COMPARATIVE STUDY BETWEEN THE EFFECT OF CALCIUM HYDROXIDE MIXED WITH NIGELLA SATIVA-L OIL VERSUS BIOGLASS ON HEALING OF INDUCED MANDIBULAR BONE DEFECTS IN RATS


ABSTRACT

Fourty albino rats were divided into four equal groups. All animals were subjected to the same surgical procedures, to induce a surgical defect (about 3 mm) in the right mandible. Group I: control group, where the bony defect was left empty. Group II: bony defect was filled with calcium hydroxide powder mixed with saline. Group III: bony defect was filled with calcium hydroxide mixed with Nigella sativa L.oil. Group IV: The bony defect was filled with ready made bioactive glass particulates. Sacrificing half of the animals after 3 weeks post operatively, the other half was sacrificed after 6 weeks. Results revealed that, there were no new bone formation in groups (I, II, III) after 3&6 weeks, while in group (IV), osteoid tissues was laid down in minute amount, after 3 weeks, but after 6 weeks, more fibrous tissues was seen & the borders of the defects were of normal thickness, smooth and lined by a well defined osteoblastic layer.

INTRODUCTION

Bone defects and irregularities, are major problems for dental implants and periodontal therapies. Bone repair is a major challenge of reconstructive surgery. Bone availability is the key to successful placement of endosseous implants. Various materials have been used for regenerating bone defects, like autogenous bone graft, heterogenous bone graft, and alloplastic material (Marx, 1994).

There are three types of alloplastic substances in clinical use today: hydroxy apatite, other ceramics, and polymers. Bioactive glass is a type of vitreous materials that belongs to the family products including calcium phosphate. These materials elicit a biological response with bone at the
interface, resulting in the formation of direct bond between the material and bone, without intervening soft tissue layer (Bendall, et al., 1998). As a matter of fact, the bioactive glasses possess many favorable physical properties in addition to their affinity to attachment of osteoblasts, the particles well retained and confined in the cavities, and has desirable haemostatic effect and form cohesive mass (Schepers & Ducheyene, 1997).

**Calcium hydroxide (Ca(OH)₂)**

Calcium hydroxide has been used extensively in the dental field for its antimicrobial effect. The mode of action of calcium hydroxide, could be related to one of the following factors: Firstly, the pH change from acidic inflammatory medium to basic one after its application. Secondly, the calcifying potential of calcium hydroxide, which could be capable of building up bone, in the periapical lesions. Lastly, the caustic action of calcium hydroxide that burns the residual chronically inflamed tissues (Weine, 1982).

**Nigella sativa L:**

Many investigations were published about the effect of Nigella sativa L-oil on healing of hard & soft tissues.

Nigella sativa was found to possess an anti-pyretic, analgesic, cardiovascular, neurologic, reproductive, respiratory, hematologic, anti-allergic, immunologic, anti-inflammatory, metabolic, diuretic, anti-microbial, anti-fungal, as well as anti-helminthic. It has been found to play a role in protection of tissue against toxic damage, as well as repair and healing of injured tissue. It also has an anti-neoplastic and anti-oxidant activity. Studies have also demonstrated low toxicity of Nigella sativa, which explains its popularity and widespread use over many centuries (Kasule, 2005).

The purpose of this study was to identify the effect of calcium hydroxide powder mixed with Nigella sativa L.oil, versus bioglass on the healing of induced bone defect in mandible of rats.

**MATERIAL & METHODS**

The present study was conducted on forty male adult albino rats weighing 200-250 grams, aged 90-120 days. The albino rats under investigation were divided into four equal groups of ten animals each. All animals were subjected to the same surgical procedures, in the right mandible, to induce a surgical defect (about 3 mm). The same defect was induced in the left side, in one animal of each group, served as self control.

**Group I:** Control group, where the bone defect was left without filling material.

**Group II:** The bone defect was filled with calcium hydroxide, mixed with saline.

**Group III:** The bone defect was filled with calcium hy-
droxide mixed with Nigella sativa L-oil (NSO)

**Group IV:** The bone defect was filled with ready made bioactive glass particulates.

**Surgical Procedures:**

General anaesthesia was performed through intra-muscular injection of ketamine hydrochloride (Ketlar 0.5%) , ketamine hydrochloride (Ketlar 0.5%) was performed through intra-muscular injection of ketamine hydrochloride (Ketlar 0.5%) , 0.20 mg / kg body weight, and xylazine (1 mg / kg body weight) (Hillyer & Quesenberry, 1997).

After routine disinfection, and preparation of surgical field by betadine, an extra-oral submandibular incision was performed at the right inferior border to raise a full thickness skin flap. The masseter muscle was incised and dissected from periosteum to expose the body of the mandible. By using low-speed round bur no 2, with copious irrigation, a surgical bony defect of ≈ 3 mm. in diameter, was carried out according to Srouji et al., (2005) followed by the debridement & irrigation of the surgical field. Surgical defects were filled by materials as mentioned before, according to each group.

Sacrificing half number of the animals was done after 3 weeks post operative, the other half was sacrificed after 6 weeks, modified from (Srouji et al, 2005)

The rat mandibles were dissected free, and placed in 10% neutral formalin solution, then subjected to radiographic & histologic examination.

Digora system was used for evaluating changes in bone density, for all cases of four groups. Results were tabulated for statistical analysis (One Way ANOVA test).

**RESULTS**

**Radiographic evaluation by Digora System:**

The X-ray films were scanned, recorded and analyzed by Digora system. The density of bone, in the surgical defect for each specimen, was recorded in minimum and maximum values.

The mean density of all specimens were analyzed statistically by "One Way ANOVA" test, results are presented in Table (1).

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1 Calcium hydroxide powder (Sultan Chemists Inc. Englewood, NJ 076331)
2 Sodium Chloride .9% (El-Nasar Pharmaceutical Chemical Co, Egypt)
3Nigella sativa L-oil (Pharco Medical Co.)
4bioactive glass (Dawaa – Smart. Pharma Group, Cairo, Egypt)
Particle size (150-450µm) M.O.H Reg.No.268/98
5 Ketlar 0.5% (Amoun Medical Co.)
6xylazine ADWI A 10th of Ramadan city.
7 Digora X ray Imaging System from Sordex Orion Corp., Finland.
Table (1): Bone densities in different groups (Mean±SD), 3 and 6 weeks after surgery, as measured by Digora.

<table>
<thead>
<tr>
<th>Follow up period</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 weeks</td>
<td>31.59±1.59</td>
<td>38.50±4.85</td>
<td>41.94±3.64</td>
<td>72.98±23.13</td>
</tr>
<tr>
<td>6 weeks</td>
<td>29.97±1.16</td>
<td>29.83±0.65</td>
<td>34.31±2.79</td>
<td>127.03±9.95*</td>
</tr>
</tbody>
</table>

*Statistical significance at p-value < 0.05

Only at 6 weeks after surgery bone density, in group IV (treated with bony glass) showed significant increase (p < 0.05), while Group I (control), Group II (treated with calcium hydroxide and saline), and Group III (treated with calcium hydroxide and NSO) showed no significant differences in bone densities. At 3 weeks, there were no significant differences in bone densities among the four groups.

Fig. (1): Histogram showing bone densities in different groups at 3 & 6 weeks after surgery.
**Histopathological Results:**

**Group I:** Where the bony defects were not filled by any material. At 3\(^{rd}\) week, No evidence of connective tissue (C.T) or new bone formation was seen, only empty cavity. No sings of inflammation or bone resorption at the periphery, as well as no osteoblastic activity (Fig. 2). At 6\(^{th}\) week, scanty connective tissue filling the cavity, with a layer of osteoblast-like cells lining the cavity (Fig. 3).

**Group II:** Where the bony defect was filled with calcium hydroxide powder + saline. At 3\(^{rd}\) week, the cavity was filled by considerable amount, of more dense cellular connective tissue. Empty space represent dissolved material during decalcification process (Fig. 4). At 6\(^{th}\) week, Considerable soft tissue mass with tendency toward osteoblastic differentiation was observed. However, focal area of sequestration surrounded by inflammatory cellular infiltrate (lymphocytes & few PMNLs) were detected, At 6\(^{th}\) weeks the hyperplastic cellular tissue was extending to the surface of surrounding bone (Fig.5).

**Group III:** Where the defects were filled with calcium hydroxide powder + Nigella sativa oil.

At 3\(^{rd}\) week, smooth broders of an empty cavity with no signs of inflammation, and no evidence of osteoblastic activity were seen. The bone boundaries were of normal contour as in Gr I (Fig.6). At 6\(^{th}\) week, scanty, less cellular fibrous tissue, with tendency for osteoblastic differentiation. The borders were smooth, with normal contour, and no evidence of inflammation or bone resorption (Fig.7).

**Group IV:** Where the defects were filled with bioglass. At 3\(^{rd}\) week, considerable amount of cellular tissue with osteoblast – like cell differentiation, infiltrating the pores of provisional filling material. Osteoid is laid down in minute amounts. The bone at the periphery was lost by the proliferating cellular tissue (Fig.8).

At 6\(^{th}\) week, considerable amount of cellular fibrous tissue with evident osteoid being laid down. The borders of the defects are normal in thickness, smooth and lined by a well defined osteoblastic layer (Fig.9).
Fig. (2) : Control group after 3 weeks.

Fig. (3) : Control group after 6 weeks.

Fig. (3) : Group II (Ca(OH)₂ + saline) after 3 weeks.

Fig. (5) : Group II after 6 weeks.

Fig. (6) : Group III after 3 weeks.

Fig. (7) : Group III after 6 weeks.

Fig. (8) : Group IV after 3 weeks.

Fig. (9) : Group IV after 6 weeks.
DISCUSSION

Although, radiography is an important, noninvasive, diagnostic tool, used for assessment of bone formation, implanted materials may give radio-opacities, up to the degree of sclerosis as seen with calcium hydroxide (Carrotte, 2004).

Histological examination is more accurate to explain the exact situation with different implanted materials, and degree of bone formation.

Clinical results, of the present work, revealed an overall good performance of all animals, except 2 animals in group II (calcium hydroxide + saline) where an abscess had appeared at the site of operation, after 10 and 15 days of surgery, and resolved after 3 days following one dose of ampicilline 250 mg. Such finding may due to self scratching by animal nails at site of operation. Absence of infection or inflammation, in all other animals, may be attributed to the bacteriostatic effect of saline in Gr II, as well as the known antibacterial effect of calcium hydroxide, in Gr II & III. This effect was reported by other investigators (Mohamed, 1987; Torabinjade, 1995; Abou-Serihe, 1999; El-Hag, 2002; Carrotte, 2004). Also, NSO is well known to have an antibacterial, antifungal as well as antiviral activity (Kosule, 2005). In group IV, absence of infection and/or inflammation, may be attributed to the bacteriostatic effect of bioglass, as reported by Peltola et al., (1999). Yassin (2002) reported there was an improved wound healing with no signs of infection or inflammation, and no foreign body reaction, when bioglass was implanted in the created defects in dog’s mandible.

The histologic findings in Gr II & Gr III (Ca hydroxide + saline or NSO) indicated that Ca hydroxide is prone to dissolution, as reported by Carrotte, 2004, and hence replaced by fibrous tissue, i.e. did not reveal an osteoconductive property, at least for the 6 weeks period of the experiment. Contrary to the well known property of calcium hydroxide, that can induce calcified tissue formation when placed in an area of bone or dentine or when used as pulp capping material.

Calcium hydroxide in Gr II appeared to be resorbed from its periphery (at 3 weeks) that was replaced by loose and vascular C.T (at 6 weeks), with incomplete resorption of the surrounding bone (Fig.4,5), and evidence of osteoblastic differentiation. In Gr IV, bioglass appeared to induce more cellular infiltration to the deeper layers (center of the defect) with complete peripheral bone resorption (at 3 weeks). The cells acquired the osteoblastic morphological features, with tendency to form an osteoblastic material (Fig.8). An ill defined RO appearance, explains the merging of proliferating osteoblast-like cells, from the surrounding marrow spaces with initial de-
mineralization of bone periphery. At 6 weeks, the soft tissue (osteoblast-like cells) was apparent at the center, with bone regeneration at the peripheries, that completely surrounded the cavity, to the point of restoring the original outline of the induced defect at day zero. New bone was formed buccolingually, as well. This new bone was evident grossly as lighter, hard bony tissue (Fig.9). This result assures the osteoconductive property of bioglass, that seems to be incorporated into surrounding bone, without fibrous capsulation, in agreement with Nasar, (2000). This finding is parallel to the well-known stimulatory effect of bioglass on surrounding tissue, to induce formation of calcified material. Although, bioglass is a resorbable material, the time interval in the present work, was not enough for all material to be completely removed and replaced by bone.

Greenspan, (1999), had explained the mechanism of bioglass effect based on a rapid and extensive formation of bioactive layer, which is hydroxyl carbonate apatite layer (HCA), at the surface between original bone and bioglass particulates. The findings of the present work, may be further supported by the observation of El-Ghannam, et al., (1999), who stated that the generated calcium phosphorus rich layer, promoted the osteoblastic function through enhancement of osteoblast attachment apparatus, that containing fibronectin, i.e. favoring attachment to calcium phosphate rich layer.

As Digora system was introduced to assess bone density (Abedl-Azim, 2004), the present findings indicate that the system assess other materials that contain calcium, as calcium hydroxide or bioglass. Although the difference in readings between Gr II & Gr III, was not statistically significant, it appears that NSO added to the density of calcium hydroxide over saline, or had delayed the dissolution of calcium hydroxide compared to group II.

Density measurement of ungrafted control defects, in group I, at 6th week, were less than that at 3rd week. This decrease in bone density, may be attributed to bone resorption, which usually precede bone healing process. This finding is in agreement with Nasar, (2000).

Although bioglass (Gr IV) gave the highest reading (in 3 weeks), it was not statistically significant, compared to Gr II & III. The highest statistically significant result at 6 weeks (Gr IV), could not be explained, depending only on the calcium content of the material, that is known to be resorbable. Rather, it reflects the new bone formation buccolingually, as well as regeneration of peripheral bone, mesiodistally, that restored the original defect size.

In conclusion, it is clear that all used materials are biocompatible, to the used animal model. However, bioglass seems to have the highest
osteoconductive and osteosimulatory effect among the used materials.

REFERENCE:


Torabinejad, M.; Hong, CU.; Pu Ford, TR . and Kariyawsam, Sp. (1995) :


الملخص العربى

دراسة مقارنة بين تأثير كلاً من هيدروكسيد الكالسيوم المخلوط بزيت حبة البركة والزجاج الحيوي على التئام العيوب المخلقة لعظم الفك السفلي للفئران

الهدف من الدراسة

هدفت الدراسة الحالية إلى المقارنة بين تأثير كلاً من مادتي هيدروكسيد الكالسيوم (المخلوط بملحى) أو بزيت حبة البركة والزجاج الحيوي على التئام العيوب المخلقة في عظم الفك السفلي لدى عينة من الفئران.

خطوات الدراسة

تم اختيار عينة من الفئران البيضاء قوامها أربعين فكاً أربعًا. قسمت إلى أربعة مجموعات متساوية تتكون كل منها من عشرة فئران:

1. عينة 1 : حث في الفك السفلي بزيت حبة البركة
2. عينة 2 : حث في الفك السفلي بزيت حبة البركة المخلوط بريفونات كولسيوم مكسيم
3. عينة 3 : حث في الفك السفلي بالزجاج الحيوي
4. عينة 4 : بحث في الفك السفلي بالزجاج الحيوي المخلوط بالريفيونات كولسيوم مكسيم

بعد الإجراءات المعقدة التدبيرية اللازمة، تم رصد التغييرات الفيزيولوجية في العينات، حيث تم قياس عدد الأمعاء ومدة التئام العيوب المخلقة بعدcluirية في الفك السفلي.
2. تعرضت جميع الفئران نفس خطوات الجراحة بغض النظر عن حفرة في الناحية اليمنى لعظم الفك السفلي يبلغ حجمه 3 مم.

3. تم ترك المجموعة الأولى بدون أي معالجة (المجموعة الضابطة) ، بينما تعرضت المجموعة الثانية للمعالجة باستخدام هيدروكسيد الكالسيوم مخلوطاً بمحلول ملحى ، وتعرضت المجموعة الثالثة للمعالجة باستخدام هيدروكسيد الكالسيوم مخلوطاً بزيت حبة البركة ، ومعالجة المجموعة الرابعة بمادة الزجاج الحيوي.

4. وكانت مدة التجربة ثلاثة أسابيع للفئران من كل مجموعة ، وستة أسابيع للنصف الآخر.

5. تم رصد النتائج عن طريق أشعة اكس والدراسة المجهرية.

6. تم رصد البيانات وتحليلها باستخدام الأسلوب الإحصائي (تحليل التباين احادي الاتجاه).

نتائج الدراسة

أسفرت النتائج عن وجود فروقات دالة إحصائياً بين المجموعات الأربعة لصالح المجموعة الرابعة بعد المعالجة لفترة ستة أسابيع ، في حين لا توجد فروقات دالة إحصائياً بين المجموعات بعد المعالجة لفترة ثلاثة أسابيع.

التوصيات

نظراً لأن مادة الزجاج الحيوي كانت من أفضل المواد المستخدمة فأنه يوصى باستخدامها في عمليات إعادة بناء عظام الفك ، حيث تعطي نتائج جيدة خلال فترة زمنية قصيرة وذلك مقارنة بالمواقع الأخرى ، إلا أنه من المتوقع استخدام (كالسيوم هيدروكسيد + محلول ملحى أو كالسيوم هيدروكسيد + زيت حبة البركة) في عمليات بناء عظام الفك مع ضرورة توفير فترة زمنية أطول ، حتى يتسنى الحصول على نتائج أفضل ، وذلك لأن هذه المواد لم تثير أو تنشئ أي تفاعلات مضادة.