ORIGINAL PAPER

THE INFLUENCE OF APPLICATION OF CONCENTRATED GROWTH FACTORS ON POST-OPERATIVE COMPLICATIONS – *IN VIVO* SPLIT-MOUTH STUDY

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ABSTRACT

Introduction: New methods of tissue regeneration revealed a new preparation called CGF – concentrated growth factors. CGF belong to the group of platelet-reach plasma preparations, obtained by blood collecting and its centrifugation.

Objectives: The aim was to evaluate the influence of CGF application in post-operative soft tissue and bone defects on post-operative complications and wound healing.

Material and methods: The research was conducted in twenty patients with a symmetric single tooth (single or multi-root) qualified for extraction. On one side of the dental arch, the post-operative region was filled with CGF material, while on the other side it was left to heal by standard primary intention. Pain, swelling, alveolar osteitis, use of antibiotics, analgesics intake and wound healing were evaluated in the post-operative period.

Results: The risk associated with the occurrence of pain classified higher on the Numeric Rating Scale (NRS) than the given category increased 3.4 times if the alveolus was not filled with CGF ($p < 0.05$). The risk of swelling on a given day after surgery dropped about 44 times when CGF was applied ($p < 0.05$, $p < 0.01$, $p < 0.05$). In cases where the alveolus was filled with CGF, the use of the membrane reduced the risk of edema by 7.5 times in the seven-day period after surgery. Filling the alveolus with CGF resulted in a 5.7-fold decrease in the risk of having to use antibiotics after tooth extraction ($p < 0.01$). The possibility for complete healing of the alveolus up to the seventh day after surgery was about 5.5 times lower when the alveolus was not filled with CGF ($p < 0.05$).

Conclusion: The filling of a post-operative defect with CGF reduces the frequency and intensity of post-operative pain, swelling, antibiotic therapy intake and the occurrence of facial edema, which improves the quality of life in the post-operative period.

Key words: augmentation, centrifuge, NRS scale, platelet preparations, socket healing.

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INTRODUCTION

New methods of tissue regeneration revealed a new preparation called CGF – concentrated growth factors. CGF belong to the group of platelet-reach plasma preparations (PPP), obtained by blood collecting and its centrifugation.

In 1998, Lynch established the triad of three factors indispensable for a proper and efficient tissue regeneration process [13]. These three factors are related. The first one is a tissue scaffold such as collagen or bone mineral. The second factor is cells filling the space around the scaffold such as fibroblasts, osteocytes or indifferent pluripotential cells. The last one is elements

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of extracellular matrix (ECM) such as molecules taking part in the signaling process between cells or genes: growth factors, morphogens, adhesins, hormones and vitamins.

The main advantage of CGF as an autogenic material over xenografts is high platelet concentration in small volume, the accumulation of growth factors, the presence of CD34+ cells and a fibrin scaffold [2]. Due to these facts the regenerative potential of CGF increases and promotes tissue healing. It is important to use methods which stabilize the clot and thereby decrease the risk of wound healing impairment [8]. As is commonly known, wound healing is a combined process, supported by growth factors released from blood platelets. The main growth factors taking part in the wound healing process are fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1) and -β2 (TGF-β2). VEGF and TGF-β1 are present in the red blood cell layer of centrifuged plasm [10]. TGF-β, FGF, VEGF and PDGF are considered to be the ones which support bone regeneration in the mosteoconduction process [12].

In comparison with other well-known PPP, such as PRF, the CGF obtaining method also does not require any tubes with anticoagulants. The main technical difference in obtaining CGF and PRF is centrifugation speeds. CGF consists of fibrin blocks, denser, larger and richer in growth factors compared to PRF. It leads to better regenerative potential of CGF material. CGF is described by four phases: 1) superior platelet poor fraction; 2) interim fibrin block; 3) liquid phase rich in growth factors; 4) lower red portion containing red blood cells [9].

Tooth extraction leads to the same sequence of healing as in mucosal wounds, i.e. inflammation, epithelialization, fibroplasia, and remodeling. The socket heals by secondary intention. During the inflammatory phase white blood cells remove bacteria and debris from the socket. The fibroplasia stage also starts during the first week after tooth removal, with fibroblast and capillary growth. Epithelium migrates down the socket wall until it contacts the epithelium from the other side of the socket. At the end of the first week, osteoclasts gather along the crestal bone. During the second week, granulation tissue fills the socket and osteoid precipitates along the alveolar bone lining the socket. The process lasts during the second, third and fourth week of the socket healing process. The resorption of the cortical bone continues and new bone is formed. At the time when bone fills the socket, the epithelium reaches the alveolar crest gingiva level [5].

Pain scales are used to describe the intensity of pain, particularly after surgical procedures. From the few most popular scales in dentistry, the NRS pain scale seems to have good sensitivity and to be convenient for statistical analysis. The NRS is an eleven-point scale, where 0 describes no pain and 10 describes severe pain. It is divided into three rating ranges: 1-3 describes mild pain, 4-6 moderate pain and 7-10 severe pain [6].

MATERIAL AND METHODS

The research was carried out in 20 patients and obtained the agreement of the Bioethical Committee of Medical University of Lodz No. RNN/258/17/KE. The inclusion criterion was patients with symmetric teeth qualified for extraction (single or multi-root in maxilla or in mandible), with their roots covered with bone minimally for 1/2 the root length. The exclusion criteria were patients with systemic diseases, pregnant and/or lactating women, patients taking any drugs known to affect the number or function of platelets and patients with abnormal platelet counts. On one side after an extraction, CGF material was applied into alveolus while on the other side no treatment was attempted. In order to choose the side of the dental arch for CGF application the rules of randomized controlled trials were used; subsequently sides of the dental arch were allocated by chance. In local anesthesia teeth were extracted. Curettage of bone was performed.

CGF material was produced in special centrifuges, which use different centrifuge speeds in one cycle. After collecting blood into 9 ml plastic tubes from the region of antecubital fossa vessels, the special centrifuging protocol was introduced: 30 seconds of acceleration, 2 minutes at 2,700 rpm, 4 minutes at 2,400 rpm, 4 minutes at 2,700 rpm, 3 minutes at 3,000 rpm, 36 seconds of deceleration and stopped. A Medifuge MF200 centrifuge by Silfradent was used in the above procedure. After centrifuging, CGF material was taken out from plastic tubes using special surgical tools and it was specially prepared by cutting the red blood cell layer, preserving a thin layer on the border with proper, platelet-rich CGF. Then, such prepared material was placed into the post-extraction bone defect. Depending on the particular case, CGF membrane was used to cover the soft tissue defect. The wound was sutured. Filling the post-extraction cavity up to the alveolar ridge with CGF material was the criterion of the number of applied plasma tubes. The wound was covered with CGF membrane in cases where soft tissue flaps were not used to close the wound.

On days 1, 4 and 7 after surgery, the following factors were evaluated: post-operative pain in terms of the NRS pain scale, post-extraction swelling, presence of local osteitis, post-operative antibiotic therapy and analgesic therapy. The last four factors were evaluated using the zero one system. The healing of soft tissue was evaluated on days 7 and 30 after surgery, based on our own classification of the epithelialization (Table 10).

To examine the relationship between two categorical variables, in the case of independent observations, the Fisher test (comparison within the control or stud-

ied group) was used while for dependent observations (comparison of studied group with control) the Bhapkar test was used. For numerical variables, statistical significance of the differences between two groups was tested with the Mann-Whitney *U* test, while for three or more independent groups the Kruskal-Wallis test was used. All calculations were made using R software, version 3.5.1. A multivariate analysis was performed. For this purpose, several models were created to explain the occurrence of particular ailments depending on predictors such as age, gender, filling the alveolus, or the use of a membrane covering the alveolus. In order to describe the intensity of postoperative pain, ordinal logistic regression with mixed effects was used, while in the case of occurrence of edema, logistic regression with mixed effects was used. The other models were based on logistic regression. In the case of mixed models, only the fixed effects are presented.

RESULTS

The relationship between intensity of pain after surgery and filling the alveolus with platelet-rich plasma was significant on the first day after the procedure (Bhapkar test, $p < 0.05$). For the fourth and seventh day no such relationship was demonstrated (Table 1). The risk associated with the occurrence of pain classified higher on the NRS scale than the given category increased 3.4 times if the alveolus was not filled with CGF material (Table 2). The value of mean pain on the NRS-11 scale in both groups was classified in the range 1-3.

There was no dry socket occurrence in any of the patients in the study and control group.

The relationship between the occurrence of swelling on the face of patients and belonging to the test or control group was significant on the first, fourth and seventh days after the procedure (Bhapkar test, $p < 0.05$, $p < 0.01$, $p < 0.05$). On the first day after surgery, edema appeared in 35% of patients in the studied group and 65% in the control group. On the fourth day, it was seen in 35% of patients in the control group and in the studied group in none of the patients. On the seventh day after the procedure, swelling occurred in 20% of patients in the control group, while in the studied group it did not appear (Table 3). The risk of swelling on a given day after surgery (up to seven days after it) dropped about 44 times when this type of filling was used. On the other hand, every day after surgery the risk of edema decreased by 2.5 times. In cases where the alveolus was filled with platelet-rich plasma, the use of the membrane reduced the risk of edema by 7.5 times in the seven-day period after surgery (Table 4).

TABLE 1. Intensity of pain

Variable/Intensity	Overall ($n = 40$)	Studied ($n = 20$)	Control ($n = 20$)	Test	*p*-value
Postoperative pain (1st day)					
Lack	2.5% ($n = 1$)	5% ($n = 1$)	0% ($n = 0$)	Bhapkar	0.0138
Low	72.5% ($n = 29$)	85% ($n = 17$)	60% ($n = 12$)		
Moderate	25% ($n = 10$)	10% ($n = 2$)	40% ($n = 8$)		
Postoperative pain (4th day)					
Lack	30% ($n = 12$)	40% ($n = 8$)	20% ($n = 4$)	Bhapkar	0.1712
Low	67.5% ($n = 27$)	55% ($n = 11$)	80% ($n = 16$)		
Moderate	2.5% ($n = 1$)	5% ($n = 1$)	0% ($n = 0$)		
Postoperative pain (7th day)					
Lack	45% ($n = 18$)	60% ($n = 12$)	30% ($n = 6$)	Bhapkar	0.0821
Low	52.5% ($n = 21$)	35% ($n = 7$)	70% ($n = 14$)		
Moderate	2.5% ($n = 1$)	5% ($n = 1$)	0% ($n = 0$)		

TABLE 2. Occurrence of pain

	Estimate	Odds ratio	Std. error.	*z*	*p* (> |*z*|)
Lack | low	–2.931	–	0.608	–4.821	0
Low | moderate	1.583	–	0.5018	3.154	0.0016
Moderate | high	4.381	–	1.089	4.022	1e–04
Day	–0.5119	0.5994	0.105	–4.876	0
Control group	1.212	3.359	0.4287	2.826	0.0047

TABLE 3. Occurrence of edema

Variable/Parameter	Overall ($n = 40$)	Studied ($n = 20$)	Control ($n = 20$)	Test	p-value
Swelling (1st day)					
No	50% ($n = 20$)	65% ($n = 13$)	35% ($n = 7$)	Bhapkar	0.0361
Yes	50% ($n = 20$)	35% ($n = 7$)	65% ($n = 13$)		
Swelling (4th day)					
No	82.5% ($n = 33$)	100% ($n = 20$)	65% ($n = 13$)	Bhapkar	0.001
Yes	17.5% ($n = 7$)	0% ($n = 0$)	35% ($n = 7$)		
Swelling (7th day)					
No	90% ($n = 36$)	100% ($n = 20$)	80% ($n = 16$)	Bhapkar	0.0253
Yes	10% ($n = 4$)	0% ($n = 0$)	20% ($n = 4$)		

TABLE 4. Risk of edema

	Estimate	Odds ratio	Std. error.	z	$p(>\|z\|)$
Intercept	–0.6931	–	0.6124	–1.132	**0.2577**
Membrane	–2.015	0.1333	0.8547	–2.357	**0.0184**

TABLE 5. Use of antibiotic therapy

Variable/Parameter	Overall ($n = 40$)	Studied ($n = 20$)	Control ($n = 20$)	Test	p-value
Antibiotic therapy (1st day)					
No	72.5% ($n = 29$)	90% ($n = 18$)	55% ($n = 11$)	Bhapkar	**0.0062**
Yes	27.5% ($n = 11$)	10% ($n = 2$)	45% ($n = 9$)		
Antibiotic therapy (4th day)					
No	72.5% ($n = 29$)	90% ($n = 18$)	55% ($n = 11$)	Bhapkar	**0.0062**
Yes	27.5% ($n = 11$)	10% ($n = 2$)	45% ($n = 9$)		
Antibiotic therapy (7th day)					
No	70% ($n = 28$)	85% ($n = 17$)	55% ($n = 11$)	Bhapkar	**0.016**
Yes	30% ($n = 12$)	15% ($n = 3$)	45% ($n = 9$)		

TABLE 6. Risk of having to use antibiotics

	Estimate	Odds ratio	Std. error.	z	$p(>\|z\|)$
Intercept	–4.948	0.0071	1.895	–2.611	0.009
Age	0.1305	1.139	0.0686	1.901	0.0572
Control group	1.746	5.734	0.8467	2.063	**0.0391**

The relationship between the use of antibiotic therapy and belonging to a particular group was significant. On the first and fourth day after the procedure antibiotics were taken by 10% of patients from the studied group and 45% from the control group (Bhapkar test, $p < 0.01$). On the seventh day after the procedure, antibiotic therapy was used in 15% of patients from the studied group and 45% from the control group (Bhapkar test, $p < 0.05$) (Table 5). Filling the alveolus with platelet-rich plasma resulted in a 5.7-fold decrease in the risk of having to use antibiotics after tooth extraction (Table 6).

The relationship between use of analgesic medication after surgery and membership in the study or control group was statistically significant. On the first day after the procedure, painkillers were used by 70% of patients from the studied group and 95% from the control group (Bhapkar test, $p < 0.05$). On the fourth day after surgery, this percentage dropped to 30 in the studied group and

TABLE 7. Use of analgesic pharmacotherapy

Variable/Parameter	Overall ($n = 40$)	Studied ($n = 20$)	Control ($n = 20$)	Test	p-value
Analgesic treatment (1st day)					
No	17.5% ($n = 7$)	30% ($n = 6$)	5% ($n = 1$)	Bhapkar	**0.0371**
Yes	82.5% ($n = 33$)	70% ($n = 14$)	95% ($n = 19$)		
Analgesic treatment (4th day)					
No	50% ($n = 20$)	70% ($n = 14$)	30% ($n = 6$)	Bhapkar	**0.0022**
Yes	50% ($n = 20$)	30% ($n = 6$)	70% ($n = 14$)		
Analgesic treatment (7th day)					
No	62.5% ($n = 25$)	85% ($n = 17$)	55% ($n = 11$)	Bhapkar	**0.0026**
Yes	37.5% ($n = 15$)	15% ($n = 3$)	45% ($n = 9$)		

TABLE 8. Assessment of epithelialization

Variable/Parameter	Overall ($n = 40$)	Studied ($n = 20$)	Control ($n = 20$)	Test	p-value
Evaluation of epithelialization (7th day)					
1	50% ($n = 20$)	30% ($n = 6$)	70% ($n = 14$)	Bhapkar	**0.0253**
2	50% ($n = 20$)	70% ($n = 14$)	30% ($n = 6$)		
Evaluation of epithelialization (30th day)					
1	2.5% ($n = 1$)	0% ($n = 0$)	5% ($n = 1$)	Bhapkar	0.3049
2	97.5% ($n = 39$)	100% ($n = 20$)	95% ($n = 19$)		

TABLE 9. Odds for complete healing

	Estimate	Odds ratio	Std. error.	z	$p(>\|z\|)$
Intercept	0.8473	2.333	0.4879	1.736	0.0825
Control group	–1.695	0.1837	0.6901	–2.456	**0.0141**

70 in the control group (Bhapkar test, $p < 0.01$). On the seventh day after the procedure analgesic pharmacotherapy was used in 15% of patients from the studied group and in 60% from the control group (Bhapkar test, $p < 0.01$) (Table 7).

The relationship between the assessment of epithelialization on the seventh day after surgery and membership in one of the groups (studied or control) was statistically significant (Bhapkar test, $p < 0.05$). On the seventh day after the procedure, 70% of patients in the studied group were fully healed, while in the control group only 30% were fully healed (Table 8). The odds for complete healing of the alveolus up to the seventh day after surgery is about 5.5 times lower when the alveolus is not filled with platelet rich plasma (Table 9).

DISCUSSION

Healing after surgical procedures is inextricably linked to inflammation and CGF lowers the harmful effects of inflammation through correction of adverse

TABLE 10. Classification of epithelialization

Healing process	Points
Intercept	0
Min. 50% of dental socket surface epithelialized	1
Fully healed dental socket	2

effects occurring during the healing of injured tissues. The three most important mediators of inflammation are interleukin (IL)-1β, IL-6 and TNF-α. PRF is able to inhibit the inflammation process and neutralize its promoters thanks to IL-4 and initiate the healing by creating blood vessels through the VEGF. IL-4 is produced by activated lymphocytes T; it increases the proliferation and differentiation of lymphocytes B. During the inflammation its primary role is to produce the collagen and block the stimulation of metalloproteinase 1 and 3 through IL-1. As a result, the inhibition of transduction pathways of IL-1β occurs. If the macrophages are affected by IL-4, they are prevented from producing IL-1 and TNF-α. VEGF is the most powerful and most

common known stimulator of blood vessel growth. It is sufficient for initiation of angiogenesis and plays a direct role in the control of endothelial cell behavior, such as proliferation, migration, and specialization [1, 3].

The influence of CGF on soft tissue healing is already known, but its influence on bone healing is still somewhat controversial. The intricate architecture of the CGF fibrin matrix offers proper mechanical behavior due to design and elasticity provided by the cross-linked monomer units, and proves to be a good environment for regeneration of bone loss. Mesenchymal cells derive from bone marrow, are non-differentiated and can differentiate into many types of cells, contributing to healing of every type of bone tissue [14].

Manzoor *et al*., based on their own research, claim that filling the intraosseous defect after enucleating the cyst with PRF can shorten the healing from 6 to even 2 months. Even though there is no certain research confirming the osteoconductive feature of PRF, it is efficacious clinically and radiologically in treatment of intrabony defects after enucleation of various periapical lesions [4].

Due to the lack of available articles regarding the use of CGF material in healing of post-extraction defects, in a few paragraphs references to the use of PRF material were made, as both materials and their uses are similar. The lack of long-term research to date regarding the use of CGF material in alveolar healing emphasizes the clinical value of the results of the research presented in the above article.

The results of the presented research indicate that the application of CGF material in oral surgery procedures such as teeth extractions reduces the intensity of post-operative alignments. Also Singh *et al*. and Meshram *et al*. concluded that PRF offers many advantages such as decrease of the frequency of intraoperative and postoperative bleeding, promotion of more rapid soft tissue healing and rapid vascularization of the healing tissue by delivering growth factors [7, 11]. In conclusion, use of CGF in intraosseous bone defects can result in successful bone formation as it promotes faster regeneration because of gradual release of growth factors lodged in the fibrin matrix.

CONCLUSIONS

The results confirmed the presence of a positive effect of alveolar filling with CGF material in stimulating the wound healing process and in reducing the frequency and intensity of ailments such as post-operative pain, swelling, antibiotic intake and the occurrence of facial edema, which improves the quality of life in the post-operative period and leads to shortening of the convalescence period. As application of CGF is a relatively low-cost procedure, it should be implemented in everyday surgical procedures.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Almeida Varela H, Noronha Oliveira MAPP, Pereira J, Souza JCM, Pinto N, Quirynen M. Platelet-rich fibrin to incorporate bioactive graft materials. In: Nanostructured Biomaterials for Cranio-Maxillofacial and Oral Applications. Souza J, Hotz D, Henriques B, Boccaccini A (eds.). Elsevier; 2018.
2. Borsani E, Bonazza V, Buffoli B, et al. Biological characterization and in vitro effects of human concentrated growth factor preparation: an innovative approach to tissue regeneration. Biol Med 2015; 7; doi:10.4172/0974-8369.1000256.
3. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101: e56-60.
4. Dar M, Hakim T, Shah A, Najar L, Yaqoob G, Lanker F. Use of autologous platelet-rich fibrin in osseous regeneration after cystic enucleation: a clinical study. J Oral Biol Craniofacial Res 2016; 6 (Suppl 1): S29-S32.
5. Hupp JR, Ellis E, Tucker MR. Contemporary Oral and Maxillofacial Surgery: Principles of Ssurgery. 6th ed. St. Louise, MO: Elsevier; 2014.
6. Matys J, Dominiak M. Assessment of pain when uncovering implants with Er:YAG laser or scalpel for second stage surgery. Adv Clin Exp Med 2016; 25: 1179-1184.
7. Meshram VS, Lambade PN, Meshram PV, Kadu A, Tiwari MS. The autologous platelet rich fibrin: a novel approach in osseous regeneration after cystic enucleation: a pilot study. Indian J Dent Res 2015; 26: 560-564.
8. Mirkovic S, Djurdjevic-Mirkovic T, Puskar T. Application of concentrated growth factors in reconstruction of bone defects after removal of large jaw cysts: the two cases report. Vojnosanit Pregl 2015; 72: 368-371.
9. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. Platelets 2017; 28: 774-778.
10. Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech 2011; 74: 772-777.
11. Singh S, Singh A, Singh S, Singh R. Application of PRF in surgical management of periapical lesions. Natl J Maxillofac Surg 2013; 4: 94-99.
12. Tollemar V, Collier ZJ, Mohammed MK, Lee MJ, Ameer GA, Reid RR. Stem cells, growth factors and scaffolds in craniofacial regenerative medicine. Genes Dis 2016; 3: 56-71.
13. Wojtowicz A, Szostak D, Malejczyk J. Tissue engineering in oral surgery – review of new methodology. Nowa Stomatologia 2002; 1: 41-45.
14. Yamada Y, Boo JS, Ozawa R, et al. Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. J Cranio-Maxillofacial Surg 2003; 31: 27-33.