Histological evaluation of intrabony defect repair induced by white ordinary Portland cement (WOPC)

Atheer A. Ali, B.D.S. (1)
Shatha S. Mohammed, B.D.S., Ph.D (2)

ABSTRACT

Background: recently the ordinary Portland cement (OPC) has been analyzed and compared physically, chemically and biologically to mineral trioxide aggregate (MTA) and because of the similarity between OPC and MTA, so the Possibility of using Portland cement as a less expensive alternative to MTA in dental practice should be considered. In view of this, the Present study is to evaluate the biological response of the jaw bone to intraosseous ordinary Portland cement (OPC) implantation.

Materials and Methods: fifteen local breed adult male rabbits divided into three groups of five rabbits, each rabbit has receive two intrabony defects in the mandible bone, one filled with white ordinary Portland cement (WOPC), the other left empty as a control. The histological sections obtained after 1, 4 and 8 weeks postoperatively. The histomorphometric analysis including counting of bone cells (osteoblasts & osteoclasts), inflammatory cell and observation of the degree of inflammation and the type of bone reaction to OPC material.

Results: there was no significant difference in inflammatory response between OPC group and control group at all period of time, there was significant increase of osteoblasts number at one and four weeks interval of OPC group when compare with the control groups but at eight week there were no significant difference of osteoblasts number between them, control group showed highly significant increase of osteoclasts number at four and eight weeks interval when compare to OPC groups. Most of OPC group and in all period of time showed bone deposition in direct contact with ordinary Portland cement (Type I bone reaction).

Conclusions: As a result we can conclude that the OPC material show high degree of biocompatibility, induce bone healing and act as bioactive material.

Key words: Portland cement, intrabony implantation, bone biomaterial. (J Bagh Coll Dentistry 2011;23(3): 44-49).

INTRODUCTION

Ordinary Portland cement (OPC) was invented in the early 19th century. Since then, it has gained universal popularity with applications covering many different fields, primarily in civil engineering, ordinary portland cement composed of minerals, among which the most important are tricalcium silicate (3CaO.SiO2), dicalciumsilicate (2CaO.SiO2), tricalcium aluminate (3CaO.A12O3), tetracalcium ironaluminate (4CaO.A12O3.Fe2O3) and di-hydrated calcium sulfate (CaSO4.2H2O) which is on hydration produce a silicate hydrate gel(C-S-H) and calcium hydroxide (CH) (1).

In dentistry, Ordinary Portland cement (OPC) had been investigated as a potential alternative restorative material to the presently used materials in endodontics which is mineral trioxide aggregate (MTA) (2). Mineral trioxide aggregate (MTA) an endodontic material used as a viable alternative for various clinical applications, such as capping of pulp tissue, root end closure and for repairing furcal perforations (3).

Wucherpfenning et al (4) reported that both MTA and Portland cement (OPC) seem almost identical macroscopically, microscopically and by X-ray diffraction analysis. Estrela et al (5) investigated the chemical and antibacterial properties of various materials including Portland cement and MTA and found that both cements are constituted of the same elements, except for bismuth that added to MTA to provide the radiopacity. Funteas et al (6) evaluate (15) elements of MTA and Portland cement composition, the results showed similarities between the materials, except for the fact that there was no detectable quantity of bismuth in Portland cement. It was concluded that there was no significant difference between the other (14) elements in both Portland cement and (MTA). Taking into account the low cost and apparently similar properties of (OPC) in comparison to (MTA), so it is reasonable to study the biocompatibility of (OPC), various methods have been suggested to evaluate materials applied in dentistry, according to Shahi et al (7), today there are four classical methods to assess the biocompatibility of a material: (a) invitro cytotoxicity assessment, (b) subcutaneous implants, (c) intraosseous implants and (d) invivo assessment of periradicular tissue reaction in animals. Several invitro studies concerning the biocompatibility of OPC had been conducted (2,8-10) and Few invivo studies (11-13) therefore more
invivo studies are recommended for giving evidence supporting that (OPC) are biocompatible and may have potential to promote bone healing. Accordingly Portland cement may become the base of a viable dental restorative material and possibly a material for orthopedics (2).

MATERIAL AND METHOD
Portland cement: White ordinary Portland cement (WOPC), (105) grade 52.5N. It has been tested for their chemical and physical properties in the national center for laboratory and building Research in (Baghdad). (Figure 1)

Animals: Fifteen local breed adult male rabbits divided into three groups of five rabbits, each rabbit has receive two intrabony defects in the mandible bone, one filled with white ordinary Portland cement (WOPC), the other left empty as a control, these rabbits scheduled for sacrificing after 1.4.8 week postoperatively.

Surgical procedures: The surgical procedures were done under general anesthetic drugs by using atropine sulfate at dose of 0.4 ml / Kg body weight I.M. as a premedication to reduce salivary and mucous secretion, followed 10 minutes later by a mixture of ketamin hydrochloride 10% and xylazin 2% at a dose of 0.5,0.2 ml / Kg body weight respectively I.M. these were injected into the rear limb-thigh muscle of the rabbits. Application of eye ointment to prevent dryness of the cornea, Lidochange hydrochloride 2% weight and adrenaline 1:80,000 was infiltrated submucosally along the planned surgical site (intraorally) (14). An incision was made along the alveolar crest in the naturally edentulous space between the incisors & premolar teeth in the mandibular arch (lower diastema) and by using slowly running hand piece(800rpm) with round bur (no.012) cooled by a continuous stream of sterile normal saline, we perform the orifice in the bone ,then with fissure bur (no.012) the cavity deepened to hold the implanted material ,the size of the cavity approximately 3mm in diameter and 3mm in depth(15). The first hole (anterior) filled with white Portland Cement which is mixed with distilled water by ratio of 1:3 (w/c) and applied by using amalgam carrier fig(2), The second hole (posterior) remain empty as control, these two holes were separated by approximately 4mm ,the surgical flaps were reaproximated with resorbable sutures.

Histological Preparation: After sacrificing of rabbit ,the right diastema resected and dissected into two segment (control and experimental) ,the specimens fixed in 10% buffered formalin for 48 hours then subject to decalcification with solution of 10% formic acid for (1-2 week) until satisfactory decalcification is obtained then we perform embedding ,sectioning and staining with heamatoxiline and eosin stains and Van-gieson stain.

Histopathological observation: Performed by two histopathologists in a blind manner. The defects and the adjacent related area of both the control and experimental specimens were examined. In each defect five separated field within high-power of magnification (40X) were taken for cell counting, and the microscopical findings include, counting of cells (inflammatory cells and bone cells (osteoblasts and osteoclasts)), Histopathological evaluation of bone apposition , neovascularity, type of material reaction with the bone and degree of inflammation were assessed.

Statistical analysis: We find the; mean, standard deviation of cell number and the significant of difference between the groups (P-value of t-test). (Table 1-3)

RESULTS
The Specimens were harvested in three periods:
1. One week
2. Four weeks (one month).
3. Eight week (two months).

The histological examination shows the following findings:

After one week the histological finding of control bony defect shows an early stage of bone healing. The defect was filled with collagen fibers, large number of fibroblast and new blood vessel with moderate degree of inflammatory response (Figure 3), while experimental defect appears empty spaces because Portland cement removed by decalcification process, but there is a few amount of loose fibrovascular tissue found at the periphery and within the WOPC material which contains a number of blood vessels, fibroblast cell, inflammatory cells and few bone marrow spaces, the inflammatory response is moderate Osteoblasts are present at the periphery of the defect on bone surface. (Figure 4).

After four weeks the histological findings of control bony defect shows formation of bone trabecula. The space between bone trabecula was filled with the cartilaginous callus with mild degree of inflammatory response,osteoblasts present at the periphery of bone trabecula and osteoclast also seen at this period (Figure 5), while experimental defect shows bone deposition around the defect and formation of new bone trabeculae and bone sequestrum within the defect few fibrovascular tissue also present ,the inflammatory reaction is mild large number of osteoblasts present at the periphery of bone.
defect, osteoclasts is not detected at this period (Figure 6)

After eight week the control defect filled with lamellated bone with few spaces, the spaces filled with little amount of collagen fiber and few fibroblast with scant inflammatory cells, osteoblasts present at the periphery of bone trabeculae with few number of osteoclast (Figure 7), the while experimental defect shows continuous deposition of bone around the implanted material (WOPC) in form of lamellae and bone ingrowth toward the core of implanted material fibro vascular tissue still present at the periphery of defect, the inflammatory reaction is very mild, Lesser number of osteoblast then that of second period were present at the periphery of bone defect and new bone trabeculae, osteoclast is rarely detected at this period (Figure 8). More than 50% of cases of experimental group among these period of time show direct contact between bone and WOPC.

DISCUSSION

Various methods have been suggested to evaluate materials applied in dentistry. Intraosseous implantation used to evaluate material applied specifically for endodontic or intended for prolong contact with the bone. International Standard Organization (ISO) recommends bones as Tibia, femur and the mandible of laboratory animals for material implantation investigation and among small animal's rabbits, rats, guinea pigs, and cats are more popular. In present study the rabbit used other than rodent because small rodents have primitive bone structures and do not have haversian systems. Whereas rabbits, as well as dogs, have haversian systems that are similar to that of man, which is an important advantage in terms of extrapolation of results obtained with such animals for human bone repair. And unlike rodents, the rabbit's size allows multiple collections from the same bone for testing biomechanical or histopathological properties.

In this study we evaluated the bone healing following the implantation of WOPC in experimentally created intrabony defects in mandible of rabbits. It should be mentioned that the white color of the applied Portland cement reject the possibility of its tissue tattooing in endodontics. Portland cement composed mainly of tricalcium silicate and dicalcium silicate which on hydration produce calcium silicate hydrate gel and calcium hydroxide. Portland cement has been shown to have similarities to dental materials (mineral trioxide aggregate MTA, calcium hydroxide Ca(OH)₂) used in endodontic treatment for the repair of perforations, pulpotomies and retro-fill preparations.

Few studies conducted concerning the bone interaction with Portland cement after intraosseous implantation, implant Portland cement in mandible of guinea pigs, use dogs mandible and in study OPC included in the skull of rats, all of these studies supported the findings of the present investigation.

At the end of the 1st week postoperatively, Both of control and experimental groups shows moderate degree of inflammation and this degree reduced with time (4,8 weeks) the inflammatory cells measurement showed no statistical significant difference (P>0.05) between the experimental group and the control at all interval and Inflammatory response observed during the first few days after surgery in all groups seemed to be related to the surgical trauma and it has been cited by other authors.

In present study the high alkaline pH levels of WOPC paste seemed to induce low grade irritation to the surrounding tissue without harmful effect like foreign body reaction or bone necrosis. This result agree with who tested an accelerated Portland cement (APC) invitro by observing the cytomorphology of human osteosarcoma cells (SaOS-2 cells) which is "represent a highly differentiated cell line capable of inducing bone formation and are thus a model for osteoblastic behavior.

In this study we counted the bony cells (osteoblasts, osteocytes & osteoclasts) determined the level of the bone formation. At the 1st week, the t-test showed very high significant difference between the control and experimental groups (P<0.001) in osteoblasts number, the experimental groups showed large number of osteoblast cells cover the implanted material. This reaction was not observed in the normal healing process in control group, this finding suggest that WOPC seemed to induce the bone healing by supporting the proliferation and adhesion of bone-forming cells (osteoblasts), this is may be attributed to their structural characteristic and mode of action. These findings supported by who reported that MTA and Portland cement had similar properties, both of them offered a biologically active substrate for bone cell and this could be attributed to their ability for allowing good adherence and proliferation of the cells, after four weeks the t-
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While the formation of fibrous capsule in few cases might be due to the insertion of material was not in close contact with host bone or possibly due to different animal individual reveals different reaction.

REFERENCES


Figure 1: White ordinary Portland cement

Figure 2: Two holes were made the anterior filled with Portland cement the posterior remain empty as control.
Table 1: Statistic analysis for cell counting at one week interval

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<th>Group</th>
<th>Control</th>
<th>Experimental</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Inflammatory cell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>102.6 ± 31.6</td>
<td>82.8 ± 15.6</td>
<td>0.23</td>
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<tr>
<td>Moderate</td>
<td>Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoblast cell</td>
<td>0.00 ± 0.00</td>
<td>21.8 ± 7.15</td>
<td>0.000***</td>
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Table 2: Statistic analysis for cell counting at four weeks interval

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<tr>
<td>Inflammatory cell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>49 ± 12.8</td>
<td>38.4 ± 9.2</td>
<td>0.171</td>
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<tr>
<td>Mild</td>
<td>Mild</td>
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<td></td>
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<tr>
<td>Osteoblast cell</td>
<td>27.2 ± 3.8</td>
<td>35 ± 6.12</td>
<td>0.04*</td>
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<tr>
<td>Osteoclast cell</td>
<td>1.6 ± 0.5</td>
<td>0.00 ± 0.00</td>
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Table 3: Statistic analysis for cell counting at eight weeks interval

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<td>Inflammatory cell</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Degree of inflammation</td>
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<tr>
<td>Mild</td>
<td>Mild</td>
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<tr>
<td>Osteoblast cell</td>
<td>19.6 ± 5.9</td>
<td>23.2 ± 8.19</td>
<td>0.4</td>
</tr>
<tr>
<td>Osteoclast cell</td>
<td>3.2 ± 1.09</td>
<td>0.16 ± 0.16</td>
<td>0.000***</td>
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