The aim of this study was to assess the efficacy of selected surgical treatment techniques of bone defects after apectomy. A total of 106 postresection bone defects, located in maxilla and mandible were included in the study: the defects were treated with resorbable collagen membrane (BG I-26 defects), xenogenic bovine material (BOC II-30 defects) and xenogenic bovine material with platelet rich plasma (BOC/PRP III-20 defects). In the control group the defects were left to heal spontaneously. Clinical and radiological assessment was performed at 6 and 12 months after the procedures. The analysis among groups revealed higher efficiency of the method of treatment that uses guide bone regeneration in comparison to the group in both post-operative control periods. After 6 months, the differences were statistically significant for each group using the regeneration methods, but after 12 months only for the BOC/PRP group. Treatment using selected guided bone regeneration techniques proved superior to the control group in both observation periods, but after 6 as well 12 months the best results in the BOC/PRP group were observed.

**Key words:** bone defects, apectomy, treatment, guided tissue regeneration, platelet rich plasma

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**INTRODUCTION**

In some cases the conventional root canal treatment could be unreliable then the periapical surgery like e.g. the apectomy is indicated. Generally the apical surgery depends on removing pathologic tissue from the periapical region and elimination any source of infection, but recently the attention to the regeneration of the destroyed periapical tissue is paid. In order to avoid the reparation processes after the apical surgery the guide bone regeneration methods (GBR) are in use. The main problem in determining the way of treatment of bone defects after apical surgery in maxilla and mandibula is specifying the conditions in which guided bone regeneration is unnecessary, and the defect will be filled with normal bone tissue spontaneously. However, in the case of defects requiring guided bone regeneration the choice of an optimal GBR technique is crucial in terms of the clinical conditions and limitations resulting from the use of a particular material.

There is considerable controversy regarding the issue of regenerative treatment in defects classified as Ia in the von Arx and Cochran classification (1), i.e. periapical defects without accompanying defects in the marginal periodontium where there is no tunelling of alveolar process. According to the authors of the classification these defects do not require regenerative management (1). The above thesis has been confirmed by the results of studies conducted on human and animal subjects and employing different materials, e.g. by Taschieri et al. (2) (Bio-Oss Spongiosa, Bio-Gide), Santamaria et al. (3) (polytetrafluoroethylene barrier, polylactide barrier), Garrett et al. (4) (Guidor) Maguire et al. (5) (polylactide membrane, hOpe-1). These studies revealed that regenerative techniques are not superior either with regard to the speed or the quality of healing. However, other studies do not support these findings. In the opinion of Pecora et al. (6) using (PTFE, Tobon et al. (7) (PTFE, OsteoGen) and Yoshikawa et al. (8) (PTFE, calcium sulphate) conventional treatment results were less predictable in comparison with the cases where regeneration methods were used. According to Yoshikawa et al. (8) guided bone regeneration methods, in class Ia defects, have a beneficial effect on the bone healing process. However, optimal biomaterial choice consideration of its limitations are crucial (9-11). Therefore, if there was no advantage to be gained from using regenerative methods over the traditional technique, this does not necessarily mean that GBR was unnecessary. In the surveys mentioned above all that it may indicate but may indicate is a sub-optimal choice of regeneration materials.

The limitations in use of xenogenic osteoconductive biomaterials arise from its processing in which proteins are eliminated followed by growth factors locally activating osteogenesis. In cases of a good blood supply to the operated region, their osteoconductive activity is not impaired, in other cases there is no regeneration guarantee and the application of innovative methods is necessary (12). Despite the fact that polyethylene growth factors are widely applied in oral surgery, their profitable effect on regenerative processes inside the bone defects is controversial. For that reason continued investigations on this topic are indicated.

The purpose of this study was to analyze the efficacy of periapical defects after apical surgery after normal healing and
with the use of guided bone regeneration techniques at 6 and 12 months postoperatively.

MATERIAL AND METHODS

The studies were conducted on a group of 106 patients (72 females and 34 males), aged 9 to 60 years (mean age 37.5 years). On the basis of the clinical signs and symptoms and radiological appearance, chronic periapical changes were found in these patients. Routine endodontic treatment of the changes was not possible for anatomic or iatrogenic reasons. The patients were informed of the surgical treatment method proposed for the treatment of their intrabony defects, gave their written informed consent and signed protocols approved by the Bioethics Committee (No KB-378/2003 and KB-1002/2005). The 106 defects qualified for treatment were located in the maxilla and mandible. Treatment consisted of 167 procedures of apectomy involving 120 incisors, 19 canines, 18 premolars and 10 molars; 82 defects were in the maxilla, and 24 in the mandible. They belonged Ia according to von Arx and Cochran (1), class II by Dietrich et al. (13), B and C by Rubinstein and Kim (14) and B and C by Kim and Krachman (15). The patients recruited were in good general health and with no systemic diseases which might impair the healing process. Patients with poor oral hygiene, periodontal disease and/or advanced caries were excluded from the study. Maximum depth of gingival pockets at the affected tooth did not exceed 4 mm. The study material was randomly allocated to the four study groups.

Prior to the procedure the teeth qualified for apectomy underwent endodontic treatment (using the lateral gutta-percha condensation method) and oral cavity sanation. An 8 ml full venous blood sample was collected immediately before the procedure. The collected blood was subjected to thrombopheresis in order to obtain platelet-rich plasma (MPW 223-b, MPW Instruments, PRP-Kit – Sarstedt Monovetten). Platelet separation was a two-stage procedure performed in accordance with the manufacturer’s protocol: soft spin (t=10 min, v=2400 rpm). 10% calcium chloride and thrombin were used in order to initiate the coagulation process. Platelet poor plasma (PPP) was used to obtain the bandage. The procedures carried out under local anaesthesis. Reimmollers’s incision was made in all cases. Retrograde filling was performed using glass ionomer cement (Ketac-cem®). The flap was stabilized with simple interrupted sutures, using 5-0 synthetic threads (Premilene-Braun®). The study material was divided into four groups (Table 1).

After the procedures antibacterial a shield in the form of Amoxicillin® 0.625 mg every 12 hours for 7 days. In addition Trilac® (every 8 hours for 7 days), Ketonal® (50 mg every 8 hours for 3 days) and Solcoseryl dental adhesive paste® were used after the procedure the oral cavity was irrigated with 0.12% chlorhexidine digluconate (twice a day for 10 days). The sutures were removed 14 days after the procedure.

The preoperative clinical assessment involved taking a dental history (presence of subjective symptoms or spontaneous pain (SP) and measurements of selected dental and oral hygiene indices (P1, API, GI, SBI). Other measurements for the teeth scheduled for apectomy included gingival pocket depth (PD), a percussion examination and assessment of the Owinsky's apico - pressure test (OWU).

Intraoperative clinical assessment included measurements of height, width and depth of the periapical defects. The wound healing process and possible early postoperative complications were assessed at 7 and 14 days.

Radiological assessment was performed at 6 and 12 months post-operatively. The radiographs were taken using a Trophy Elity® intraoral x-ray machine (60 kVp, 4 mA, 0.08 s) using aright angle technique device (Rinn, USA). The images were imported as graphic files (JPEG), archived and assessed, using x-ray analysis and Digora for Windows (2.0 version) processing software.

The first step in the radiological analysis of intrabony defect healing was the evaluation of the degree of bone regeneration using the scale described by Molven et al. (16) and modified by Taschieri et al. (2) and Dietrich et al. (13). These degrees are:

degree I - complete healing, degrees II/III - incomplete healing or uncertain healing, degree IV- failure. The radiographs were separately and independently assessed by the authors. If there were divergent opinions regarding group allocation, the radiograph was re-assessed jointly by all three observers and this result was deemed valid (16). The second step in the radiological assessment of bone defect healing process was a densitometric analysis of the tissue in the operated area. Densitometric measurements were performed, expressed as a ratio of the tissue density in the operated periapical area (apical periodontitis (AP)) to normal bone density in the region adjacent the operated area (normal bone (N)).

In the clinical and radiological assessment a 3-degree scale was used. This was a compilation of radiological and clinical results as proposed by Gutmann and Harrison (17) and modified by Taschieri et al. (2) to meet the needs of regenerative surgery. The scale were:

S - clinical succes, succesful - degree I by Molven et al. (16) and lack of pathological clinical signs and symptoms;
D - doubtful, clinical questionable - degree II or III by Molven et al. (16) and/or presence of pathological clinical symptoms;
F - failure – degree IV by Molven et al. (16) and/or presence of pathological clinical signs.

Defects allocated to the S group were defined as healed (H), defects from groups D and F were defined as non-healed (N).

Mean values (x), medians (m), range (min-max) and standard deviations (SD) were calculated for the continuous parameters. Mean parameter hypothese were verified by means of the non-parametric Kruskal-Wallis rank sum test. For the discrete parameters the frequency of a trait in groups was analysed by means of the χ²df test with Yates’ correction with the correct number of df degrees of freedom and when the expected value in the cell was less than 5 with Fisher’s test. The significance level was set at p≤0.05 for all the tests. Statistical analysis was performed using the EPIINFO software package (3.3.2.version).

RESULTS

Histopathological examination of material from the 106 investigated defects revealed the presence of dental root cysts in

Table 1. Characteristic of the examined groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>I BG</th>
<th>II BOC</th>
<th>III BOC/PRP</th>
<th>IV C</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of treated defects</td>
<td>26</td>
<td>30</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

I BG - defects treated with resorbable collagen membranes (Bio-Gide®) immobilized with resorbable pins (Resor-Pin®). II BOC - 30 defects filled with xenogenic Bio-Oss Collagen material®. III BOC/ PRP-25 defects filled with xenogenic Bio-Oss Collagen® material in combination with platelet rich plasma (PRP). IV C - 25 defects left to heal without using guided bone regeneration techniques (control group)
33 cases, and fibrous, granular or fibrogranular lesions were found in 73 specimens. The mean dimensions were width 8.38 mm, height 9 mm and depth 8.40 mm. Assessment of soft tissue healing over the treated bone defects was performed at 7 and 14 days after the procedure. No healing complications were observed, and accordingly all the operated defects were qualified for further assessment.

On the basis of clinical and radiological examination at 6 months after the procedure, 69 defects were defined as healed (H) and 37 as non-healed (N). The healed defects (H) comprised 23 defects of the BOC group, 19 of the PRP group, 18 of the BG group and 9 of the control group. In all non-healed cases the treatment result was considered doubtful (D). On the basis of clinical and radiological assessment carried out after 12 months on a group of 105 defects, 85 defects were defined as healed (H) and 21 as non-healed (N). The healed defects (H) comprised of 25 defects from the BOC group, 23 from the PRP group, 21 from the BG group and 16 from the control group. The unhealed defects (N) comprised 20 defects defined as doubtful (D) and only in one case complications were observed (F).

Intra group analysis of radiological indices of healing (R) at 6 months after the procedure did not reveal statistically significant differences between the evaluated treatment methods. Analysis carried out after taking into account clinical and radiological indices (S/D/F and H/N), revealed statistically significant differences between the results of healing for the defects from the control group in relation to all study groups and no statistically significant differences between the groups where regeneration methods were used. Due to the fact that no complications were noted after 6 months the S/D/F and H/N values were identical. At 12 months postoperatively, on the basis of the radiological index (R) as well as clinical and radiological H/N index, statistically significant differences were found between control group and the BOC/PRP group (Table 2). As no statistically significant differences were found between the BOC/PRP group and the groups BG and BOC as well as between the control group and the BG and BOC groups, additional comparison of the results of spontaneous healing with those from guided bone regeneration methods were made. Statistically significant differences were observed for the H/N index value between the control group results and all the groups where guided bone regeneration methods were used (p=0.0408).

The significant decrease of subjective pain (SP) and pain provoked by Owinsky’s apico-pressure test APT (OWU) and percussion test (P) in all the groups throughout the observation period was observed. On the basis of intergroup analysis it was stated that the end of 6-month period following the procedure, there were statistically significant differences in the frequency of all signs (SP, P, APT, OWU) between the control group and groups in which regeneration techniques were used.

However, no differences were observed between the groups in which guided bone regeneration methods were used. At 12 months, the differences between the BG, BOC and PRP groups and the control group were seen only with regard to trait SP. Densitometric analysis at the site of the defect revealed statistically significant differences in bone density between the study groups after both six and twelve months. The lowest mean values and medians were observed in the control group, next in the collagen membrane group, and highest in those groups in which the defects were augmented with biomaterial (Table 3). Intergroup analysis revealed no statistically

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Table 2. Intragroup analysis of healing process results of periapical bone defects after apicectomy at the 6 and 12 month post-operative review.

<table>
<thead>
<tr>
<th>Groups</th>
<th>C VS BG</th>
<th>BOC VS PRP</th>
<th>C VS BOC</th>
<th>C VS PRP</th>
<th>BOC VS BG</th>
<th>BOC VS PRP</th>
<th>BG VS PRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 6</td>
<td>p=0.216</td>
<td>0.46</td>
<td>p=0.184</td>
<td>1.76</td>
<td>p=0.488</td>
<td>0.48</td>
<td>p=0.115</td>
</tr>
<tr>
<td>I/II/III</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=12.6)</td>
</tr>
<tr>
<td>S/D/F 6</td>
<td>p=0.0056</td>
<td>2</td>
<td>p=0.0056</td>
<td>1</td>
<td>p=0.0361</td>
<td>4.39</td>
<td>p=0.0103</td>
</tr>
<tr>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
</tr>
<tr>
<td>H/N 6</td>
<td>p=0.0056</td>
<td>2</td>
<td>p=0.0056</td>
<td>1</td>
<td>p=0.0361</td>
<td>4.39</td>
<td>p=0.0103</td>
</tr>
<tr>
<td>(\chi^2=12.6)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
<td>(\chi^2=7.67)</td>
</tr>
<tr>
<td>R 12</td>
<td>p=0.469</td>
<td>5.61</td>
<td>p=0.195</td>
<td>3.27</td>
<td>p=0.175</td>
<td>3.48</td>
<td>p=0.0352</td>
</tr>
<tr>
<td>I/II/III/IV</td>
<td>(\chi^2=5.61)</td>
<td>(\chi^2=3.27)</td>
<td>(\chi^2=3.48)</td>
<td>(\chi^2=3.48)</td>
<td>(\chi^2=3.48)</td>
<td>(\chi^2=3.48)</td>
<td>(\chi^2=3.48)</td>
</tr>
<tr>
<td>S/D/F 12</td>
<td>p=0.585</td>
<td>4.69</td>
<td>p=0.198</td>
<td>3.24</td>
<td>p=0.309</td>
<td>2.35</td>
<td>p=0.0535</td>
</tr>
<tr>
<td>(\chi^2=4.69)</td>
<td>(\chi^2=3.24)</td>
<td>(\chi^2=2.35)</td>
<td>(\chi^2=2.35)</td>
<td>(\chi^2=2.35)</td>
<td>(\chi^2=2.35)</td>
<td>(\chi^2=2.35)</td>
<td>(\chi^2=2.35)</td>
</tr>
<tr>
<td>H/N 12</td>
<td>p=0.0892</td>
<td>6.51</td>
<td>p=0.184</td>
<td>1.76</td>
<td>p=0.304</td>
<td>1.06</td>
<td>p=0.0405</td>
</tr>
<tr>
<td>(\chi^2=6.51)</td>
<td>(\chi^2=1.76)</td>
<td>(\chi^2=1.06)</td>
<td>(\chi^2=1.06)</td>
<td>(\chi^2=1.06)</td>
<td>(\chi^2=1.06)</td>
<td>(\chi^2=1.06)</td>
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</tbody>
</table>

R6 – radiological assessment after 6 months on the basis of modified Molven’s scale (I—complete healing, II/III- incomplete healing or uncertain healing).
S/D/F6 – clinico-radiological assessment after 6 months on the basis of three degrees scale (S - clinical success, D – doubtful, F- failure).
H/N6 – clinico-radiological assessment after 6 months on the base of two degree scale (H-healed defects, N-non-healed defects).
R12- radiological assessment after 12 months on the basis of modified Molven’s scale (I—complete healing, II/III incomplete healing or uncertain healing, IV- failure).
S/D/F12 - clinico-radiological assessment after 12 months on the base of three degrees scale (S - clinical success, D – doubtful, F- failure).
H/N6 – clinico-radiological assessment after 12 months on the base of two degree scale (H-healed defects, N-non-healed defects).
significant differences between groups BG and C at 6 months (p=0.317) and between groups BOC and BOC/PRP at 12 months (p=0.101). Intergroup analysis revealed statistically significant differences between the mean density value at 6 months (83.7) and 12 months (90.9) after the procedure for group BG only (p=0.0022).

**DISCUSSION**

In the course of this investigation regeneration techniques were found to be superior to normal healing at the two observation periods. However, differences between the control and experimental groups were more evident after a 6-month period than later. The differences in healing observed after 6 months were statistically significant, mainly with regard to the clinical indices (APT, OWU, P, SP) as well as the clinical and radiological indices (S/D/F-6, H/N-6). The absence of statistically significant differences in the radiological assessment (R) may have been related to biomaterial remodelling processes in the defects, which prohibited them from being assigned to Molven’s class I (2). The absence of statistically significant differences in the radiological assessment using the Molven classification between the healing results in groups BG and C after a 6-month period, may be explained by the fact that the effect of the defect being filled with new bone at the initial treatment stage may not be recognizable in standard radiographs. This effect can only be noticed moment of new bone mineralization as well as the formation and maturation of the lamellar bone (18). Also, densitometric assessment, conducted after a 6-month period, did not show a statistically significant difference between group BG and the control group, although the mean bone density value was higher in the BG group.

It follows that the absence of significant differences between these groups, with bone density slightly higher for group BG ought to be interpreted as a beneficial effect in the context of bone regeneration with bone characteristics closely resembling those of natural bone. Statistically significant differences in bone density were observed after a 12-month period. In this period newly formed bone underwent complete mineralization and the significant difference observed may indicate that bone that was formed following the use of barriers has natural bone characteristics, alike that occurring with spontaneous healing. Moreover, in contrast to the control group statistically characteristic changes in optic density were noted in group BG at 6 and 12 months postoperatively constitutes a measurable property of osteogenesis in the bone defect.

In this study we found that the use of purely xenogenic material resulted in the greater increase in bone density. On the one hand, this value may indicate normal bone regeneration in the region analyzed and, on the other, incomplete remodelling of the xenogenic material, resulting in excessive density at the site. However, this effect was observed in the BOC group in 9 cases out of 30. The differences between our observations and those of Kozakiewicz et al. (18) may be due to the type of biomaterial used. In our study we used deproteinized bovine bone with 10% collagen which is characterized by a relatively short resorption time and this may have lead to a smaller increase in bone density in the operated region. This seems to be a positive trait of the biomaterial used, enabling bone regeneration (19). On the radiographs in our studies, the presence of parts of the implanted mineral were observed in the PRP group and in 5 cases an increase in bone density was noted over time. However, the mean values of bone density achieved at the two observation periods were closest to normal bone tissue density.

After 12 months bone density close to the optimal value also was observed in the case of collagen membrane use. Nevertheless, it appears that xenogenic material use, together with polypeptide growth factors, can result in optimal bone structure regeneration and, at the same time does not cause an excessive increase in bone density.

It is known that the activity of the polypeptide growth factors is maintained for a short time, it means for 7-40 days (20) and after that period the regenerative function being taken over by the xenogenic osteoconductor itself. However in practice the profitable healing effect after the usage of PRP could be observed even after longer time. The presented study indicates that using of PRP might influence on optimum bone density, accelerate bone healing and increase the predictability of regeneration processes inside bone defects (21). The received results can indicate that the optimal conditions of obtainment and application of platelet rich plasma in this study were applied.

In the opinion of Hatakeyama et al. (22) the controversies with regard to PRP action and the polypeptide growth factors may be explained in that way, thus standardization of the method of obtainment and application should be elaborated.

**CONCLUSIONS**

1. The efficacy of treatment proved to be superior after the use of three types guided bone regeneration methods in comparison with the control group in the two observation periods.
2. All types of guide bone regeneration methods lead to acceleration of the process of bone defect healing compared to that in controls.
3. Bone density after the use of xenogenic material containing platelet rich plasma was closest to that of natural bone.

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