

## Improved treatment of mandibular odontogenic cysts with platelet-rich gel

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**Background.** Platelets play a central role in hemostasis and healing processes. By concentrating platelets, platelet-rich plasma (PRP) with higher levels of growth factors can be obtained, which might stimulate healing processes. After platelet degranulation, massive release of growth factors and active substances occurs and gelatinous matrix-platelet-rich gel (PRG) is formed.

**Objective.** In this double-blind study, we report the influence of PRG on healing of mandibular odontogenic cysts.

**Study design.** We examined 23 cases divided into control (no PRG treatment) and experimental (PRG-treated) groups. Each participant was followed on a regular basis with clinical examinations, roentgenograms, and dual-energy x-ray absorptiometry (DEXA) examinations.

**Results.** Clinical observations showed that oral mucosa healed faster in patients treated with PRG compared with cases where gel was not added. Roentgenograms and DEXA examinations showed considerable enhancement of bone regeneration beginning from the 5th week and continuing during subsequent periods after implantation of PRG in the experimental group compared with the control group.

**Conclusions.** In our opinion, PRG possesses inductive properties that could stimulate the healing processes. The use of autologous growth factors from platelet gel seems to be one of the most promising methods of treatment of bone, cartilage, and soft tissue defects. (*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;xx:xxx)

Platelets play a central role in hemostasis and healing processes. Upon their activation, platelet  $\alpha$ -granules release over 30 cytokines, including platelet-derived growth factor (PDGF), transforming growth factor  $\beta$  (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF),<sup>1,2</sup> as well as active substances such as serotonin, catecholamines, von Willebrand factor, proaccelerin, osteonectin, and antimicrobial proteins.<sup>3-6</sup> By concentrating platelets, platelet-rich plasma (PRP) with higher levels of growth factors might be reached, which could stimulate the healing processes. The activator for PRP is a mixture of thrombin and calcium chloride. After connecting these substances, a platelet-rich gel (PRG) is formed. Apart from

converting fibrinogen into fibrin, thrombin can directly stimulate cells, e.g., by inducing cell proliferation.<sup>3,7</sup>

Whereas some authors have reported significant increase in bone formation after application of PRG,<sup>8</sup> others did not observe any improvement.<sup>9,10</sup> Despite controversies in the literature regarding the added benefit of this procedure, recent investigations have confirmed osteoinductive properties of PRG in vitro.<sup>11,12</sup> Over the past decade autologous platelets have been shown to promote tissue repair as a source of healing factors in several clinical situations in maxillofacial surgery and orthopedics.<sup>8,13-15</sup>

In the present study, we report the influence of PRG on healing of mandibular odontogenic cysts. To our knowledge, this is the first report of PRG application without grafts in cystic lesion sites.

### MATERIAL AND METHODS

From October 2003 to January 2005, 28 consecutive patients from I Department and Clinic of Oral and Maxillofacial Surgery in Zabrze, Poland, were involved in the study.

The following criteria were used for inclusion in the study:

Odontogenic mandibular cyst greater than 3 cm<sup>2</sup>, diagnosed on the basis of clinical, radiologic, and histopathologic examinations

No previous surgical treatment of the cyst site

Supported by the Committee for Scientific Research, Polish Ministry of Health, no. N403 070 32/4120.

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Received for publication Apr 17, 2007; returned for revision Jul 11, 2007; accepted for publication Jul 23, 2007.

1079-2104/\$ - see front matter

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doi:10.1016/j.tripleo.2007.07.039

**Table I.** Increase of bone mineral density (BMD) in the control group

No.	Gender	Age	Bone defect (cm <sup>2</sup> )	BMI	DEXA BMD (%)						
					After operation	3rd week	5th week	8th week	12th week	18th week	24th week
1	F	41	3	24.933	0	9	19	28	35	41	48
2	M	57	3.6	22.985	0	7	16	25	37	45	52
3	M	46	3	23.082	0	13	16	22	34	39	43
4	F	35	7.5	27.434	0	7	12	14	21	27	33
5	F	69	6	35.156	0	8	17	26	35	39	41
6	F	41	3	22.985	0	9	19	28	35	41	48

BMI, Body mass index; DEXA, dual-energy x-ray absorptiometry; F, female; M, male.

**Table II.** Increase of bone mineral density (BMD) in the PRG group

No.	Gender	Age	Bone defect (cm <sup>2</sup> )	BMI	DEXA BMD (%)						
					After operation	3rd week	5th week	8th week	12th week	18th week	24th week
1	M	18	3.75	19.151	0	13	52	80	82	87	105
2	M	23	6	22.683	0	10	36	58	66	73	77
3	F	24	8.5	25.735	0	12	40	68	74	80	91
4	F	23	3.9	19.738				n/c			
5	M	46	8.5	23.082	0	12	28	41	59	65	72
6	F	38	7.5	21.855	0	11	32	40	55	63	69
7	F	41	3.6	23.423	0	9	29	38	47	51	58
8	F	38	8.25	27.671				n/c			
9	M	35	3	23.357				n/c			
10	M	53	8.25	24.622	0	6	6	8	16	26	31
11	F	58	7.5	26.112	0	9	31	45	51	63	68
12	F	62	9	32.744	0	13	48	59	70	75	81
13	M	55	3.4	28.628				n/c			
14	M	57	3	25.059				n/c			
15	F	81	3	19.819	0	7	30	35	39	59	63
16	F	85	4.5	31.501	0	7	30	37	41	50	59
17	M	79	3.6	20.954	0	5	26	33	41	52	60

PRG, Platelet-rich gel; n/c, no concern; other abbreviations as in Table I.

No evidence of inflammation or infection

A state of good health

Regular attendance at control visits.

Patients were divided into 2 groups. The control group consisted of patients treated operatively without PRG (Table I). Mean cyst size was 4.26 cm<sup>2</sup>. In the experimental group, PRG was injected into the bone defect after cyst removal (Table II). Mean cyst size was 5.6 cm<sup>2</sup>. Patients were blinded with regard to which group they were selected. The postoperative examinations were performed by an independent physician for the perioperative patient treatment to prevent bias during the mandibular assessment. Each participant was followed on a regular outpatient basis with clinical examinations, roentgenograms, dual-energy x-ray absorptiometry (DEXA) examinations, and functional evaluations. All examinations were performed at 3 days and 3, 5, 8, 12, 18, and 24 weeks after operation. Radiographs were taken in posteroanterior view. The DEXA examination was performed with a Lunar DPX

scanner once per visit. Scan regions were located at the defect site (Fig. 1, A). The standard regions of interest covered the width of the bone defect. To perform investigation, University Ethics Committee approval was obtained.

One patient from the control group and 4 patients from the PRG group did not attend control visit more than 2 times and were excluded from statistical analyses. Another 5 patients did not agree to undergo DEXA investigation.

### PRG preparation procedure

Fifty-four milliliters of whole blood with 6 mL anticoagulant (sodium citrate) was drawn into a sterile tube and centrifuged for 12 minutes at 3200 rpm (GPS Platelet Concentration System; Biomet, Warsaw, IN). After centrifugation, blood was separated into 3 basic components: red blood cells, PRP, sometimes referred to as "buffy coat," and platelet-poor plasma (PPP). Six milliliters of PRP was obtained and mixed with 1.5 mL 1600 U/mL bovine thrombin in a 10% calcium chloride

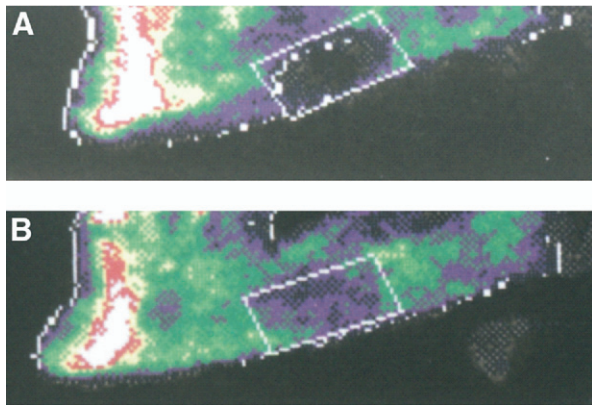


Fig. 1. Dual-energy x-ray absorptiometry examination of patient 1, platelet-rich gel group. **A**, Mandibular defect on 3rd day after operation. **B**, Mandibular defect at 24th week after operation.

solution at room temperature to form PRG. The average whole blood platelet counts and PRP platelet counts were  $244 \pm 68 \times 10^9/L$  and  $1830 \pm 489 \times 10^9/L$ , respectively. The mean leukocyte numbers were  $5,875 \pm 2,011 \times 10^9/L$  in whole blood and  $45.2 \pm 15.3 \times 10^9/L$  in PRP.

### Surgical procedure

The surgical procedure was carried out in the operating room under general anesthesia. All patients were operated on by the same experienced surgical team. Cystectomy was performed. In the experimental group, an 18-gauge needle was subsequently introduced immediately into the bone cavity and PRG was injected to fill up the empty space completely. In the control group, PRG was not given and the defect was empty. The second injection into the bone defect or operation with bone grafts was not performed. In the control group, PRG was not given.

### Statistical analysis

Statistical analysis was performed using Statistica for Windows version 6.1 (Statsoft, Kraków, Poland). Statistical differences were evaluated using the Mann-Whitney *U* test and  $\chi^2$  test with Yates correction. To estimate correlation between parameters, Spearman ratio (*r*) was used. Probability values of  $P \leq .05$  were considered to be significant.

Normal distribution of the parameters analyzed between groups was confirmed. The difference between the number of women and men was not significant. In the control group, the patient age ranged from 35 to 69 years (average 49.6 years). In the experimental group, the patient age ranged from 18 to 85 years (average

**Table III.** Correlation between the increase of bone mineral density (BMD) between groups

	<i>Mann-Whitney U test</i>	<i>P value</i>
BMD increase, 3rd week	26.0000	.6733
BMD increase, 5th week	5.0000	.0084
BMD increase, 8th week	5.0000	.0084
BMD increase, 12th week	5.0000	.0084
BMD increase, 18th week	5.0000	.0084
BMD increase, 24th week	5.0000	.0084

48.0 years). The difference between the groups was not significant. Also the difference of bone defect size was not significant. The values of the body mass index (BMI;  $kg/m^2$ ) in the groups were comparable: in the control group it ranged between 22.985 and 35.156, and in the experimental group from 19.151 to 32.744.

### RESULTS

Operations were carried out in all patients without complications. In cases where PRG was used, decrease of bleeding from bone and soft tissue was noted during surgery. The average hospitalization was 3.0 days.

Clinical observations showed that oral mucosa healed faster in patients after using PRG compared with cases where gel was not added. In both groups the wound margins were red-colored 1 week postoperatively, owing to inflammatory reactions, but in the PRG group this was less pronounced. The sutures were removed in the experimental group and the control group on the 5th and 7th days, respectively. After 2 weeks, no signs of inflammation could be found. A small line of scar tissue remained after wound healing. No infection complicating wound healing was observed.

Radiographic results showed considerable enhancement of bone regeneration at the 5th week after implantation of PRG in the experimental group compared with the control group. In subsequent periods the differences between groups were also distinguished on radiographic films. All patients exhibited an increase in bone mineral density (BMD) on DEXA examinations. In the 3rd week after operation, BMD increase in the control group ranged from 7% to 13% (Table I) and in the experimental group from 5% to 13% (Table II). From the 5th week after operation, DEXA showed significant differences in BMD increase between the control and experimental groups (Table III). In this period, BMD increase in the control group ranged from 12% to 19% and in the experimental from 6% to 52%. At the 12th week after operation, the difference was still significant: 21% to 37% in the control group versus 16% to 82% in the experimental group. A maximum value in all cases was reached in the last period (24th week; Fig.

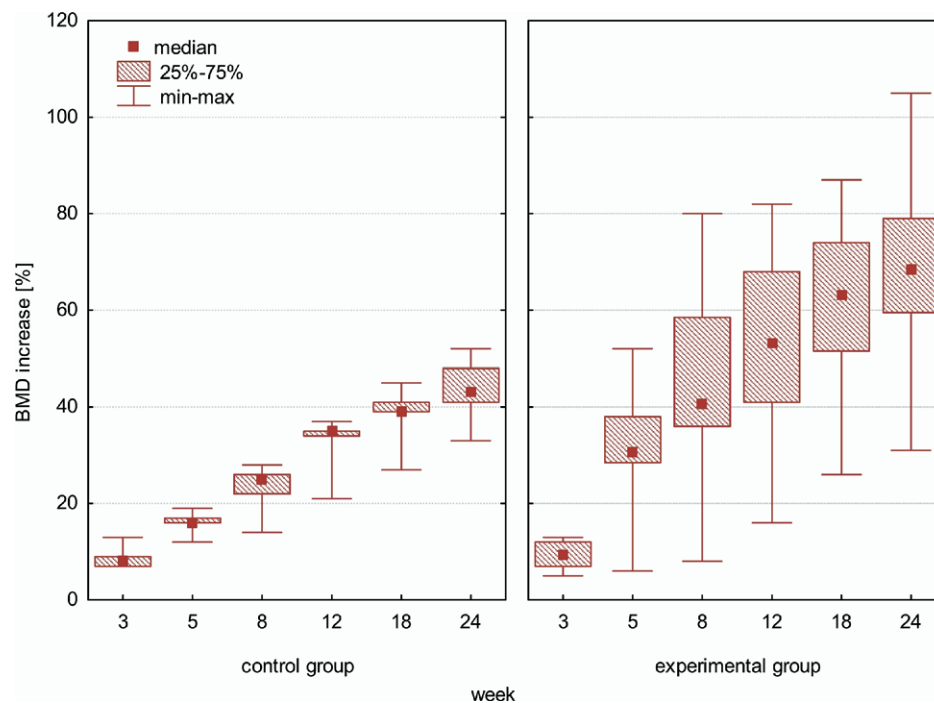


Fig. 2. Bone mineral density (BMD) increase in control and experimental groups.

**Table IV.** Correlation between age of patients and increasing bone mineral density (BMD) in both groups

	Control group		PRG group	
	<i>R</i> correlation ratio	<i>P</i> value	<i>R</i> correlation ratio	<i>P</i> value
Age-BMD increase, 3rd week	0.0513	.9347	-0.5951	.0412
Age-BMD increase, 5th week	0.3591	.5528	-0.4799	.1144
Age-BMD increase, 8th week	0.4000	.5046	-0.6364	.0261
Age-BMD increase, 12th week	0.6669	.2189	-0.6935	.0124
Age-BMD increase, 18th week	0.4104	.4925	-0.6095	.0354
Age-BMD increase, 24th week	0.3000	.6238	-0.5734	.0513

PRG, Platelet-rich gel.

1, B). Bone density in the control group ranged from 35% to 50% (average 43.4%) and in the experimental group from 31% to 105% (average 69.5%; Fig. 2).

No correlation between BMD increase and concentration of platelets in blood and PRP was observed. A significant correlation between the age of patients and increasing BMD was observed in the experimental group (Table IV). In older patients, lower BMD increase and slower bone healing processes were noted. No significant differences between the increase in BMD and cyst size were observed. No correlation was found also between BMI and BMD increase.

## DISCUSSION

Platelet-rich gel is an autologous modification of fibrin glue that has recently been described and used in various

clinical applications.<sup>6,8,13-15</sup> Platelet-rich plasma is an autologous source of PDGF and TGF- $\beta$  that is obtained by sequestering and concentrating platelets by gradient density centrifugation. This technique produces a concentration of human platelets from 540% to 770% greater than that found in blood.<sup>6,8,10,12</sup> We found markedly increased platelet counts (more than sevenfold) compared with baseline levels. Nevertheless, no correlation between BMD increase and concentration of platelets in blood and PRP was observed.

The advantages of PRG over previously described biologic sealants include safety and convenience for the patient and improved support for tissue healing. Marx et al. reported on 88 cancellous cellular bone marrow graft reconstructions of mandibular continuity defects larger or equal to 5 cm. In that study, 44 patients

received grafts with PRG, with 44 additional cases serving as a control.<sup>8</sup> Bone marrow graft with addition of PRG showed a radiographic maturation rate that was 1.62 to 2.16 times that of the grafts without PRG. In the present experiments, where PRG was introduced into the mandibular defect after cyst removal, the regeneration process was faster than in patients treated without PRG at the 5th week, as determined with radiographs. In subsequent periods the differences were also distinguished. To exactly estimate bone healing processes we used DEXA examination. Many authors have reported that DEXA is an accurate, reproducible, and noninvasive technique for measuring the mineral density.<sup>16,17</sup> This examination is widely used to measure BMD to predict the risk of potential pathologic fractures.<sup>18</sup> Limited published data show that the BMD measurement is also useful to assess bone healing processes.<sup>19</sup> The healing processes of mandibular bone defects are most frequently examined by radiovisiographic images.<sup>20,21</sup> However, standard plain radiographs, even after digital processing, can not always reflect the real state of the bone, whereas with DEXA examination even smaller changes in bone density could be assessed quantitatively.<sup>22</sup> We are not aware of any study using DEXA examination to estimate healing processes in mandibular cysts, and the present study indicates that such investigation might be useful to follow bone healing processes.

In the experimental group, a higher increase in BMD was observed compared with the control group. This difference was significant from the 5th week onward. Unfortunately, we did not receive approval from the University Ethics Committee to perform more than 1 DEXA examination during each visit, so we cannot estimate a precision error and repeatability of this examination.<sup>18</sup> In the investigations of Marx et al., histomorphometry at the 6th month demonstrated a higher bone density in PRG-treated sites (74.0%) than in the sites where PRG was not added (55.1%).<sup>8</sup> We obtained 69.5% and 43.3% of BMD increase, respectively. Because we applied PRG without bone marrow graft, it is not surprising that increase in bone density was lower in our study. In addition, the present study involved exclusively patients with odontogenic mandibular cysts, whereas in Marx's investigation mandibular defects after cancer operations were also included.

In the present investigation, we observed that placing PRG in the regenerative site of the mandible improved the mineral density of the bone. This process was dependent on patient age. In the group with PRG, bone healing processes were slower with age. In the control group, we did not find such correlation. We cannot explain this correlation. Weibrich et al. reported that

with age the levels of growth factors were similar except for lower concentration of IGF in older patients.<sup>23</sup> It is possible that the dynamics of bone healing processes is decreasing with age, which might correlate with concentrations of different cytokines.

The ideal concentration of growth factors in PRG to induce bone ingrowth is unknown. Weibrich et al. tried to address this problem.<sup>24</sup> They compared 3 concentrations of PRP and noted that the difference in fluorochrome-labeled bone between the treated and control subjects was significant for the intermediate platelet concentration group (2×-6×). The use of highly concentrated platelet preparations (6×-11×) appeared to have an inhibitory influence on osteoblast activity. The authors hypothesized that growth factors at such high concentrations might exert paradoxical inhibitory and cytotoxic effects.<sup>25,26</sup> They also mentioned that the size of the group (6 samples) was not large enough and that false negative results cannot be excluded.<sup>21</sup> In the present investigation, platelet counts were increased by 750% on average. We did not observe any inhibitory effects on healing processes. In all cases BMD increase was found. Nevertheless, the slowest BMD increase (31% at the 24th week) was noted in a patient from the PRG group.

In the present investigation, we did not observe any significant correlation between the rate of bone healing processes and defect size. The literature appears to indicate that PRG may be more effective in smaller than in larger bone defects when they are grafted with autologous bone.<sup>14,27</sup> Some authors reported that PRG might not produce the desired stimulatory response when autologous bone is not present in the graft or when the graft is of large volume, because vital bone cells are needed for this stimulation to occur.<sup>27</sup> We did not fill up the defect with autologous bone or bone marrow grafts. However, bleeding occurring during cyst removal and the wall curettage jointly with PRG can release growth factors stimulating the progenitor cells which are localized in bone matrix. Our observations showed that PRG application is sufficient to increase bone healing processes in smaller and bigger cysts despite the lack of osteogenic cells from harvested bone grafts.

The importance of platelet-derived growth factors in the repair of soft tissues has been widely documented.<sup>28,29</sup> Platelets contain a number of substances, most of which are released into the wound sites. Glover et al. made a retrospective study evaluating the wound healing and limb salvage outcomes over a 4-year period in 3,830 patients.<sup>29</sup> Their study showed that patients treated with comprehensive wound care plus topical use of autologous platelet release had significantly higher

rates of wound healing and increased limb salvage than those treated with comprehensive care alone. In the present experiments, based on clinical examination, we observed that oral mucosa healed faster in patients from the PRG-treated group than in control patients.

Autologous bone and bone marrow grafts are the most valuable biomaterials containing progenitor cells. However, the numbers of osteoprogenitor cells in aspirated bone marrow are highly variable between the patients. Hernigou et al. noted that it could range between 12 and 1224 progenitors/cm<sup>3</sup>.<sup>30</sup> The autologous percutaneous bone marrow injection offers the advantage of decreased morbidity associated with the classic open grafting techniques. Additional advantages are decreased costs and duration of hospitalization. Although autologous bone marrow harvesting is thought to be a relatively simple procedure, it has not been without complication. Donor site problems include bleeding, hematoma, and risk of infection.<sup>30</sup> In contrast, the preparation of PRG is easy and atraumatic, and application of PRG lasts only 30-60 seconds.

The present results showed that autologous PRG grafting is an effective and safe method for accelerating bone healing processes. In our opinion, PRG possesses osteoinductive properties that stimulate healing processes. The use of autologous growth factors from platelet gel seems to be one of the most promising methods for the treatment of bone, cartilage, and soft tissue defects in the future.

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