The Journal of Oral and Maxillofacial Surgery

The Osteoinductive Effect of PuraBone on Bone Regeneration After Cyst Enucleation

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Abstract

Purpose: The investigators hypothesized the osteoinductive properties of PuraBone on bone regeneration. It is the nanocrystalline form of Puerarin being a phytoestrogen, derived from a wild leguminous creeper, Kudzu. This research was made to evaluate the effect of Purabone with PLATELET RICH FIBRIN (PRF) carrier as a graft material on bone regeneration after cyst enucleation in the mandible. Materials and Methods: Eighteen patients with mandibular cysts were included in the study. They were divided equally into 3 Groups. Group A (Study group) included patients grafted with 1cc of Purabone with Platelet Rich Fibrin carrier. Group B (Positive Control) included patients grafted with Platelet Rich Fibrin only. Group C (Negative Control) included patients with no graft (Control). Radiographic follow up was made using Digital Panoramic X ray at 0 and after 6 months. Follow up included measuring bone density changes and changes in height and width of cystic bone cavity after enucleation. Results: Panoramic Radiographic examination showed a statistical significant increase in bone density and decrease in cyst dimensions in the Study group than the Control Groups. Conclusions: It can be concluded that Purabone has an osteoinductive effect on bone regeneration.


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1. Introduction

1.1 Background

A cyst has been defined as “a pathological cavity having fluid, semifluid or gaseous contents and which is not created by the accumulation of pus” (Kramer, 1974). Classification of jaw cysts has been carried out by many authors. Thoma and Goldman classified jaw cysts into two main groups, Odontogenic and nonodontogenic, depending on the suspected origin of the epithelial lining (Thoma and Goldman, 1949). Other authors classified them according to the presence or absence of epithelial lining into true and pseudo cysts. Most of cysts in the oral region are true cysts (Meara et al., 1998; Wali et al., 2012).

Enlargement of cysts can lead to facial asymmetry, displacement of teeth and alteration in occlusion, losing of associated or adjacent teeth and displacement of denture which will be diagnosed during clinical examination. However, pathological fracture can occur in the mandible where the cystic lesion has caused resorption and weakening of most of the bone. The treatment of choice is conditioned by a number of factors such as the size of the lesion, its location, the possible involvement of neighboring anatomical structures, or damage to dental structures, among other (Ledesma et al., 2000; Camacho et al., 2002; Matsumoto et al., 2004; Escobar et al., 2007) .

Cysts of the jaws are treated by one of the following methods: (1) enucleation, (2) marsupialization, (3) a staged combination of the two procedures, and (4) enucleation with curettage. Enucleation is the process by which the total removal of a cystic lesion is achieved. By definition, it means a
shelling-out of the entire cystic lesion (Ellis, 2002; Riachi and Tabarani, 2009). Some authors have described the process of repair in bony cavities. They concluded that, these cavities heal primarily, by the organization of the extravasated blood and its replacement by an osteogenic granulation tissue. It has been reported that blood clots, especially in large bone defects cannot maintain themselves due to their size, also, contamination from the oral cavity may impede the normal process of healing (Thoma and Sleeper, 1948; Rowe and Kelly, 1955). Many researchers had emphasized the importance of maintaining and protecting the blood clot during the process of bone regeneration (Melcher and Drever, 1962; Weinmann and Sichor, 1955). There have been, accordingly, many efforts to accelerate the formation of new bone by using various types of implants either of biological origin (as bone grafts), or non biological substances (alloplastic materials) (Weinmann and Sichor 1955).

1.2 Platelet Rich Fibrin (PRF)
Platelet-rich fibrin (PRF), described by Choukroun et al. 2006 is a second-generation platelet concentrate which consist of fibrin membranes enriched with platelets and growth factors that originate from anticoagulant-free blood harvest (Choukroun et al., 2006). Thorat MK et al aimed in a study to investigate the clinical and radiological effectiveness of autologous PRF in the treatment of intra-bony defects of chronic periodontitis patients. The use of PRF as a graft exhibited pocket reduction clinical attachment after 3 and 6 months were observed (Thorat et al., 2011). Peck et al 2011 presented a case where PRF was successfully used in alveolar ridge preservation (ARP). Unlike other ARP procedures, the use of (PRF) is a simple method that requires minimal cost and reduces the need for specialized grafting material. Because it is a completely autologous product, no risk of disease transmission and graft rejection is existed (Peck et al., 2011). In 1997 Garner and Anderson, made a retrospective study on the special class of plant-derived molecules known as phytoestrogens. It was so named because of their partial estrogen agonism with estrogen receptors in mammalian systems (Garner and Anderson, 1997).

1.3 Phytoestrogens
Phytoestrogens are often good antioxidants and anti-inflammatory agents. These estrogen receptor (ER)-independent properties of genistein, resveratrol and other isoflavones, indicate that they have the potential to affect a wide array of intracellular signaling mechanisms important for regulating cellular growth and protection. populations. **Puerarin** is one of the major phytoestrogens isolated from the root of a wild leguminous creeper, **Pueraria lobata** (Kudzu, Willd.) .This is a commonly used traditional Chinese medicine known as **Gegen** (Rabie and Wong, 2007). In 2007 Rabie and Wong studied the use of Puerarin as a bone grafting material locally. It resulted in an increase in bone formation by 554% of new bone formation more than the control group grafted with collagen carrier only (Rabie and Wong, 2007). In 2015 Rabie and Khashaba proved that Puerarin was comparable to autogenous bone graft when applied in maxillary sinus augmentation (Rabie and Khashaba, 2015).

**Objective of the Research**
The aim of this study was to evaluate the osteoinductive effect of Purabone with PLATELET RICH FIBRIN (PRF) carrier as a graft material after cyst enucleation in the mandible.

**Justification of the Research**
The fact that Pura Bone is the nanocrystalline form of the osteoinductive plant derivative Puerarin increases its uses as bone graft material . This could help in decreasing the usage of autogenous bone grafts and reducing the drawbacks of harvesting bone from donor sites in addition to adding the osteoinductive qualities to a bone graft material and accordingly improving the bone quality and quantity during bone regeneration.

2. Materials & Methods

2.1 Materials
Purabone (212mg/cc, Tyjito Biotech Ltd, Hong Kong) is a nanocrystalline form of Puerarin. The maximum daily dose for using Puerarin in systemic application is 200-400 mg. The Study aimed studying the effect of the minimal permissible dose 1cc which is equivalent to 200mg.

This study included Eighteen patients with cystic lesion with age ranges from 18-45 years, who were selected from the outpatient clinic of Oral and Maxillofacial Department, Faculty of Oral and Dental Medicine, Cairo University. This study followed the Declaration of Helsinki on medical protocol and ethics and the regional Ethical Review Board of the Research Ethics Committee that approved the study. The inclusion criteria included cystic lesions in the anterior or the posterior regions of the mandible, freedom from any systemic disease that may affect bone healing and that the cyst to be of a moderate size. All cysts were treated by enucleation and the patients were divided into 3 groups according to grafting.

**Group A (Study Group)**: composed of 6 patients; the cyst cavity was grafted with Purabone + PRF after cyst enucleation.

**Group B (Positive Control)**: composed of 6 patients; the cyst cavity was grafted with PRF after cyst enucleation.
Group C (Negative control group): composed of 6 patients; the cyst cavity was left with no graft material after cyst enucleation (natural healing).

Careful clinical examination was made for the hard and soft tissue areas involving the cyst for any signs of swelling, inflammation, ulceration, or fistula formation. Bone covering the cyst area was palpated for hardness, egg shell crackling or perforation. (Figure.1) Aspiration biopsy was taken to examine the intralesional fluid to help in diagnosis of the type of the cyst. Vitality test was done to teeth involved in the lesion.

Proper oral hygiene measures were made for all the patients and they were instructed with daily instructions. All the teeth related to the cystic lesion were subjected to root canal treatment. Preoperative digital panoramic radiograph was obtained for each patient to evaluate the cyst lesion regarding its size, pattern, presence or absence of impacted teeth or odontome, and relation to vital structures. (Figure 2)

Figure 1: Photograph showing mucoperiosteum covering the cyst area

Figure 2: Preoperative Panoramic Xray showing presence of cyst in the mandible
2.2) **Surgical Phase:**
The surgical procedure was performed under local anesthesia using Mepivicaine-L* 2% with 1:200000 Levonordefrin vasoconstrictor were administrated to the patient few minutes before surgery.

2.3) **Platelet rich fibrin preparation (PRF):**
Platelet rich fibrin was prepared from patient own blood before starting the surgery. Chourkroun's platelet rich fibrin is defined in autologous leukocyte- and platelet rich fibrin biomaterials. Ten cc of blood was withdrawn in glass-coated plastic tubes without anticoagulant and immediately centrifuged at 3000 rpm for 10 minutes (Figure 3, Figure 4). Within few minutes the absence of anticoagulant caused activation of platelets contained in the sample, thus triggered coagulation cascade. At first fibrinogen was concentrated in the upper part of the tube, until thrombin transformed it into fibrin. Fibrin clot was the result and located in the middle of mass of cellular plasma, with maximum number of platelets caught in the mesh of fibrin. Therefore the blood sample was divided in to three layers: a base of red blood at the bottom, acellular plasma at the top and a clot of PRF in middle (Figure 5).

**Figure 3:** Photograph showing 10 cc of blood withdrawn from the patient.

**Figure 4:** Photograph showing the blood sample divided into three layers: a base of red blood at the bottom, acellular plasma at the top and a clot of platelet rich fibrin in the middle.

**Figure 5:** Photograph showing the clot of Platelet Rich Fibrin.

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2.4 Surgical Procedure:
- After administration of local anaesthesia a gingival incision was made around the teeth related to the cyst using Bard Parker blade #15. It was extended one tooth before and after the radiographic limit of the cystic cavity with a vertical releasing incision performed for better visualization and exposure of the operative field. (Figure 6).
- The flap was raised to aid in better access to the cystic cavity. (Figure 7) Any intervening bone was removed to aid in the enucleation of the cyst lining using bone curettes.
- After complete separation from the surrounding tissues the cyst was taken for pathological assessment. Apicectomy was performed only to the roots of the teeth involved in the cyst that hinder the complete removal of the cyst lining.
- After complete debridement of the bony cavity, sharp bony edges were trimmed. The PRF +1cc (200mg) of Purabone were placed in Study Group (Group A) (Figure 8).
- In Group B the bony cavity was filled with PRF only (Figure 9). In Group C the cavity was left empty (Figure 10).

Figure 6: Photograph showing the gingival incision.

Figure 7: Photograph showing reflection of the mucoperiosteal flap and full exposure of the lesion.

Figure 8: showing the bony cavity filled with PRF + PURABONE.

Figure 9: Photograph showing the PRF filling the surgical cavity.

Figure 10: Photograph showing the surgical cavity left with no graft material (group C).
2.5 Postoperative Care:
- Post operative instructions were given to all patients. Clindamycin Hydrochloride 300mg antibiotic (Clindam 300mg, SIGMA pharmaceutical industries, Egypt. SAE) was prescribed three times a day and Diclofenac Potassium 50mg was prescribed three times a day to the patients for one week post operatively. Sutures were removed after 7 days.
- Postoperative Follow up assessment included clinical examination to asses healing, infection, bleeding or inflammation. The Radiographic follow up included Digital panoramic radiographs that were taken preoperatively, immediate and 6 month postoperatively. All Data were collected and statistically analyzed.

3. Results
Clinical assessment showed good results as shown in no postoperative complications as periapical inflammation, infection, dehiscence, bleeding, fistulation or edema. No signs of tenderness on palpation of the endodontically treated teeth. No side effects towards the graft material. Only one case showed swelling related to cyst area, which completely disappeared after one month postoperative.
3.1 Radiographic Assessment:
Digital Panoramic radiograph imaging was done preoperatively, immediate and at six months postoperative. Density was assessed using The Romexis’ software. The mesiodistal length and the superoinferior height were assessed using the Digora” software.

- Radiographic examination showed well defined radiolucency related to periapical area of the related teeth. There were no odontomes or impacted teeth found in all lesions of both groups. No vital structures were involved in any lesion of all cases except one case where the inferior alveolar nerve was in a close relationship to the cyst lesion.

A comparison was done between digital panoramic radiographs which are taken immediate postoperatively and six months postoperatively. (Figure 11, Figure 12)

Radiographic analysis revealed that there was gradual increase in bone density. Also, there was gradual reduction in size of bone defect in all cases during follow up period.

Statistical analysis was performed by Microsoft Office 2013 (Excel) and Statistical Package for Social Science (SPSS) version 20. Data were presented as mean and standard deviation (SD) values. The significant level was set at P ≤ 0.05. Kolmogorov-Smirnova and Shapiro-Wilk tests was used to assess data normality and data was assumed normally distributed. Paired t test was used to compare between follow up period within groups. Independent t test was used to compare between case and control groups.

Group A (Study Group):
As shown in Table (1):
- The minimum bone density in the study group in the immediate postoperative radiograph was 1600 Hu and the Maximum was 1867 Hu and they had a mean of 1734 Hu.
- The minimum bone density 6 months postoperative was 2000 Hu and maximum was 2579 Hu and they had a mean of 2313.31 Hu (Figure 13).

The bone density change after Six months in the study group has P value of 0.002.

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* Planmeca Romexis Software 2.9.2.R.
** Digora software for windows 2.5 Rev.
1.Manufactured by SOREDEX, FINLAND.
Table 1: Bone density of the PRF + PuraBone group. (Group A)

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1600.0</td>
<td>1867.79</td>
<td>1734.0050</td>
<td>104.22386</td>
<td>0.002*</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>2000.0</td>
<td>2579.50</td>
<td>2313.3133</td>
<td>271.67039</td>
<td></td>
</tr>
</tbody>
</table>

Figure 13: A bar chart showing an increase in the bone density in the Group A between immediate postoperative readings and after 6 months.

As shown in Table (2):
The mean mesiodistal width of cystic bony cavity in the immediate postoperative period was 3.421 mm and after Six months was 0.783 mm (Figure 14).
The decrease in the width of the cystic bone cavity is an indication of bone regeneration which statistically significant of a P value 0.004.

Table 2: CHANGES in width of the Cyst cavity PRF + PuraBone (Group A)

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1.50</td>
<td>5.52</td>
<td>3.4217</td>
<td>1.66366</td>
<td></td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>.10</td>
<td>2.50</td>
<td>0.7833</td>
<td>0.85829</td>
<td>0.004*</td>
</tr>
</tbody>
</table>
**Figure 14:** a bar chart showing a decrease in the width of the cyst cavity in the Study group after 6 months. There was decrease in bone width after 6 months and difference was statistically significant.

![Bar Chart](image1)

As shown in Table (3):
The mean superoinferior height of cystic bony cavity in the immediate postoperative period was 1.67 mm and after Six months was 0.48 mm (**Figure 15**).
The decrease in the height of the cystic bone cavity is an indication of bone regeneration which statistically significant of a *P value 0.004*.
Change in Superoinferior height of the cyst cavity in PRF +PuraBone GROUP immediate postoperative and after 6 months. (Group A)

**Table 3:** Changed in Superinferior Height of the Cyst cavity in PRF + PuraBone (Group A)

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1.31</td>
<td>2.71</td>
<td>1.675</td>
<td>.51392</td>
<td>.004*</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>.30</td>
<td>.70</td>
<td>.4833</td>
<td>.13292</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 15:** a bar chart showing a decrease in the height of the cyst cavity in the Study group after 6 months. There was decrease in bone height after 6 months and difference was statistically significant.

![Bar Chart](image2)
Group B (PRF ONLY) (Positive Control)

As shown in Table (4):
- The minimum bone density in the study group in the immediate postoperative radiograph was 55 Hu and the Maximum was 1244 Hu and they had a mean of 108.70 Hu.
- The minimum bone density 6 months postoperative was 289 Hu and maximum was 1341 Hu and they had a mean of 642.12 Hu (Figure 16).
- The bone density change after 6 months in the study group has **P value of 0.001**.

**Table 4: Bone density of Group B**

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>55</td>
<td>1244</td>
<td>108.70</td>
<td>29.1526</td>
<td>0.001*</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>289</td>
<td>1341</td>
<td>642.12</td>
<td>161.3745</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 16:** a bar chart showing an increase in the bone density in **GROUP B**. There was increase in bone density after 6 months and difference was statistically significant (TABLE 4).

As shown in Table (5):
The mean Mesiodistal width of cystic bony cavity in the immediate postoperative period was 2.10 mm and after Six months was 1.13 mm (Figure 17).
- The decrease in the width of the cystic bone cavity is an indication of bone regeneration which statistically significant of a **P value 0.008**.

**Table 5: Changes in the width of the cyst cavity GROUP B**

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1.12</td>
<td>7.44</td>
<td>2.108</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>0.65</td>
<td>5.047</td>
<td>1.135</td>
<td>0.628</td>
<td>0.008*</td>
</tr>
</tbody>
</table>
**Figure 17:** a bar chart showing a decrease in the width of the cyst cavity in GROUP B after 6 months. There was a decrease in bone width after 6 months and difference was statistically significant (TABLE 5).

As shown in **Table (6):**

The mean Superoinferior height of cystic bony cavity in the immediate postoperative period was 1.94 mm and after Six months was 1.13 mm (**Figure 18**).

The decrease in the height of the cystic bone cavity is an indication of bone regeneration which statistically significant of a *P value 0.002*.

**Table 6:** Change in Superoinferior height of the cyst cavity in Group B

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1.47</td>
<td>2.74</td>
<td>1.9463</td>
<td>0.481</td>
<td>0.002*</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>0.65</td>
<td>1.66</td>
<td>1.1303</td>
<td>0.325</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 18:** a bar chart showing a decrease in the height of the cyst cavity in Group B. There was decrease in bone height after 6 months and difference was statistically significant (TABLE 6).
**Group C** (EMPTY CAVITY) CONTROL (Negative Control)

As shown in **Table (7)**:

- The minimum bone density in the study group in the immediate postoperative radiograph was 60 Hu and the maximum was 670 Hu and they had a mean of 143.6 Hu.
- The minimum bone density 6 months postoperative was 85 Hu and maximum was 780 Hu and they had a mean of 170.3 Hu (**Figure 19**).
- The **Table (7)**: bone density change after Six months in the study group has **P value of 0.051**.

**Table 7**: Bone density of Group C

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>60</td>
<td>670</td>
<td>143.6</td>
<td>15.46</td>
<td>0.051</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>85</td>
<td>780</td>
<td>170.3</td>
<td>21.59</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 19**: a bar chart showing an increase in bone density in GROUP C. There was increase in bone density after 6 months and difference was statistically non significant (TABLE 7).

As shown in **Table (8)**:

The mean Mesiodistal width of cystic bony cavity in the immediate postoperative period was 2.49 mm and after Six months was 2.37 mm (**Figure 20**).
The decrease in the width of the cystic bone cavity is an indication of bone regeneration which was statistically non significant of a **P value 0.8**.

**Table 8**: Changes in WIDTH of the cyst cavity in Group C

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>0.82</td>
<td>5.51</td>
<td>2.49</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>0.78</td>
<td>5.19</td>
<td>2.37</td>
<td>0.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure 20: a bar chart showing a decrease in the width of the cyst cavity in GROUP C. There was decrease in the width of the cystic cavity after 6 months and difference was statistically non significant (TABLE 8).

As shown in Table (9):
The mean Superoinferior height of cystic bony cavity in the immediate postoperative period was 2.72 mm and after Six months was 2.45 mm (Figure 21).
The decrease in the height of the cystic bone cavity is an indication of bone regeneration which was statistically non significant of a $P$ value 0.7.

<table>
<thead>
<tr>
<th>Follow Up Period</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Postoperative</td>
<td>1.09</td>
<td>7.87</td>
<td>2.72</td>
<td>1.18</td>
<td>0.7</td>
</tr>
<tr>
<td>6 Months Postoperative</td>
<td>0.89</td>
<td>4.5</td>
<td>2.45</td>
<td>1.03</td>
<td></td>
</tr>
</tbody>
</table>

Figure 21: a bar chart showing a decrease in the height of the cyst cavity in GROUP C. There was decrease in height of cystic cavity after 6 months and difference was statistically non significant (TABLE 9).

As shown in Table (10):
- Using Paired T test to compare the 3 groups, there was a significant difference in the Bone density, Width and heights of the cystic cavities in the immediate postoperative period.
- In comparing the value of the bone density in the immediate postoperative period, the $P$ value was 0.001.
• The Statistical difference of the Mesiodistal width of the cystic cavity between the groups has a P value of 0.53.
• The Statistical difference of the Superoinferior height of the cystic cavity between the 3 groups has a P value of 0.63.

Table 10: Comparison between groups (Immediate Post Operative)

<table>
<thead>
<tr>
<th>Immediate Postoperative</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone density</td>
<td>1734.0050</td>
<td>104.22386</td>
<td>108.705</td>
<td>0.001</td>
</tr>
<tr>
<td>Width of Cyst Cavity</td>
<td>3.4217</td>
<td>1.66366</td>
<td>2.108</td>
<td>0.53</td>
</tr>
<tr>
<td>Height of Cyst Cavity</td>
<td>1.6750</td>
<td>.51392</td>
<td>1.9463</td>
<td>0.63</td>
</tr>
</tbody>
</table>

As shown in Tables (11-13): Comparing the 3 groups individually, in the immediate postoperative follow up period, showed a statistically significant difference (P value <0.05) (Figure 22, Figure 23, Figure 24).

Table 11: Comparison between All Groups in Bone Density (Immediate Postoperative)

<table>
<thead>
<tr>
<th>Bone density</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>1625.3</td>
<td>44.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A - group C</td>
<td>1590.4</td>
<td>42.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>34.9</td>
<td>13.33</td>
<td>0.0257</td>
</tr>
</tbody>
</table>

Figure 22: A bar chart comparing the bone density in the 3 Groups immediate postoperative.

Table 12: Comparison between all Groups in Width of Cystic Cavity (Immediate Postoperative)

<table>
<thead>
<tr>
<th>Width of the cyst cavity</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>1.3</td>
<td>0.8</td>
<td>0.135</td>
</tr>
<tr>
<td>Group A - group C</td>
<td>0.91</td>
<td>0.818</td>
<td>0.29</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>0.39</td>
<td>0.624</td>
<td>0.545</td>
</tr>
</tbody>
</table>
Figure 23: A bar chart comparing the width of the cystic cavity in the 3 Groups immediate postoperative.

![Bone width](image)

Table 13: Comparison between all Groups in Height of Cystic Cavity (Immediate Postoperative)

<table>
<thead>
<tr>
<th>Height of the cystic cavity</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>0.3</td>
<td>0.283</td>
<td>0.314</td>
</tr>
<tr>
<td>Group A – group C</td>
<td>1.12</td>
<td>0.523</td>
<td>0.06</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>0.82</td>
<td>0.523</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Figure 24: A bar chart comparing the height of the cystic cavity in the 3 Groups immediate postoperative.

![Bone height](image)

As shown in Tables (14-17), (Figure 25, Figure 26, Figure 27)

- Using Paired T test to compare the 3 groups, there was a significant difference in the Bone density, Width and heights of the cystic cavities after 6 months follow up period.
- In comparing the value of the bone density in the immediate postoperative period, the P value was < 0.001.
- The Statistical difference of the Mesiodistal width of the cystic cavity between the groups has a P value of 0.03.
- The Statistical difference of the Superoinferior height of the cystic cavity between the 3 groups has a P value of 0.01.
Table 14: Comparison between all Groups (After 6 months)

<table>
<thead>
<tr>
<th>After 6 months</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone density</td>
<td>2313.3133</td>
<td>271.67039</td>
<td>642.125</td>
<td>161.3745</td>
</tr>
<tr>
<td>Width of the cyst cavity</td>
<td>0.7833</td>
<td>0.85829</td>
<td>1.135</td>
<td>0.628</td>
</tr>
<tr>
<td>Height of the cyst cavity</td>
<td>.4833</td>
<td>.13292</td>
<td>1.1303</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Table 15: Comparison between groups in Bone density After 6 months

<table>
<thead>
<tr>
<th>Bone Density</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>1671.1883</td>
<td>129</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A - group C</td>
<td>2143.0133</td>
<td>111.258</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>471.825</td>
<td>66.467</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 25: A bar chart comparing the bone density in the 3 Groups after 6 months.

Table 16: Comparison between groups in Width of Cystic cavity After 6 months

<table>
<thead>
<tr>
<th>Width of the cyst cavity</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>0.355</td>
<td>0.434</td>
<td>0.43</td>
</tr>
<tr>
<td>Group A - group C</td>
<td>1.59</td>
<td>0.507</td>
<td>0.0107</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>1.235</td>
<td>0.488</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Figure 26: A bar chart comparing the width of the bony cystic cavity in the 3 Groups after 6 months.

As shown in Tables (15- 17):
Comparing the 3 groups individually, after 6 months postoperative follow up period, showed a statistically significant difference (P value <0.05) (Figure 25, Figure 26, Figure 27).

Table 17: Comparison between groups in Superoinferior Height of Cystic cavity After 6 months

<table>
<thead>
<tr>
<th>Height of the cyst cavity</th>
<th>Mean diff</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A – group B</td>
<td>0.642</td>
<td>0.1429</td>
<td>0.001</td>
</tr>
<tr>
<td>Group A- group C</td>
<td>1.96</td>
<td>0.42238</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group B – group C</td>
<td>1.32</td>
<td>0.44</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 27: A bar chart comparing the height of the cystic bony cavity in the 3 Groups after 6 months.

3. Discussion

This study showed that Purabone mixed with Platelet Rich Fibrin (PRF) increased new bone formation when used locally after cyst enucleation in the mandible. This was demonstrated by an increase in bone density and decrease in dimensions of cystic bone cavity (Figures 13, Figure 14, Figure 15).

Platelet Rich Fibrin (PRF) in the mix acted as a carrier with osteogenic properties (Dohan et al., 2006; Anitua et al., 2007). Results demonstrated that the osteoinductive ability of the mix is mostly due to the Puerarin in its nanocrystalline form. In comparing all the groups there was a statistical difference in the increase in new bone formation in Group A, being the Puerarin containing group compared to control Groups B and C (Figure 25). Puerarin, is an isoflavone phytoestrogen that stimulates osteogenesis at low concentrations (Rabie and Khashaba, 2015). It acts in a dual manner, by stimulating osteoblasts and by suppression of osteoclasts (Zang et al., 2005). In this study, the minimal permissible dose used was 212mg/cc. The amount of bone induced in Group A was significantly higher than Group B being the PRF group alone (Table 15, P value <0.001). This confirms the osteoinductive ability of Puerarin.

The osteoinductive abilities of Isoflavones were demonstrated in experimental studies by Kanno in 2004 as well as clinical studies (Kanno et al., 2004). Experimental studies revealed the increase in osteoblastic differentiation which was screened by an increase in Alkaline Phosphatase. The direct effect of Puerarin on osteoblasts was explained by Li and Yu 2003 where Puerarin was applied to osteoblasts from rat calvaria. This study demonstrated the dual action of Puerarin on increased activity of osteoblasts by Alkaline Phosphatase and the decrease in osteoclastic activity detected by Acid Phosphatase (Li and Yu, 2003).

The effect of applying Puerarin locally was demonstrated by Rabie and Wang 2007. It was applied locally in calvarial defects of New Zealand rabbits, by injecting it in a resorbable collagen sponge. It led to 554% more bone formation than the control group (Rabie and Wang, 2007). The effect of Puerarin is much attributed to its nature as an isoflavone. Isoflavone acts by targeting Bone morphogenetic protein-2 (BMP-2), a potent inducer of osteogenic differentiation. The clinical importance of bone morphogenetic protein was highlighted earlier by Barboza et al. in 2000 (Barboza et al., 2000). He used BMPs as an aid to increase bone crest height prior to the placement of implants. Furthermore, this was followed by a clinical study in 2002 by Govender et al. where the effect of Recombinant BMP-2 on opened tibial fractures was evaluated. The rhBMP-2 group had significantly fewer interventions (P = 0.026) and significantly faster fracture healing (P = 0.002).
than did the control patients (Govender et al., 2002). The use of recombinant BMP-2 was found to be comparable to the use of autogenous bone graft according to a clinical study by Jones and Bucholz in 2006 where autogenous bone grafting in the treatment of diaphyseal tibial fractures was compared to recombinant BMP-2/ allografts clearly demonstrating the importance of Bone morphogenetic proteins in bone healing and regeneration. The fact that Puerarin induces bone formation through the BMP-2 route and the fact that it is a natural plant extract, it could be considered as a viable alternative to rhBMP (Jones and Bucholz., 2006).

The clinical effect of Puerarin was applied clinically in maxillary sinus augmentation by Rabie and Khashaba in 2015 (Rabie and Khashaba, 2015). It was compared to autogenous bone grafted from the chin. Its osteoinductive effect was demonstrated by histomorphometrical analysis with no significant difference than that resulted by the autogenous bone. The results of Purabone in this study are comparable with that of nanocrystalline Ostim Hydroxyapatite paste by Canuto in 2013 (Canuto et al., 2013). The Ostim Hydroxyapatite paste was applied clinically for socket preservation, it acted by increasing the synthesis of pro-osteogenic factors as bone morphogenetics protein (BMP)-4, BMP-7, alkaline phosphatase, and osteocalcin.

This study showed the osteogenic effect of Purabone on bone regeneration. It is recommended that further studies be made using Purabone in different clinical applications. The fact that we eliminated the need to use autogenous bone and replaced it with an active ingredient from a plant extract, points to a significant clinical value.

Conclusion

This study showed the osteogenic effect of Purabone on bone regeneration. The use of Purabone highlights the use of a naturally derived bone graft material to eliminate the use of autogenous bone.

Limitations

The use of the minimal permissible dose of Purabone (200mg) with Platelet Rich Fibrin (PRF) carrier.

Research Highlights

- To outline the management of cyst and lines of treatment.
- To highlight the different modalities of treatment of cysts.

Authors’ Contribution and Competing Interests

Authors declare no any conflict of interest.

This research has been fully self funded. The present study was approved by the Ethics Committee of the Faculty of Oral and Dental Medicine, Cairo University.

Recommendations

It is recommended that various concentrations of PuraBone be used according to its permissible dose (200-400mg).

This study is recommended to be applied on larger samples of patients, for longer follow up periods and different doses.

It is recommended that PuraBone be applied in various clinical applications.

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