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# In Vitro And In Vivo Effects of Concentrated Growth Factor on Cells And Tissues

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## Abstract

This article reviews the biological outcome of the concentrated growth factor (CGF), a new platelet derivative used for tissue regeneration, in published articles related to the use of this product in basic and clinical studies. An electronic literature research using PubMed and SCOPUS was performed using combination of keywords: “concentrated growth factor” (OR “CGF”), AND “stem cells,” AND “cells” OR “cell proliferation” OR “cell migration” OR “cell differentiation,” AND “repair” OR “survival” OR

“revitalization,” AND “tissue” OR “bone.” Forty-five articles that were published between 2012 and 2020 met the inclusion criteria. These studies have used CGF as fresh solid form, freeze-dried, membrane, extract, or exudate. Most studies demonstrate the positive effects of CGF in a dose-dependent manner under certain concentrations. Studies comparing CGF with other platelet concentrates, report lower efficiency, no statistically significant differences, or better results for CGF. Combination of CGF with stem cells and biomaterials significantly improves bone regeneration and the effect of allograft or collagen membrane is better than CGF alone. For a better examination of the biological outcomes of CGF, the standardization of CGF preparation regarding the choice of the test tube material for blood collection, the required volume of blood, the necessary count of platelets in CGF, and the most appropriate type of CGF are recommended.

## 1 INTRODUCTION

Tissue engineering and regenerative medicine requiring stem cells, scaffolds, and growth factors are developing fast. In this development, the product cost, the processing time, the similarity with natural tissues, degradation of materials, and ethical issues are the most important considerations. Using stem cells is now shifting to stem cell-free concept by the administration of stem cell-derived exosomes or conditioned media (Kusuma, Carthew, Lim, & Frith, **2017**; Phinney & Pittenger, **2017**). New approaches in scaffolds consist of additive manufacturing (3D printing), decellularizing and scaffold-less strategies like self-assembling, self-organizing, and 3D bioprinting (Tatullo, Codispoti, Paduano, Nuzzolese, & Makeeva, **2019**; Thomas et al., **2016**). With great roles of growth factor in cell recruitment, proliferation, differentiation, and tissue repair, new strategic approaches to growth factors delivery are established (Mitchell, Briquez, Hubbell, & Cochran, **2016**; Nyberg, Holmes, Witham, & Grayson, **2016**). The release of growth factors is one of the challenging issues in tissue regeneration. Currently, platelet concentrates—like platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and CGF—as a reservoir of growth factors that can control their release are under many investigations (Kang et al., **2011**). PRP and plasma rich in growth factors (PRGF), as the first generation of these products, have been used in several clinical fields. However, the addition of anticoagulated agents and thrombin or calcium chloride for induction of fibrin polymerization leads to some debate about its biosafety. To overcome these drawbacks, PRF, which is the second generation of platelet concentrates, self-clotted, and set by one-step centrifugation, was presented (Raghoobar et al., **2005**). Many studies have proven the beneficial effects of L-PRF (with high number of leukocytes) and A-PRF (prepared by centrifugation at lower speed than L-PRF) in tissues regeneration (Bakhtiar et al., **2017**). Finally, repeated switch of the centrifugation speed resulted in the presentation of CGF as a modified form of PRF, with high amount of cytokines and very stiff texture, by Sacco (Masuki et al., **2016**; Qiao & An, **2017**).

Intrinsic coagulation reaction in venous blood causes activation of CGF, which contains many different growth factors like vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF- $\beta$ ), and platelet-derived growth factor (PDGF). The effects of these growth factors on tissue regeneration are approved in many studies. Also, the release of chemokines responsible for cell recruitment is another advantage of this new generation of platelets concentrates. The dense network of CGF leads to the protection of growth factors and chemokines (Lundquist, Dziegiel, & Agren, **2008**). Special centrifuge (Medifuge, Silfradent srl, Italy) used for extraction of CGF is the same device used for PRF isolation, but unlike constant speed for PRF, it utilizes altered speed rate. The process is easy and

results in a denser matrix, which has more growth factors than PRP and PRF and potentially better sustained release of growth factors as a drug delivery system (Sohn et al., **2011**). Besides platelets, the presence of CD34-positive stem cells in CGF has been established in one study (Rodella et al., **2011**). Moreover, a study on the release pattern of CGF in scaffolds, like deproteinized bovine bone mineral and intrafibrillar-mineralized collagen (IMC), showed that mixture of CGF with IMC causes a sustained release of growth factors and cytokines until 28 days (Yu, Wang, Liu, & Qiao, **2019**). It seems that addition of some biomaterials—like beta-tricalcium phosphate ( $\beta$ -TCP)—could enhance the release of some growth factors, like bone morphogenetic protein (BMP) 2 and 7, which have an important role in bone regeneration (Bonazza et al., **2018**).

In recent years, applications of autogenous growth factors prepared from centrifugation of whole blood increased in wound healing and tissue regeneration. These platelet concentrates can be used alone or in combination with other biomaterials for soft tissue healing or bone formation. Utilization of these growth factors in many clinical issues like ridge preservation and augmentation, sinus lift procedures, fracture repair, and implant stability has been reported (Mirkovic, Djurdjevic-Mirkovic, & Pugkar, **2015**; Pirpir, Yilmaz, Candirli, & Balaban, **2017**; Shyu, Fu, & Shen, **2016**). Avoiding immunoreactions, cross-contamination, and ethical concerns make CGF a very popular product, among all platelet concentrates, for tissue repair (Zhang & Ai, **2019**). Many clinical studies actually focus on the effects of CGF in bone regeneration (Brignardello-Petersen, **2020**; Doan, Reher, Duong, Wang, & Truong, **2019**; Özveri Koyuncu, Işık, Özden Yüce, Günbay, & Günbay, **2019**; Xu, Qiu, et al., **2019**). With the increasing use of CGF, questions about its FDA approval are raised. According to FDA's 21 CFR 1271 of the Code of Regulations, FDA's traditional regulatory pathway is not required for blood products; however, the device used for their preparation will need FDA clearance. Harvest SmartPrep Platelet Concentrate System (Harvest Technologies, Plymouth, MA) and the 3i Platelet Concentrate Collection System (3i Implant Innovations, Palm Beach Gardens, FL, United States) for preparing PRP, and IntraSpin L-PRF (Intra-Lock Inc., Boca Raton, FL) for preparing L-PRF, have FDA clearance (Agrawal, **2017**; Dohan Ehrenfest et al., **2014**). Off-label use of other devices is not restricted but requires high responsibility of clinicians and their awareness about the advantages and disadvantages of CGF application (Beitzel et al., **2015**; Jones, Togashi, & Thomas Vangness Jr., **2018**).

Published articles about the clinical and biotechnological application of CGF have increased; however, no review article summarizes the results. Our objective was to review the in vitro and in vivo biological effects of CGFs.

## 2 METHODS

A comprehensive search in PubMed and SCOPUS was carried out using keywords: “concentrated growth factor” (OR “CGF”), AND “stem cells,” AND “cells” OR “cell proliferation” OR “cell migration” OR “cell differentiation,” AND “repair” OR “survival” OR “revitalization,” AND “tissue” OR “bone.”

Correspondingly, references of founded articles were investigated to find other relevant articles. Only publications in the English language were considered for evaluation and extraction of data. Articles published from 2000 to 2020 were assessed based on our research question, which was addressed respecting *PICOS* elements (**P**—participants or population, **I**—intervention, **C**—comparison, control or comparator, **O**—outcome, and **S**—study design) (Table 1). Case reports and articles not considering the

control group were removed from our study. The full texts of all corresponding articles were assessed and finally, 45 studies were selected for this review.

**Table 1.** PICOS format of our reviews' question

Component	Description
Population	Studies using CGF and considering its effect on cells or tissues
Intervention	Using CGF for cell proliferation, cell differentiation, or healing damaged tissues
Comparison	Control group with no treatment or receiving other treatments
Outcome	Cell behavior or tissue response after treatment
Study design	In vitro and in vivo studies

Abbreviations: CGF, concentrated growth factor; PICOS, **P**—participants or population, **I**—intervention, **C**—comparison, control or comparator, **O**—outcome and **S**—study design.

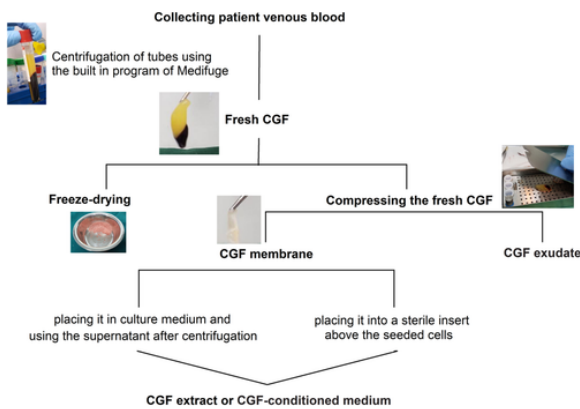
## 3 RESULTS

### 3.1 Methods of CGF preparation and application

CGF is applied as prepared (solid form), or as membrane, exudate or conditioned media. For preparing the solid form, venous blood is drowned into sterile VACUETTE tubes with a glass surface. There is no need for using synthetic or catalytic agents during the processing of CGF. Tubes are centrifuged using the built-in program (30 s acceleration, 2 min at  $408 \times g$ , 4 min at  $323 \times g$ , 4 min at  $408 \times g$ , 3 min at  $503 \times g$ , and 36 s deceleration and stop). Then, the intermediate gel layer in the final three-layered product, which is CGF, should be separated from the third layer by scissor, and could be used as prepared or freeze-dried (Borsani et al., **2018**).

For producing the CGF membrane, the fresh form of CGF will be compressed for 1 or 2 min. The release of growth factors in the resulting film is slower than the solid form (Qiao & An, **2017**). The fluid isolated from CGF clot during pressing and collected in the tray of the endo box is named CGF exudate (CGFe). The next step is centrifugation of this exudate at  $500 \times g$  for 5 min and removal of red blood cells (Li et al., **2019**). CGFe could also be prepared by mincing CGF clots, homogenizing it and storing at  $-80^{\circ}\text{C}$  for 1 hr, then using the supernatants after centrifugation (Jun, Lei, Qifang, Yuan, & Deqin, **2018**).

CGF extract or conditioned media is another form of CGF used in research. By placing CGF fresh membranes or lyophilized membrane in culture medium (5–10 ml) for one or several days (7–14 days) and centrifuging the media, the supernatant could be used as CGF extract or CGF-conditioned medium (CCM). CCM should be supplemented with fetal bovine serum and antibiotics (Hong, Li, Cai, & Jiang, **2019**; Jin et al., **2018**). Another way to produce CGF extract is placing the CGF membrane into a sterile insert above the seeded cells in the six-well plates (an insert in each well), which are immersed in culture medium (for 72 hr) (Borsani et al., **2015**). Figure 1 shows different steps in preparing the various types of CGF.



**Figure 1** Steps in preparing the various types of concentrated growth factor (CGF)

One more type of applied CGF is its mixture with biomaterials or autologous bone. For this purpose, fresh CGF will be cut into small fragments and mixed with biomaterial at a defined volume ratio. The result of the mechanical mixture is an adhesive paste that can be placed in tissue defect (Qiao, Duan, Zhang, Chu, & Sun, **2016**).

### 3.2 Effect of CGF on stem cells

Stem cells were used in 11 studies to evaluate their proliferation and differentiation under treatment with various types of CGF (Table **2**). Different types of stem cells, which were derived from PDL, dental pulp, apical papilla, bone marrow, adipose tissue, and gingiva have been used in these studies. Considering the high amount of TGF- $\beta$ , IGF, VEGF, and PDGF in CGF and their important effect on proliferation and differentiation (Borsani et al., **2015**), these characters were evaluated in almost all studies. Most of these studies reported more proliferation in a dose-dependent manner. However, four studies showed that a high dose of CGF does not have a positive effect. In the study of Honda et al., concentrations of 1–10% of CGF extract had a positive effect on proliferation and differentiation in a dose-dependent manner, while high concentration (20%) had an inhibitory effect (Honda et al., **2013**). Chen et al., by studying the effect of 5, 10, 20, and 40% CGF, demonstrated more proliferation and differentiation in 10% CGF (Chen et al., **2018**). No dose-dependent effect was also stated by Hong et al. in investigation of the effect of three different concentration of CGF–CCM (1,  $\frac{1}{2}$ ,  $\frac{1}{4}$ ) (Hong et al., **2019**). Likewise, Qiao et al. reported lower cell proliferation by using five CGFs (5 ml whole blood) in comparison to three CGFs (3 ml whole blood) (Qiao & An, **2017**). Although under treatment with CGF extracts, higher cells proliferation in a dose-dependent manner was approved by Jin et al., enhanced cell migration, and differentiation in the low doses (<50%) of CGF was noticeable (Jin et al., **2018**). Two studies compared effect of CGF with PRF, and the results show that PRF is more efficient in osteogenic differentiation of stem cells, while CGF is more efficient in vascular differentiation (Hong et al., **2018**; Hu et al., **2018**).

**Table 2.** List of studies on the effects of CGF on stem cells

Author	Stem cell	Intervention	Control group	Features tested	Assays	Outcome
Honda, Tamai, Naka, Yoshikawa, and Myoui (2013)	hTERTE6/E7 human MSCs	CGF extract, at concentrations of 1, 3, 5, 10, or 20%	Medium without CGF	Proliferation, differentiation	Cell count, ALP staining and activity, Alizarin Red, and Von Kossa staining, qRT-PCR	More proliferation, and differentiation of hTERTE6/E7 human MSCs in a dose dependent at concentrations between 1 and 10%, inhibitory effect at higher concentrations
Yu and Wang (2014)	Beagle PDLSCs	One CGFs (prepared with 1 ml fresh whole blood), three CGFs (3 ml whole blood), osteogenesis-induced fluid +1 CGF, osteogenesis-induced fluid +3 CGFs	The standard group (0 CGF), osteogenesis-induced fluid (0 CGFs)	Proliferation, osteogenic differentiation	Cell counting, MTT assay, Alizarin Red S staining, ALP activity, qPCR, western blot analysis, immunohistochemistry	More proliferation and differentiation by using CGF in a dose-dependent manner
Qiao and An (2017)	hPDLCs	One CGF (1 ml whole blood), three CGFs (3 ml whole blood), five CGFs (5 ml whole blood)	Negative control group (standard cell culture), positive control group (standard cell culture +40 ng/ml rhPDGF-AB 100 ng/ml rhTGF- $\beta$ 1)	Proliferation, ALP activity, Wnt3a expression	MTT assay, p-NPP assay, qRT-PCR	More proliferation and ALP activity and more Wnt3a mRNA expression in a dose dependent Manner in groups treated by CGFs. Optimal concentration: three CGFs
Hong, Chen, and Jiang (2018)	SCAPs	Freeze-dried CGF-CCM	PRF-CCM and normal medium	Proliferation, migration, mineralization and differentiation	Cell counting Kit-8, Transwell assays, Alizarin Red S staining qPCR	More proliferation, migration and mineralization in PRF and CGF groups than normal medium,

						more differentiation in PRF group
Jin et al. (2018)	DPSCs	Different concentrations (5, 10, 20, 50, or 80%) of CGF extracts	Blank DMEM	Proliferation, EdU assay, migration, odontoblastic differentiation, endothelial differentiation	Cell counting Kit-8, scratches, ALP assay, Alizarin Red S staining, qRT-PCR and western blot, tube formation assay	Higher cell proliferation in a dose-dependent manner. More migration, and differentiation in low doses (<50%) of CGF
Hong et al. (2019)	SCAPs	CGF-CCM in three different concentrations (1, ½, ¼)	Normal medium	Proliferation, migration, mineralized nodule formation and odontoblastic differentiation	Cell counting kit-8, Transwell Filter Inserts, Alizarin red staining, RT-qPCR	More proliferation, migration, and differentiation under treatment with CGF. No dose-dependent effect
Chen et al. (2018)	BMSCs	5, 10, 20, and 40% CGF	DMEM, osteogenesis induction medium	Proliferation, mineralization, osteogenic differentiation	CCK-8 assay, ALP-positive cell staining and mineralized nodule formation assay, RT-qPCR analysis and western blotting	More proliferation in 10% CGF. Better differentiation in this group than positive control
Hu, Jiang, Wang, Tian, and Wang (2018)	hADSCs	1 ml of CGF	Culture medium, 1 ml of PRP, 1 ml of PRF	Proliferation and vascular differentiation	Cell count, RT-PCR	Higher cell proliferation and expression of VEGF and PECAM in CGF group than other groups
Li et al. (2019)	hPDLCs stimulated by TNF-α	CGFe (concentration 50%) or CGFe + TNF-α	Culture medium, TNF-α (10 ng/ml) treatment	Proliferation, osteogenic differentiation	Cell counting Kit-8 assays, Alizarin Red S staining, ALP activity, western blotting, and qPCR	More proliferation and differentiation in CGFe groups, even in the presence of TNF-α-induced inflammation



Xu, Qiao, et al. (2019)	hDPSCs	CCM extracted by freeze-dried method	Cell treated with or without LPS	Proliferation, migration, differentiation	CCK-8, Transwell assays, Alizarin Red staining, ALP activity, qPCR, HE	Positive effect of CGF on the proliferation, migration, and differentiation of hDPSCs exposed to LPS
Chen, Chen, et al. (2019)	GMSCs	CGF extract: 5, 10, 20, and 40%	DMEM; osteogenesis induction medium	Cell proliferation, mineralization, differentiation	MTT, CCK-8 assay. ALP staining, RT-qPCR, western blotting	Proliferation and osteogenic differentiation of GMSCs treated with CGF

Abbreviations: ALP, alkaline phosphatase; BMSCs, bone marrow-derived mesenchymal stem cells; CCK, cell counting kit; CCM, conditioned medium; CGFe, concentrated growth factor exudate; DMEM, Dulbecco's modified Eagle medium; DPSCs, dental pulp stem cells; GMSCs, gingiva-derived mesenchymal stem cells; hADSCs, human adipose-derived stem cells; HE, hematoxylin and eosin stain; hPDLs, human periodontal ligament cells; LPS, lipopolysaccharides; MSCs, mesenchymal stem cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PECAM, platelet endothelial cell adhesion molecule; pNPP, *p*-nitrophenyl phosphate; PRF, platelet-rich fibrin; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SCAPs, human stem cells of the apical papilla; TNF- $\alpha$ , tumor necrosis factor; VEGF, vascular endothelial growth factor.

**Table 3.** List of studies on the effects of CGF on adult cells or cell lines

Author	Cell	Intervention	Control group	Features tested	Assays	Outcome
Borsani et al. (2015)	Three different types of human cell lines: NHDF, HUVEC, HOB	Basal medium + CGF, growth medium + CGF	Basal medium, growth medium	Proliferation	FACS	Higher proliferation in growth medium + CGF
Takeda, Katsutoshi, Matsuzaka, and Inoue (2015)	Rat bone marrow cells	CGF from rat blood	PPP gel	Morphology, proliferation, differentiation	Immunofluorescence, WST-1 assay, ALP activity	More proliferation and differentiation in CGF-coated disks
Masuki et al. (2016)	Human alveolar bone-derived periosteal cells	CGF extract	PRP, PRGF, A-PRF extract	Proliferation	Photographed and counted in three randomly selected views using image-PRO plus software	More proliferation in PRP > CGF > A-PRF > PRGF

Qin, Wang, Sun, et al. (2016)	SCs	CGF extract from rats	DMEM	Proliferation, cell cycle analysis, expression of NGF and GDNF	CCK-8 assay, flow cytometry, RT-qPCR, Western blot	More proliferation and secretion of neurotrophic factors in CGF-treated SC
Qin, Wang, Zheng, et al. (2016)	SCs	CGF extract from rats	DMEM	Migration, expression of integrin $\beta$ 1	Scratch wound-healing assay, Western blot, integrin $\beta$ 1 gene silencing, RT-qPCR	More migration in CGF group through higher expression of integrin $\beta$ 1
Borsani et al. (2018)	Human osteoblasts treated with bisphosphonates	CGF, CGF + RSV 10, CGF + AL 5, CGF + ZOL 5, CGF + AL 5 + RSV 10, CGF + ZOL 5 + RSV 10	Complete medium ( <i>OGM</i> )— Control OGM + RSV 10, OGM + AL 5, OGM + ZOL 5, OGM + AL 5 + RSV 10, OGM + ZOL 5 + RSV 10	Proliferation and differentiation	MTT, ELISA, immunohistochemistry, Alizarin Red S staining	Protective role of CGF + resveratrol on osteoblasts treated with bisphosphonates
Yilmaz et al. (2018)	SaOS-2 osteoblast-like cells cultured on titanium discs	CGFe	Untreated control group	Cell counts, cell proliferation, OCN levels and ALP activity	MTT, ELISA	More proliferation and differentiation in CGF group
Jun et al. (2018)	Human dental pulp cells and HUVECs	CGF extracts (2, 5, 10, and 15%)	DMEM	Proliferation, migration, angiogenesis-associated mRNA and protein expression, angiogenic capacity	CCK-8 assay, cell cycle assay, Transwell assay, qRT-PCR, Western blotting, tube formation assay	More proliferation, differentiation, and migration in CGF groups in a dose-dependent manner
Chen, Jiao, et al. (2019)	NHDFs	Four concentrations of CCM: 5, 10, 15, and 20%	Normal, and cellular photoaging models	Cell viability, migration, amount of oxygen free radicals and	MTT, ROS assay, SOD assay, scratch assay	Positive effect of CGF on scavenging ROS, improving SOD, and increasing

				antioxidative enzymes		migration rates of cells
Zhang and Ai (2019)	Rabbit PDCs	CGF membranes or CGF conditioned media	Untreated control group	Cell proliferation, osteogenic differentiation and angiogenic potential	CCK-8, ALP activity, immunohistochemistry, ELISA, qPCR, Western blot	Increased proliferation and osteogenic differentiation and angiogenic potential of PDCs treated with CGF
Tian et al. (2019)	DPCs	Different dose of CGF membrane	Untreated control group	Proliferation, migration, odontogenic differentiation	CCK-8, Transwell assay, Alizarin Red staining, alkaline phosphatase activity, Western blot and qPCR	Increase proliferation, migration and differentiation with application of CGF
Borsani et al. (2020)	Human SH-SY5Y neuronal cells	CGF or CGF+ retinoic acid	(1) Untreated control group and (2) differentiated control (supplemented with retinoic acid)	Cell proliferation, cell viability, cell differentiation	Automated cell counter; MTT assay; immunocytochemistry, Western blotting, ELISA	Decrease of cell proliferation and increase of cellular differentiation in the presence of CGF

Abbreviations: AL, alendronate; ALP, alkaline phosphatase; CCK-8, cell counting kit-8; CCM, conditioned medium; A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factor; CGFe, CGF exudate; DMEM, Dulbecco's modified Eagle medium; DPCs, dental pulp cells; ELISA, enzyme-linked immunosorbent assay; FACS, flow cytometry; GDNF, glial cell-derived neurotrophic factor; HOB, human osteoblast; HUVECs, human umbilical vein endothelial cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NHDF, normal human dermal fibroblasts; NGF, nerve growth factor; OCN, osteocalcin; PDCs, periosteum-derived cells; PPP, platelet-poor plasma; PRGF, plasma rich in growth factors; PRP, platelet-rich plasma; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species assay; RSV, resveratrol; SCs, Schwann cells; SAOS-2, sarcoma osteogenic; SOD, superoxide dismutase assay; ZOL, zoledronate or zoledronic acid.

**Table 4.** List of studies on the effects of CGF on bone regeneration

Author	Study	Intervention	Control group	Assays	Outcome
Honda et al. (2013)	Animal study ( <i>Rattus norvegicus</i> calvaria defect model)	CGF alone, CGF + BMSCs	Unfilled defect	Micro-CT and histological analysis	Completely repair of critical-size bone defects with CGF + BMSCs

Kim, Kim, Sandor, and Kim (2014)	Animal study (rabbit-skull defect)	CGF	PRP, PRF, and void	Micro-CT and histomorphometries	Similar effect of PRP, PRF, and CGF on bone formation
Takeda et al. (2015)	Animal study (rat calvaria bone defects)	CGF	PPP gel or left unfilled	Micro-CT and histological analysis	More bone formation in defects treated with CGF
Yang, Liu, Chen, Xie, and Wu (2015)	Human study (reducing bone resorption in immediate implant)	CGF	Bio-Oss	CBCT	No significant bone regeneration in using CGF alone
Durmuslar et al. (2016)	Animal study (healing of peri-implant bone defects in New Zealand white rabbits)	CGF, AB + CGF	Untreated defect, AB	Histomorphometrically	Best bone regeneration in CGF + AB
Park et al. (2016)	Animal study (femur defect in adult dogs)	CGF	Unfilled defect, PRF, synthetic bone	Histomorphometry evaluation, detection of TGF- $\beta$ and VEGF, SEM	More bone formation in allograft group. Better results in CGF than PRF
Qiao et al. (2016)	Human study (intrabony defect treatment)	CGFs + BPBM	BPBM alone	Radiographic measurements	Improved clinical effectiveness in CGFs + BPBM
Pirpir et al. (2017)	Human study (implant placement)	CGF membrane in implant cavity walls, CGF fluid on the surfaces of implants	Conventional implant placement	RFA with the Osstell device	Positive effects of CGF on implant stabilization and osseointegration
Wang, Sun, He, and Wang (2017)	Animal study (maxillary sinus floor augmentation in beagle dogs)	CGFs/Bio-Oss (1:1)	BMSCs/Bio-Oss construct, or Bio-Oss alone	Micro-CT, microhardness test, histological examination, and histomorphometry	Increase bone formation in combination of Bio-Oss/CGFs
Shetty, Kalra, and Hegde (2018)	Human study (sinus elevation)	CGF	No graft material	CBCT	Higher bone densities and lower bone formation in CGF-treated group

Huang, Zou, He, Ouyang, and Piao (2018)	Human study (alveolar cleft)	Alveolar bone grafting combined with CGF	Acellular dermal matrix film combined with alveolar bone grafting using iliac crest bone grafts	CBCT	Improvement of bone density in CGF-treated groups
Chen et al. (2018)	Animal study (male Wistar rats with calvaria defects)	CGF + BMSCs; CGF	Collagen + BMSCs; collagen; blank	Micro-CT, histological and immunohistological analysis	Highest level of bone regeneration in CGF + BMSC group
Yilmaz et al. (2018)	Animal study (rabbit)	Masquelet technique + CGF	Masquelet technique as control groups	Histopathology and immunohistochemistry	Improvement of the quality of the membrane with addition of CGF to the Masquelet technique
Isler, Soysal, Ceyhanli, Bakirarar, and Unsal (2018)	Randomized clinical trial (peri-implantitis)	Bone substitute + CGF	Bone substitute + CM	Radiographic evaluation	Better results in collagen membrane + bone substitute
Lei et al. (2019)	Human study (retrospective cohort study for GTR)	CGF + GTR	A-PRF + GTR, or GTR surgery	Radiographic evaluation, recording PD and CAL	No difference between A-PRF and CGF, more favorable clinical result in both groups than the control
Özveri Koyuncu, İçpınar Çelik, Özden Yüce, Günbay, and Çömlekoğlu (2019)	Human study (implant stability)	Dental implant socket covered with CGF membrane	Conventionally prepared socket	Implant stability measured by RFA	Not significant difference between groups
Xu, Qiu, et al. (2019)	Human study (implant stability)	Flap surgery + CGF (a), flap surgery + CGF + Bio-Oss (b)	Flap surgery alone (c), flap surgery + Bio-Oss (d)	Recording PD and CAL change	No significant difference between Groups b and d

Kizilaslan, Karabuda, and Olgac (2020)	Diabetic rat model (calvaria defects)	(1) Empty bone defect, (2) xenogenous graft, (3) CGF, (4) CGF + xenogenous graft	Healthy control group	Histomorphometric analysis	Highest rate of bone healing in CGF + xenogenous graft group
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Abbreviations: AB, autogenous bone; A-PRF, advanced platelet-rich fibrin; BMSCs, bone mesenchymal stem cells; BPBM, bovine porous bone mineral; CAL, clinical attachment level; CBCT, cone beam computed tomography; CGF, concentrated growth factor; CM, collagen membrane; GTR, guided tissue regeneration; micro-CT, microcomputed tomography; PD, probing depth; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RFA, resonance frequency analysis; SEM, scanning electron microscope; TGF- $\beta$ , transforming growth factor beta; VEGF, vascular endothelial growth factor.

**Table 5.** List of studies on the effects of CGF on regeneration of other tissues than bone

Author	Study	Tissue or disorder	Intervention	Control group	Assays	Outcome
Bozkurt Dogan, Ongoz Dede, Balli, Atalay, and Durmuslar (2015)	Split-mouth randomized clinical trial	Multiple adjacent GRs	CGF membrane + CAF	CAF alone	CRC, MRC, RD, PD, RW, CAL, KGW, GT, GR depth, PD	Did not provide additional benefits in RD, CRC and MRC by using CGF + CAF, but significant increase in KGW and GT
Qin, Wang, Sun, et al. (2016)	Animal study (rat model)	Sciatic nerve crush injury	CGF membrane	Blank control	Functional nerve recovery using an SFI	Increase of the sciatic functional index by CGF
Topkara, Ozkan, Ozcan, Oksuz, and Akbulut (2016)	Animal study (New Zealand white rabbits)	Diced cartilage grafts	Diced cartilage wrapped with CGF	Bare diced cartilage; diced cartilage wrapped with fascia; diced cartilage wrapped with fenestrated fascia; diced cartilage wrapped with blood glue	HE staining, Masson's trichrome, Toluidine blue, Safranin-O staining, immunohistochemical staining of GFAP	Improvement of the viability of diced cartilage grafts by CGF
Wang et al. (2017)	Animal study (goat)	TMJ-OA	CGF membrane	Physiologic saline was applied to the left	Histopathologic observations with HE	Tissue repair and regeneration in CGF-repaired condyle

				joints (unrepaired group)		
Hu et al. (2018)	Animal study (nude mice)	Fat graft	1 ml of CGF	1 ml of PRP, PRF	H&E and immunohistochemical analysis	More normal adipocytes and fewer fat vacuoles in the CGF group
Xu, Qiao, et al. (2019)	Beagle dogs	Immature teeth	CGF used as root canal filling agent	Positive control (no treatment), negative control (only root canal preparing)	Radiography, H&E, Masson trichrome technique, immunohistochemical analysis	Dentine-pulp complex regeneration in the immature teeth in the presence of CGF

Abbreviations: CAF, coronally advanced flap; CAL, clinical attachment level; CGF, concentrated growth factor; CRC, complete root coverage; GFAP, glial fibrillary acidic protein; GRs, gingival recessions; GT, gingival thickness; H&E, hematoxylin and eosin; KGW, keratinized gingiva width; MRC, mean root coverage; PD, probing pocket depth; PRF, platelet rich fibrin; PRP, platelet-rich plasma; RD, recession depth; RW, recession width; SFI, sciatic functional index; TMJ-OA, temporomandibular joint osteoarthritis.

### 3.3 Effect of CGF on adult cells or cell line

Adult cells or cell lines used for assessment of CGF are NHDF, HUVEC, HOB, Rat bone marrow cells, Schwann cells, SaOS-2, periosteal cells, and dental pulp cells (Table 3). Positive effects of CGF on proliferation, migration, and differentiation were reported in these studies; however, Borsani et al. reported the decrease of cell proliferation and increase of cellular differentiation in the presence of CGF (Borsani et al., 2020). Only one study in this group compared CGF extract with other platelet concentrate including PRP, PRGF, and A-PRF extract and reported more proliferation in PRP-treated cells (Masuki et al., 2016). While in the study of Jun et al. on CGF extracts (2, 5, 10, and 15%), a dose-dependent manner in CGF groups was reported, study of Chen et al. on the effects of 5, 10, 15, and 20% concentrations of CCM showed better effect of 5% CGF in comparison to other concentrations (Chen, Jiao, et al., 2019; Jun et al., 2018).

### 3.4 In vivo effect of CGF

Since the introduction of CGF by Sacco in 2006, many studies have evaluated the osteoinductive and regenerative properties of CGF in clinical studies as case series, animal studies, or clinical trials. In this regard, with excluding case series (without a control group), 18 studies on bone regeneration and six studies on regeneration of other tissues are listed in Tables 4 and 5. Two articles were randomized clinical trials and one was a cohort study in which the treated pathologies were peri-implantitis, guided tissue regeneration (GTR), and gingival recessions (Bozkurt Dogan et al., 2015; Isler et al., 2018; Lei et al., 2019). Most studies stated improvement of tissue healing or regeneration in the presence of CGF, yet three studies investigating its effect on peri-implantitis, implant stability, and immediate implants reported no significant difference or lower effect of CGF (Isler et al., 2018; Özveri Koyuncu, İçpınar Çelik, et al., 2019; Yang et al., 2015). Combination of CGF with stem cells or grafts resulted in better results than CGF alone (Chen et al., 2018; Durmuslar et al., 2016; Honda et al., 2013; Kizilaslan et al., 2020; Qiao et al., 2016; Wang et al., 2017). Three studies compared CGF to other platelets concentrates; one study reported no significant difference between CGF, PRP, and PRF on bone formation in rabbit-skull defect (Kim et al., 2014), another study also confirmed no significant difference in GTR between A-PRF and CGF (Lei et al., 2019); while Park et al. indicated better bone formation of CGF than PRF in femur defect of adult dogs (Park et al., 2016).

## 4 DISCUSSION

The aim of the present study was to summarize the biological effects of CGF. Stem cells and primary cells (which are taken directly from tissues) or continuously grown cells/cell lines are commonly used in in vitro studies. The similarity of the characteristics of primary cells to in vivo conditions makes them a good candidate for cell culture experiments. However, continuous cell lines, due to their genetic stability, are better for the standardization of in vitro assays (Kaur & Dufour, 2012).

As seen in the result of this review, CGF was used alone in in vitro studies, but in in vivo studies, the combination of CGF with stem cells or biomaterials has been reported (Chen et al., 2018; Durmuslar et al., 2016; Masuki et al., 2016; Qiao et al., 2016; Qiao & An, 2017; Rodella et al., 2011). Tissue engineering is a complex of stem cells, scaffold, and growth factors. Fiber network in CGF can play a role as bioscaffold by securing growth factors in its fibrin fibers (Kobayashi et al., 2012); however, its combination with another scaffold could change the dynamic of growth factor release. Architecture



and components of different scaffolds may provide more interface area for release or affect its cell attachment (Yu et al., **2019**). Bonazza et al. showed that the mixture of CGFs with  $\beta$ -TCP could increase the release of BMP-2 and BMP-7 due to the presence of Ca ions in  $\beta$ -TCP (Bonazza et al., **2018**).

Cell migration, adhesion, proliferation, differentiation, and angiogenesis are the sequential events in regeneration; various growth factors in CGF, along with its 3D network structure, can induce these events (Rodella et al., **2011**). Most studies listed in Tables **2** and **3** have focused on proliferation and osteogenic differentiation. However, the effects of CGF on migration and endothelial differentiation or angiogenesis were also under investigation in four studies included in this review (Hong et al., **2019**; Jin et al., **2018**; Jun et al., **2018**; Qin, Wang, Sun, et al., **2016**). Improvement of cell migration is the result of a high concentration of chemotactic factors, such as PDGF-BB and bFGF in CGF. The effects of PDGF-BB and bFGF on cell homing have been reported in previous studies (Takeuchi et al., **2015**; Zhang et al., **2017**). TGF and IGF-I have also been shown to facilitate the migration of cells (Maucksch et al., **2013**; Takai et al., **2012**). On the other hand, the presence of CD34 positive cells in CGF explains its angiogenesis feature (Kikuchi-Taura, Soma, Matsuyama, Stern, & Taguchi, **2006**). Also, migration of endothelial cells can be stimulated by VEGF content of CGF (Morales-Ruiz et al., **2000**).

There were some controversies in reporting the optimal concentration of CGF in different studies. Some studies reported the positive effect of CGF on proliferation in a dose-dependent manner, but such results were not reported in all studies (Hong et al., **2019**). This could be related to the non-standardization of platelet count in CGF, which may affect the pH value of the medium. Change in pH value by platelet concentrates disturbs cell proliferation (Liu, Kalen, Risto, & Wahlstrom, **2002**). Also, different concentrations of CGF among the donors, different blood volumes used for CGF preparation, and different periods used for preparing CGF extract could disturb the results. Another agent is the test tube used for blood collection, which its material may interfere with the properties of CGF (Bonazza et al., **2016**; Tsujino et al., **2019**). The release dynamics of growth factors should be considered before obtaining the extract. Although Borsani et al. assumed that release of growth factors from CGF continue for almost 8 days (Borsani et al., **2015**), more sophisticated research showed that some of the growth factors are released during 8 hr, while the release of some others continues throughout 13 days (Dohan Ehrenfest, de Peppo, Doglioli, & Sammartino, **2009**; Honda et al., **2013**; Zumstein et al., **2012**). Based on these differences, the optimum concentration of CGF could vary in studies.

Most studies have focused on the osteogenic differentiation effect of CGF. Two studies have evaluated odontoblastic differentiation under treatment with CGF (Hong et al., **2019**; Jin et al., **2018**), and two others, neuropathic differentiation (Qin, Wang, Sun, et al., **2016**; Qin, Wang, Zheng, et al., **2016**). It should be noted that differentiation is related to growth factors as well as inflammatory cytokines, which might have synergistic or antagonistic effects in CGF (Liu et al., **2016**).

The result of animal and human studies demonstrates that, presently, most studies are about the effectiveness of CGF in bone regeneration. Applications of CGF in filling extraction sockets, sinus lift procedure, ridge augmentation surgeries, gingival recession, mixture with autologous bone or biomaterials, or application in implants as a membrane or liquid coating are the most common reports in bone regeneration (Table **4**). In most of the cases, the improvement of bone repair in the presence of CGF was demonstrated. The comparison between CGF alone and the combination of CGF with stem cells show the highest regeneration in their combination. Also, the results in this review highlight the

fact that the combination of CGF and biomaterials like BPBM, Bio-Oss or other bone substitute improve tissue regeneration. Only one study reported the mixing ratio (1:1) (Wang et al., **2017**). The optimal mixing ratio should be clarified in future studies.

Common assays for evaluating the result of in vivo studies were micro-CT or CBCT, histological and immunohistological staining. Additionally, six studies have shown that CGF is beneficial in the treatment of other tissues like nerve injury, cartilage, TMJ, and fat graft (Table 5).

One of the limitations of research on platelet derivatives is the nonstandard process of collecting blood, which could influence the resulting outcome. Consequently, a distinct prospect for evidence-based dentistry is the standardization of the CGF preparation methods, consideration of intraindividual differences and comparison of in vitro and in vivo studies. Meanwhile, considering all the beneficial effects of CGF, awareness of dental practitioner about this natural biomaterial and its advantages should be improved (Prakash, Ganapathy, & Mallikarjuna, **2019**).

## 5 CONCLUSION

Overview of 45 articles, regarding the in vivo and in vitro studies on CGF, reveals the beneficial effects of CGF as a natural scaffold and reservoir of growth factors in regenerative medicine. The material of test tube used for blood collection, different concentrations of CGF among the donors, different blood volumes used for CGF preparation, platelet count in CGF, different periods used for preparing CGF extract, and different types of CGF used for application (fresh, freeze-dried, extract, exudate, membrane) could explain different results in reporting better effects of CGF than PRF or no significant difference between them. More well-designed studies could help in standardization of CGF protocols.

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