factors and bioactive molecules.[3] Platelet-derived growth

factor BB (PDGF-BB) and vascular endothelial growth factor (VEGF) are two major molecules associated with pulp regeneration.[4] Concentrated growth factor (CGF) is a relatively new platelet concentrate, that is not investigated enough in regenerative endodontics. Therefore, the aim of this study was to compare the concentration of PDGF-BB and VEGF, in two different platelet concentrates PRP and CGF, and in addition to evaluate whether the age of the patients affects the above-mentioned concentrations.

Materials and Methods

Twenty healthy volunteers (12 females and 8 males) participated in this study. All volunteers were informed regarding the purpose of this study and signed an informed consent prior to their inclusion. The study protocol was reviewed and approved from Ethical Committee of Dental School AUTH (57/13-06-2019). The clinical process was conducted at AHEPA University Hospital and preparation of platelet concentrates and quantification of growth factors at the Laboratory of Biological Chemistry of Medical School. Donors with systemic diseases, patients receiving drugs known to affect platelet functions and women on pregnancy were excluded. The participants were divided into two groups. The first group consisted of donors with an age between 20 and 30 years old, while the second group consisted of donors with an age ranging between 40 and 50 years old. 10 mL of venous whole blood was drawn from each volunteer, divided into two different tubes of 5 mL each.

PRP preparation

5 mL of each donor's blood was collected in a sterile BD Vacutainer EDTA tube (Becton-Dickinson Company, Plymouth, United Kingdom), containing a liquid solution of K3 Potassium Salt of EDTA (Ethylene Diamine Tetra Acetic acid) as an anticoagulant. Then, the tubes were centrifuged at 270g for 15 min (SV8 RH, Firlado), which resulted in the separation of two layers. The upper layer was plasma, platelets, and white blood cells (WBC), followed by the red blood cell (RBC) layer at the bottom. Then, the top layer was aspirated with a Pasteur pipette and centrifuged at 1000g for 7 min (Eppendorf centrifuge 5417R). Two basic fractions were obtained: platelet poor plasma (PPP) on the top, followed by PRP (a mixture of platelet concentrates and a small amount of red blood cells and white blood cells) at the bottom. Most PPP was aspirated according to platelet concentrations and the remaining PPP volume was mixed with PRP and stored at -80°C for the measurements of the contents of growth factors.

CGF preparation

5 mL of each donor's blood was collected in a sterile BD Vacutainer tube (Becton-Dickinson Company, Plymouth, United Kingdom) without anticoagulant solutions. These tubes were then immediately centrifuged (Eppendorf centrifuge 5417R) using a program with the following characteristics: 30 sec acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 4 min at 2700 rpm, 3 min at 3000 rpm, and 36 sec deceleration and stop. At the end of the process, three blood fractions were created: the upper layer consisted of the serum (blood plasma without fibrinogen and coagulation factors, platelet-poor plasma, PPP); the middle layer consisted of a large fibrin block containing the CGF, white blood cells and stem cells; and the bottom red blood cell (RBC) layer. The middle layer was obtained as CGF. Then, the obtained CGF was minced into two pieces of 2-3 mm, transferred into two sterile Eppendorf tubes and stored at –80°C for the measurement of growth factors.

Quantification of growth factors

Growth factors extracted from the CGF were prepared according to previously reported protocols (Zheng et al. 2015, Hong 2018). In brief, the frozen CGF clot was placed in a lyophilizer overnight and was grounded into powder. Then the CGF powder was immersed into the appropriate volume of Dulbecco's Modified Eagle's Medium (DMEM; Biowest , USA) without serum at 37 °C for 24 h. The medium was collected and centrifuged at 2000 g for 10 min, and the supernatant was filtered by a 0.2 lm strainer. Finally, the extracted solution, named CGF-conditioned medium (CCM), was investigated by enzyme-linked immunosorbent assay (ELISA). Growth factors from the PRP were warmed until room temperature and investigated by enzyme-linked immunosorbent assay (ELISA). The levels of two major growth factors in platelets, platelet derived growth factor (PDGF-BB) and vascular endothelial growth factor (VEGF) were evaluated using double antibody sandwich high sensitivity ELISA kits (Boster Bio, Pleasanton, CA, USA). PRP and CGF samples were plated on pre-coated 96 well plate in duplicates and growth factor concentrations were measured according to the manufacturer's instructions. OD values were measured under 450 nm wavelength absorbance using microplate reader (Stat Fax -2100, Awareness Technologies INC).

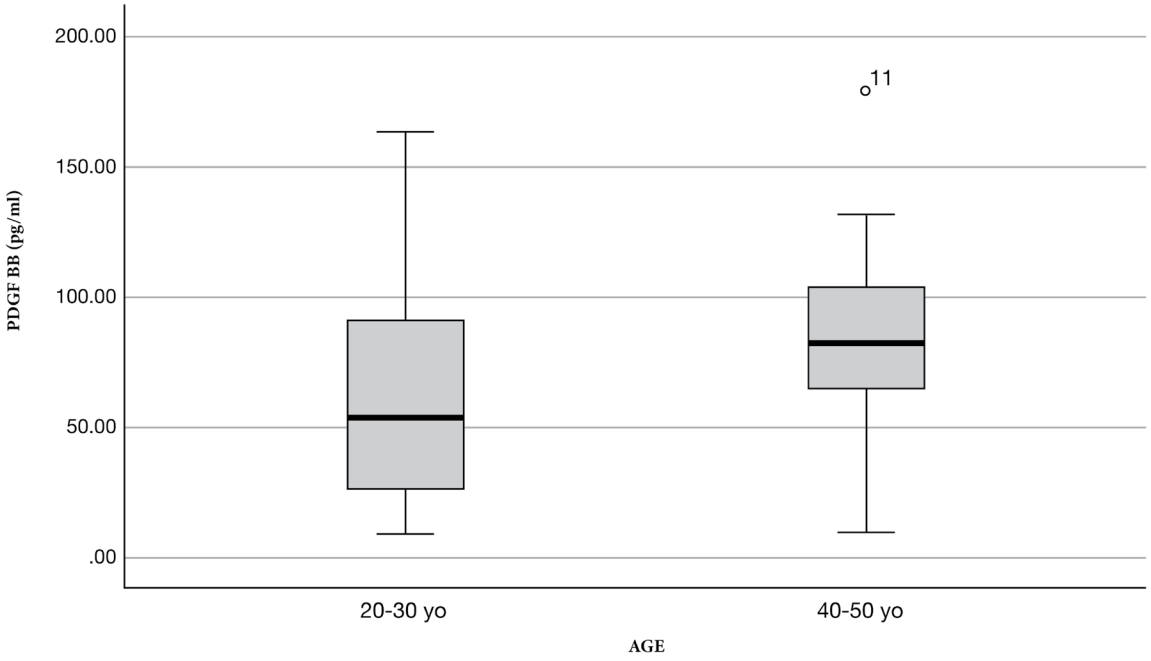
Statistical analysis

All reported values are the means of duplicate samples, and tests were repeated twice. Data were analysed using SPSS version 10.0 (Chicago, IL, USA). Statistical analysis of the data was performed by Student's t-test and Mann Whitney test. The levels of growth factors in PRP and CGF were compared with significance assigned at the $p \leq 0.05$ level.

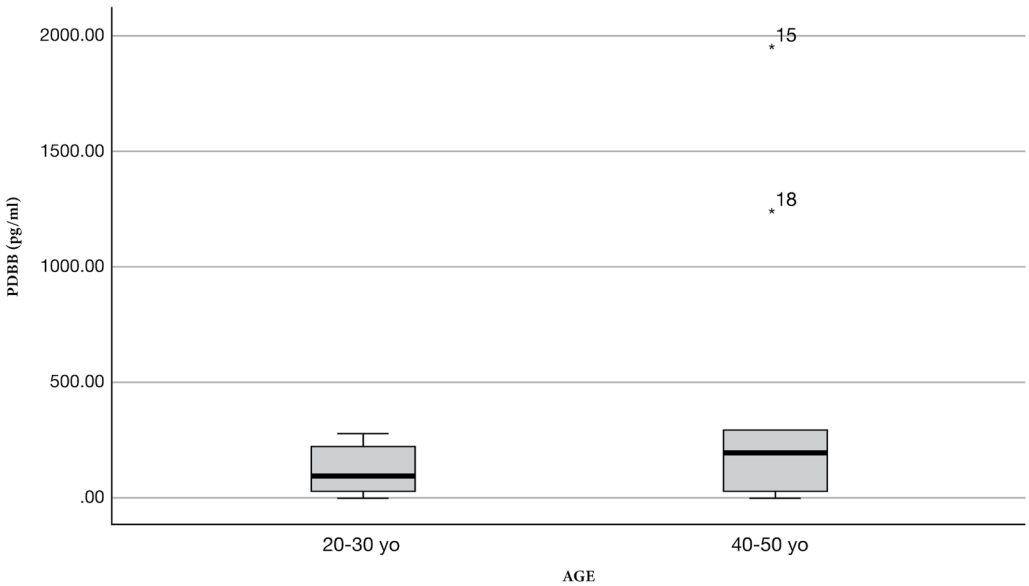
Results

The PDGF-BB levels

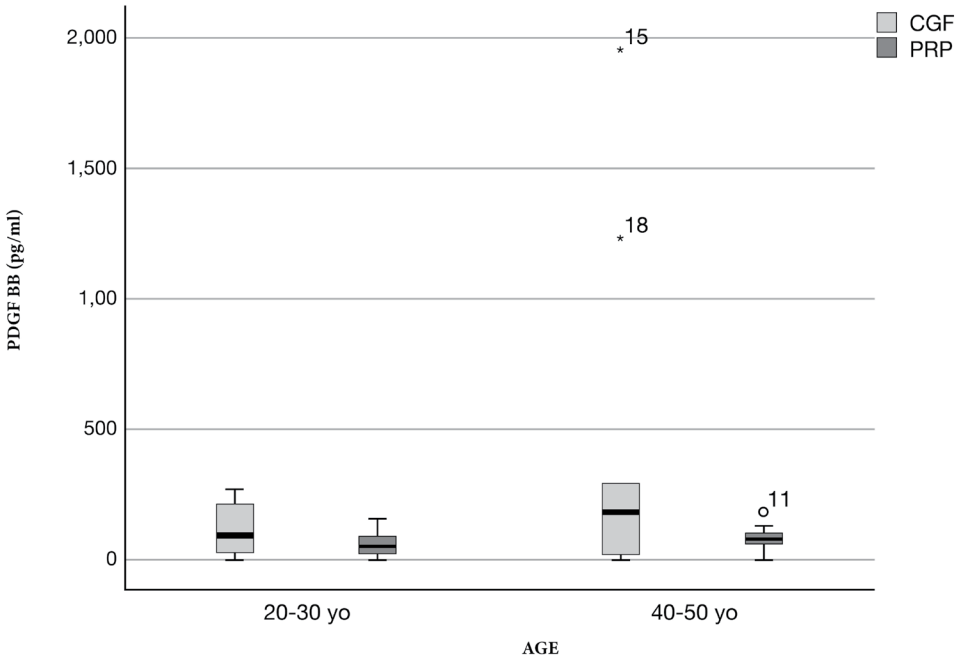
There was no significant difference between the levels of PDGF-BB in PRP from each group ($p=0.227>0.05$). In 20-30 yo group the mean PDGF-BB concentration was 3437,25 ± 693,65 pg/mL, while in 40-50 yo group the mean PDGF-BB concentration was 2871,5 ± 751,55 pg/mL (Figure 1).



There was no significant difference between the levels of PDGF-BB in CGF from each group (p=0.705>0.05). In 20-30 yo group the mean PDGF-BB concentration was 1295,4 ± 1099 pg/mL, while in 40-50 yo group the mean PDGF-BB concentration was 1384,65 ± 860,93 pg/mL (Figure 2).

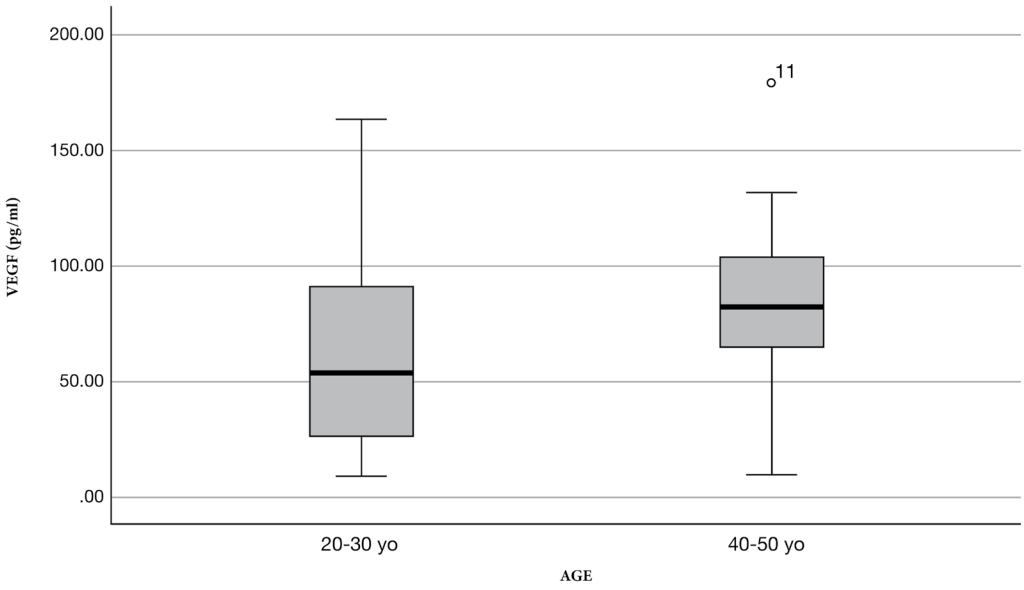


There was statistically significant difference between the levels of PDGF-BB in PRP and CGF (p=0.000<0.05). The mean PDGF-BB concentration in PRP was 3154,38 pg/mL, significantly higher than that in CGF, which was 1340,03 pg/mL(Figure 3).

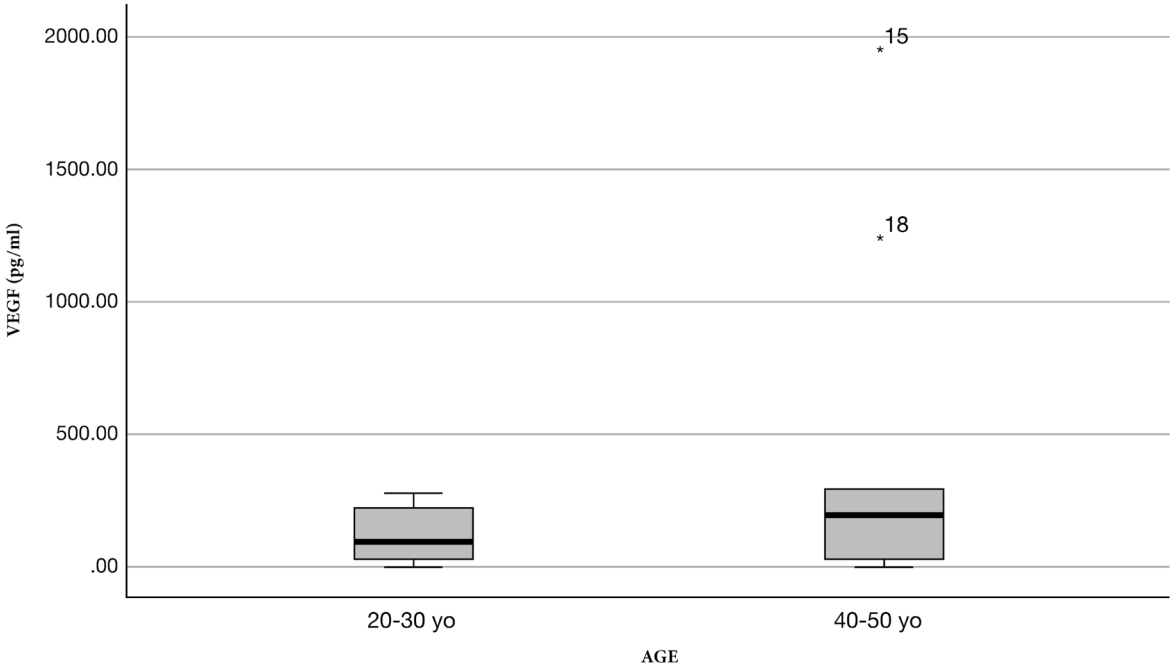


The VEGF levels

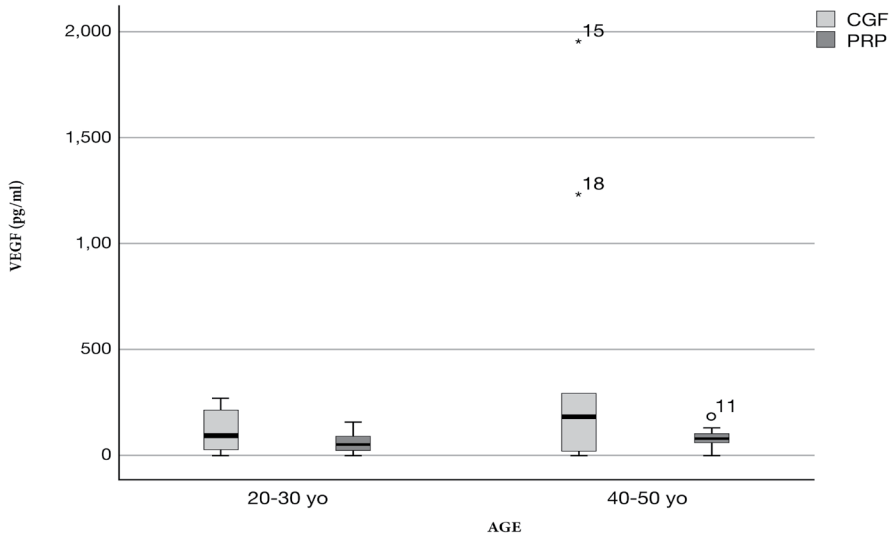
There was no significant difference between the levels of VEGF in PRP from each group (p=0.351>0.05). In 20-30 yo group the mean VEGF concentration was 63,33 ± 34,2 pg/mL, while in 40-50 yo group the mean VEGF concentration was 84,06 ± 35,03 pg/mL (Figure 4).



There was no significant difference between the levels of VEGF in CGF from each group (p=0.495>0.05). In 20-30 yo group the mean VEGF concentration was 116,50 ± 77,02 pg/mL, while in 40-50 yo group the mean VEGF concentration was 419,49 ± 465,04 pg/mL (Figure 5).



There was no significant difference between the levels of VEGF in PRP and CGF (p=0.73>0.05) (Figure 6).



Discussion

Regenerative dentistry consists a new evolving field of research and affects many dental specialties including endodontics. REP are defined as 'the biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex'.[5] They are applied in immature permanent teeth with necrotic pulp with or without apical periodontitis, as an alternative to apexification with calcium hydroxide or mineral trioxide aggregate, with promising results, as resolution of periapical lesions, completion of root formation, and, in some cases, reestablishment of tooth sensation.[6-8]

REP, as a form of tissue engineering, are based in the use of stem cells, scaffold and growth factors.[2]

Stem cells available for regenerative endodontic procedures are apical papilla stem cells (SCAPs)[9,10], periodontal ligament stem cells (PDLSCs)[11], bone marrow stem cells (BMSCs)[12] and in case of vital pulp remnants dental pulp stem cells (DPSCs)[13].

The most common scaffold used in routine clinical practice of REP is blood clot, provoked by a push and pull motion of a file beyond the apex. However, apical bleeding is considered as a potential factor of intracanal calcifications following REP.[14] Martin et al suggest that apical bleeding delivers PDLSCs and BMSCs inside the root canal resulting to cementogenesis and ectopic bone formation.[15] Digka et al confirmed that the mineralized tissues formed as a result of REP are mostly cementum-like and in some cases bone-like tissues. Additionally, blood provocation can cause trauma and detachment of the apical papilla. Furthermore, the amount of stem cells inserting in the root canal cannot be estimated.[16]

Lately, platelet concentrates, such as platelet-rich-plasma (PRP) and platelet-rich fibrin (PRF), have been used as alternative scaffolds in many cases. PRP is the first-generation platelet concentrate and it has been found to contain five × amounts of platelets than the blood clot and therefore higher concentrations of growth factors and bioactive molecules.[17,18] It has been histologically shown that PRP alone, as a scaffold for REP, can successfully lead to pulp-like tissue formation.[19] PRF as a second generation concentrate, shows very promising in vitro as well as clinical results, with a more simple preparation process.[20-22]

CGF is the latest generation platelet concentrate, which has been mainly used in vitro and has been applied clinically only in one animal study for pulp regeneration. It appears to be able to induce dentinal wall thickening and soft tissue ingrowth with odontoblasts and new blood vessel formation.[23] Research data suggest that CGF can promote the proliferation, migration and differentiation of SCAPs in vitro but there has been found no significant difference to PRP.[24]

Growth factors are bioactive signaling molecules capable of stimulating a variety of cellular processes such as cellular growth, proliferation and differentiation. VEGF is the most potent angiogenic growth factor. It promotes the migration, proliferation and expansion of endothelial cells. In addition, it has been shown to induce stem cell differentiation into endothelial cells and to promote dentin formation.[25]

PDGF-BB has, also, angiogenic potential, as it induces VEGF secretion.[4] Additionally, PDGF-BB acts as a chemotactic agent for mesenchymal stem cells and promotes tissue regeneration. Zhang et al[4] demonstrated that PDGF-BB strongly promotes pulp-dentin complex regeneration with newly formed dentin-like tissues and vessel formation.

Angiogenesis plays a key role for pulp regeneration, as adequate blood supply is essential for the new tissue to be formed without scar tissue into it.[25]

Here, it is demonstrated that PDGF-BB levels are higher in PRP, while VEGF levels show no significant difference. Qiao et al[22] proved that both PDGF-BB and VEGF levels did not differ significantly between PRP and CGF. On the other hand, Masuki et al suggested that CGF is more potently capable of inducing angiogenesis and promote tissue regeneration than PRP[26].

The stemness of stem cells is affected by age.[27] Stem cells are subjected to cellular senescence, losing their capacity for proliferation, differentiation and supporting tissue regeneration.[28] The effect of age in the concentration of growth factors, one of the crucial elements for tissue regeneration, has never before until now evaluated. In that notion, the concentration levels of two major growth factors PDGF-BB and VEGF from two different concentrates (PRP and CGF) were estimated in two age groups (A: 20-30 years old and B: 40-50 years old). The results of this study indicated that age does not play a significant role in the concentration and release of growth factors.

Further research with larger variety of growth factors is needed in order to expand the level of knowledge in this area.

Conclusion

PRP contains higher concentrations of PDGF-BB than CGF. VEGF levels do not significantly differ between PRP and CGF. The age of the patient does not affect the concentration of PDGF-BB and VEGF.

Declaration of Interest

The authors confirm that they have no conflicts of interest.

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