The effect of Nanobone® in combination with platelet rich fibrin on bone regeneration following enucleation of large mandibular cysts

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Abstract

Objective: This study was performed to evaluate the healing kinetics of combining Nanobone® and PRF following enucleation of large mandibular cysts.

Materials and methods: 15 patients with large mandibular odontogenic cysts were treated by enucleation and grafting the bone defects by a combination of Nanobone® and PRF. Post-operative clinical and radiographic follow-up were performed. The change in the surface area and bone density of the bone defects was measured using cone beam computerized tomography (CBCT) immediately post-operatively, and after 6 and 9 months.

Results: Accelerated wound healing was observed in all cases without any signs of post-operative complications. On the 9th month post-operatively the surface area decreased by 51% and there was an increase of 50.8% in bone density.

Conclusion: Nanobone®/PRF combination accelerated bone healing and improved the quality and quantity of regenerated bone.

Keywords: Large mandibular cysts; PRF; Nanobone; Bone regeneration; Enucleation

1. Introduction

Odontogenic cysts are the most common form of cystic lesions that affect the oral and maxillofacial region [1]. Their origin, mechanism of growth, as well as treatment related problems have been often discussed. What remains often problematic is the healing of bone defects following the surgical removal of cysts [2–5]. This is a complicated process particularly after the removal of medium and large cysts due to the significant risk of pathological fracture of the weakened bone, requiring long-term postoperative care [6].

Maxillofacial reconstruction is one of the great challenges faced in clinical research for the development of bioactive surgical additives regulating inflammation and increasing healing [7]. A variety of treatment modalities including the use of autogenous bone grafts and bone substitutes materials, guided
tissue regeneration (GTR) with the use of barrier membranes, and growth factors have been proposed to promote bone regeneration [8].

The drawbacks associated with autogenous bone grafts have led to the production of a large number of alternative bone substitute materials. Their biological behavior depends upon their chemical composition and physicochemical structure. A group of these synthetic biomaterials are termed osteoinductive biomaterials. These bone graft substitutes are able to induce the in vivo environment to form bone [9]. This also refers to their ability to stimulate and support the proliferation and differentiation of mesenchymal progenitor cells of the host tissue when implanted in ectopic sites, together with the induction of bone formation [10–12].

One of these osteoinductive biomaterials is the NanoBone®, which is a recently developed granular material consisting of nanocrystalline hydroxyapatite (nCHA) embedded in a silica gel matrix, which offers several of the advantages of nanostructural biomaterials [8,13–16]. The nCHA has an extremely large internal surface and a material porosity of about 60%. Animal experiments using this nCHA in the mini pig critical size defect model showed a significantly higher rate of bone formation compared to other HA and tricalcium phosphate (TCP) materials or gelatin sponges and a nearly complete resorption 8 months after implantation [17,18].

On the other hand, platelet growth factors are a well-known source of healing cytokines, usable for clinical applications. Numerous techniques of autologous platelet concentrates have been developed and applied in oral and maxillofacial surgery. The platelet rich fibrin (PRF) was first developed in France by Choukroun et al., in 2001. This second generation platelet concentrate eliminates the risk associated with the use of bovine thrombin used in the preparation of platelet rich plasma (PRP) [19,20].

The biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on the healing process. PRF is not only a platelet concentrate but also an immune node able to stimulate defense mechanisms. It is likely that the significant inflammatory regulation noted on surgical sites treated with PRF is the outcome of retro control effects from cytokines trapped in the fibrin network and released during the remodeling of this initial matrix [21–23].

In the present study an innovative idea of combining PRF with NanoBone® was used. Indeed, several studies have reported clinical success using both these materials separately. Therefore, the aim of this study was to evaluate the healing kinetics of combining the NanoBone® with the PRF following enucleation of large mandibular cysts.

2. Materials and methods

This study was conducted on 15 patients, their ages ranged between 24 and 49 years, suffering from large mandibular odontogenic cysts (more than 3 cm in either vertical or horizontal dimensions). The diagnosis was based on clinical and radiographic examinations. All patients were treated in the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University. This study was approved by the institutional review board, and an informed consent was obtained from all patients before their inclusion in the study.

2.1. Nanobone® bone graft

The new ceramic NanoBone® used in this study, consists of hydroxyapatite crystallites with an average size of 60 nm in each crystallographic direction that are embedded in a matrix of silica gel. It is produced by a sol–gel-technique at temperatures below 700 °C, avoiding sintering of the nanocrystalline hydroxyapatite. In the transition process from sol to gel, a loose connection of hydroxyapatite crystals with the silicon dioxide molecules takes place. This connection is responsible for a nanoporous structured bone substitute. The biomaterial is characterized by numerous open bonds, which are responsible for an internal surface of up to 84 m²/g in size. The pore size distribution within the silica gel ranges from 10 to 20 nm in diameter. Macroscopically, the cone shaped NanoBone granules possess an average length of 2 mm and an average diameter of 0.6 mm with a porosity of 60%—80%.

2.2. Pre-operative phase

A full medical history was conducted for all patients, as well as the presence or absence of a history of trauma related to the affected site. Any missing or decayed tooth was recorded. Sensitivity tests were performed for teeth involved in the lesion using an

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electrical pulp tester and endodontic treatment of savable non-vital teeth was performed pre-operatively.

An orthopantomogram was performed to confirm the diagnosis (Fig. 1), followed by aspiration of the cystic fluid, although it was difficult to perform in areas where the buccal cortical plate was thick.

A cone beam computerized tomography scan (CBCT) was also performed pre-operatively to measure the exact size and extension of the lesion and to detect the approximation of the lesion to the inferior alveolar canal and the mental nerve (Fig. 2).

### 2.3. Operative phase

All patients were treated under general anesthesia. The surgical procedure included reflection of a full thickness mucoperiosteal flap through a sulcular incision and relaxing vertical incisions, followed by careful removal of bone covering the lesion using rotary and hand instruments (Figs. 3 and 4). Careful enucleation of the cyst with the capsule and extraction of impacted or non-savable teeth were performed at the same time (Fig. 5). This was followed by proper debridement of the defect site and irrigation with sterile saline solution (Fig. 6). The cystic lesions were sent for histopathological examination.

### 2.4. Protocol for PRF preparation

Twenty ml of blood were collected without anticoagulant from the brachial vein. The blood was transferred and equally divided in two 10 ml sterile glass tube, and was immediately centrifuged using a table centrifuge at 3000 rpm for 10 min. The absence of anticoagulant implies the activation of most platelets in contact with the glass tube walls in a few minutes and the release of the coagulation cascades. Fibrinogen is initially concentrated in the high part of the tube, before the circulating thrombin transforms it into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and the acellular plasma at the top. The success of this technique depends entirely on the speed of blood collection and transfer to the centrifuge. Quick handling is the only way to obtain a clinically usable PRF clot. After centrifugation, each PRF clot was separated from the red blood cell base (Fig. 7a and b).

The NanoBone® granules were mixed with the PRF and the mixture was placed into the defect site (Fig. 8). The mucoperiosteal flap was repositioned and stabilized using 3-0 black silk suture. All lesions were sent for histopathological examination to confirm the diagnosis (Fig. 9).

### 2.5. Post-operative and follow-up phase

Antibiotics were prescribed to all patients in the form of 1 gm of Amoxicillin and Clavulenate potassium\(^2\) twice a day for five days post-operatively and non-steroidal anti-inflammatory drug in the form of Ibuprofen\(^3\) 400 mg three times daily after meals for four days and 0.2% chlorhexidine gluconate solution as a mouth rinse for a period of five days.

Patients were followed-up weekly for the first month, then monthly for 9 months to assess potential post-operative complications and to evaluate pain and tissue healing. Sutures were removed 10 days post-operatively.

Cone beam computed tomography was performed immediately post-operatively and on the 6th and 9th month post-operatively for the assessment of healing of the bony defects regarding surface area and bone density using the Galileos software.

### 3. Results

The present study involved 15 patients suffering from large mandibular cysts; 8 female patients (53.3%) and 7 male patients (46.7%). Their age ranged between 24 and 49 years with a mean/SD of 35.13 ± 7.97 years. Ten patients had cysts within the anterior region of the mandible (66.7%), while five patients had cysts in the posterior region of the mandible (33.3%). The results of the histopathological diagnosis confirmed that 9 patients had inflammatory radicular cysts (60%), while 6 patients had dentigerous cysts related to an impacted mandibular third molar (40%) (Tables 1 and 2).

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\(^2\) Augmentin\(^{TM}\), GlaxoSmithKline, Egypt.
\(^3\) Brufen\(^{®}\), Abbott, Egypt.
3.1. Clinical follow-up

Healing was uneventful in all cases and normal color of the overlying mucosa was detected throughout the follow-up period. Furthermore, the patients did not complain of any unusual or severe pain. There were no signs of infection, abnormal reaction, wound dehiscence or extrusion of the material in any of the patients. Only one patient suffered from post-operative parasthesia that gradually disappeared during the first month, due to the encroachment of the cyst on the inferior alveolar nerve.

3.2. Radiographic follow-up

The surface area and the bone density were calculated immediately post-operatively and at 6 and 9 months post-operatively (Figs. 10 and 11). The mean surface area/SD of the lesions immediately post-
operatively was $487.5 \pm 32.0 \text{ mm}^2$, on the 6th month there was 31% size reduction of the surface area with a value of $336.3 \pm 57.6 \text{ mm}^2$, and on the 9th month the surface area was recorded as $238.8 \pm 56.5 \text{ mm}^2$ with a 51% size reduction. By comparing the results obtained throughout the follow-up period, the decrease of the surface area of the defect was statistically significant (Table 3).

Regarding the bone density, it was calculated using the Hounsfield Unit through the ROI (Region Of Interest) within the software. By selecting a $45 \times 45 \text{ mm}$ square placed every time within the center of the defect, the bone density was calculated and recorded. The mean bone density/SD was $153.95 \pm 15.04 \text{ HU}$ immediately post-operatively, which increased by 22.2%—$188.17 \pm 17.33 \text{ HU}$ by the 6th month and continued to increase by 50.8% reaching $226.90 \pm 33.10 \text{ HU}$ by the 9th month. The increase in bone density was statistically significant throughout the different follow up periods (Table 4).

4. Discussion

Advancements in mandibular reconstruction have continued to develop over the past decades. Many researches advocate grafting critical-size bone defects following enucleation of odontogenic cysts or tumors to accelerate bone healing and improve the quality and quantity of the regenerated bone [24,25]. The most
commonly used technique for regeneration is the use of bone replacement grafts. These grafts can promote tissue or bone regeneration through a variety of mechanisms. Bone grafting materials include autografts, allograft, xenografts, and alloplasts. Alloplasts such as osteoconductive hydroxyapatite have been widely used in periapical surgery to enhance new bone formation [13].

Among the variety of grafting materials, PRF has become a focus of current studies due to its potential to accelerate and improve the healing process [26]. Therefore, in the present study, we evaluated hydroxyapatite Nanobone® in combination with PRF in bone defects following the enucleation of large mandibular cysts.

The initial diagnosis and treatment planning were performed using orthopantomogram and CBCT. The radiographic findings were compared to the clinical picture and the surgical biopsy report which established the confirmatory histopathological diagnosis of the cystic lesions. The follow-up was performed for the assessment of the treatment outcome following cyst enucleation and grafting of the bone defect, using the gray scale value measurements of the CBCT.

The use of the CBCT for diagnosis in this study was highly successful in outlining the boundaries of the mandibular canal and the mental nerve to avoid surgical nerve damage. This is in accordance with Stoezzer et al. [27] in 2013, where they concluded that the CBCT was superior to conventional CT in detecting cortical bone involvement and delineating the mandibular canal. They stated that when there is resorption of the cortex of the mandibular canal and the cyst lining is in close proximity to the inferior alveolar nerve, enucleation can damage the nerve and lead to postoperative paraesthesia.

All cases in this study demonstrated accelerated wound healing without any signs of post-operative

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Distribution of studied cases according to demographic data (n = 15).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Min. – max.</td>
<td>24.0–49.0</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>35.13 ± 7.97</td>
</tr>
<tr>
<td>Median</td>
<td>34.0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of studied cases according to location of cyst (n = 15).</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Location of cyst</td>
<td></td>
</tr>
<tr>
<td>Anterior region</td>
<td>10</td>
</tr>
<tr>
<td>Posterior region</td>
<td>5</td>
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</tbody>
</table>
complications. There was a 31% reduction of the surface area of the bone defects on the 6th month and 51% size reduction on the 9th month post-operatively. Regarding the bone density, there was an increase of 22.2% on the 6th month, reaching 50.8% by the 9th month.

The results of this study showed that the Nanobone/PRF mixture accelerated bone healing and improved the quality and quantity of the regenerated bone. The rate of increase of the bone density and decrease in the surface area of the bone defects is significantly superior to other studies where the defects were left to heal spontaneously without bone graft. This could be demonstrated by the study performed by Pradel et al. [24] in 2006, where the bone density showed an increase of 48% after 12 months as compared to the immediate postoperative values following the enucleation of large mandibular cysts without grafting. Also,

Table 3
Distribution of studied cases according to surface area of the defect.

<table>
<thead>
<tr>
<th>Surface area of the defect</th>
<th>Immediately post-operative (n = 15)</th>
<th>6 months (n = 15)</th>
<th>9 months (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. − Max.</td>
<td>420.2−542.6</td>
<td>262.5−462.8</td>
<td>177.6−359.8</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>487.5 ± 32.0</td>
<td>336.3 ± 57.6</td>
<td>238.8 ± 56.5</td>
</tr>
<tr>
<td>Median</td>
<td>490.1</td>
<td>315.5</td>
<td>220.1</td>
</tr>
<tr>
<td>% of reduction</td>
<td>31.0</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>$p_1$</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>$p_2$</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p_1$: $p$ value for paired $t$-test for comparing between immediately post-operative period and each other studied periods.

$p_2$: $p$ value for paired $t$-test for comparing between the 6th month and the 9th month.

*: Statistically significant at $p \leq 0.05$. 
Table 4
Distribution of studied cases according to bone density within the defect.

<table>
<thead>
<tr>
<th>Bone density within the defect</th>
<th>Immediate postoperative (n = 15)</th>
<th>6 months (n = 15)</th>
<th>9 months (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>135.6–180.1</td>
<td>165.80–214.80</td>
<td>188.40–298.80</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>153.95 ± 15.04</td>
<td>188.17 ± 17.33</td>
<td>232.14 ± 33.10</td>
</tr>
<tr>
<td>Median</td>
<td>148.70</td>
<td>187.30</td>
<td>226.90</td>
</tr>
<tr>
<td>% of increase</td>
<td>22.2</td>
<td>50.8</td>
<td></td>
</tr>
<tr>
<td>p1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p1: p value for paired t-test for comparing between immediate postoperative and each other studied periods.
p2: p value for paired t-test for comparing between 6 months and 9 months.
*: Statistically significant at p ≤ 0.05.

Chiapasco et al. [28] in 2000 reported that the computed analysis of postoperative radiographs showed mean values of reduction in the size of the residual cavity of 12.34% after 6 months and 43.46% after 12 months, while the increase in bone density was 37% after 6 months and 48.27% after 12 months.

In two studies performed by Götz et al. [17] in 2010 and Harms et al. [29] in 2012, they reported a high osteoconductivity of nanocrystalline HA. They reported that the presence of silicate ions appears to promote the process of bone formation and remodeling at the bone–HA interface, as well as induction of angiogenesis, as adequate blood supply is a prerequisite for cellular activity. In contrast to what was observed with other HA-based bone substitute materials, the rapid osseointegration of nanocrystalline HA seemed to prevent its complete degradation. The Nanobone® particles were completely and firmly embedded within newly formed bone without a detectable fibrous interface and with no indication of an adverse host reaction to the material.

Clinical trials suggest that the combination of bone grafts along with the growth factors in the PRF may be suitable to enhance the bone density [30]. Kim et al. [31] in 2012 used TCP, PRF + β-tricalcium phosphate (β-TCP), and recombinant human bone morphogenetic protein2 (rhBMP-2)–coated TCP in the augmentation of the maxillary sinus in rabbits. The animals were killed at 3 days and at 1, 2, 4, 6, and 8 weeks. The PRF + β-TCP group showed greater area of bone formation compared with the TCP and the rhBMP-2–coated TCP groups during the evaluation period.

In another study performed by Jayalakshmi et al. [32] in 2012 where PRF and β-TCP allograft were used for augmentation of a periapical bone defect, they concluded that the addition of PRF to β-TCP accelerates the regenerative capacity of bone, and that when used in combination they give a predictable clinical and radiographic evidence of bone formation.

Moreover, Bölükbasi et al., in 2013 [26] conducted a histologic and histomorphometric study to evaluate the effect of adding PRF to β-TCP on bone regeneration. They created 5 mm bone defects in both tibias of 6 sheep. The defects were left empty or grafted with β-TCP, PRF, or PRF/β-TCP. The animals were sacrificed at 10, 20 and 40 days. Their study revealed that the PRF/β-TCP group showed the highest ratio of new bone formation.

Furthermore, Shivashankar et al. [33] in 2013 used PRF in conjunction with conventional HA crystals for the augmentation of a bone defect following the enucleation of a periapical lesion in the maxilla. They stated that the PRF/HA might have accelerated the resorption of the graft crystals and would have induced the rapid rate of bone formation.

5. Conclusion

On the basis of the results obtained from this study, it can be concluded that the combined use of Nanobone® and PRF for bone regeneration following the enucleation of large mandibular odontogenic cysts induced accelerated bone healing and improved the quality and quantity of regenerated bone.

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References


