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

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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, 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 received 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 compared 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 compared 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 shows high degree of biocompatibility, induce bone healing and acts as bioactive material.

Key words: Portland cement, intaosseous implantation, bone biomaterial. (J Bagh Coll Dentistry 2011;23(1):22-27).

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), dicalcium silicate (2CaO.SiO2), tricalcium aluminate (3CaO.Al2O3), tetracalcium iron aluminate (4CaO.Al2O3.Fe2O3) and di-hydrated calcium sulfate (CaO.SO3.2H2O) 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) intrasosseous implants and (d) invivo assessment of periapical tissue reaction in animals. Several invitro studies concerning the biocompatibility of OPC had
been conducted\(^{(2,8,9,10)}\) and few invivo studies\(^{(11,12,13)}\), therefore more invivo studies is 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\(^{(19)}\).

**MATERIALS AND METHODS**

**Portland cement:** White ordinary Portland cement (WOPC), (197-1 CEM I) 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 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, Lidocaine hydrochloride 2% with 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.010) 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 (Figure 2). The second hole (posterior) remain empty as control, these two holes were separated by approximately 4mm ,the surgical flaps were reapproximated with resorbable sutures.

**Histological Preparation:** After sacrificing of rabbit, the right diastema resected and dissected into two segment (control and experimental). The specimens were fixed in 10% buffered formalin for 48 hours then subjected to decalcification with solution of 10% formic acid for (1-2week) until satisfactory decalcification is obtained then we perform embedding, sectioning and staining with hematoxiline and eosin stains and Vangieson 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, 2, 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 trabeculae. The space between bone trabeculae was filled with the cartilaginous callus with mild degree of inflammatory response, osteoblasts present at the periphery of bone trabeculae 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 fibrovasecular 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 than 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.

Figure 1: White ordinary Portland cement
Figure 2: Two holes were made the anterior filled with Portland cement the posterior remain empty as control.
Intraosseous implantation used to evaluate materials 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 CaOH) 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 intrasosseous implantation, implanted Portland cement in mandible of guinea pigs, use dogs mandible and in study by 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

**DISCUSSION**

Various methods have been suggested to evaluate materials applied in dentistry. Intraosseous implantation used to evaluate materials 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.

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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 (2) 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."(28) with the presence of the APC and the effect of this material on the expression of bone remodeling markers, demonstrated evidences that these materials are non-toxic does not cause cell death and may have potential to promote bone healing.

Counting the bony cells (osteoblasts, osteocytes & osteoclasts) determined the level of the bone formation (26). 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 are supported by Saidon et al (11) 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-test showed significant difference between the control and experimental groups (p<0.05) with greater number in experimental group than that of control group but at eight week interval the osteoblast mean number in both groups decrease than that of (second period), the t-test showed there is no significant difference between the control and experimental groups (p=0.4), This is in agreement with (27) who showed as time elapsed there were reduction in the production of calcium hydroxide as a by-product of cement hydration reaction thus affect the proliferation of bone-forming cells (osteoblasts) so there is reduction of these cells number. Control group showed highly significant increase of osteoclasts number at four and eight weeks interval when compare to WOPC groups (p<0.001), this is indicating that the WOPC material is not resorbable and decreasing in size due to biodegradation, this is compatible with (28) who documented that postoperative radiographs taking to root perforation of dogs teeth sealed with OPC and MTA after 90 days revealed that both of them were totally or partially dissolved in some cases with new cementum formation on root perforations of dogs’ teeth.

In more than 50% of cases of WOPC intraosseous implantation there were new bone appositions in direct contact with Portland cement, this is supported by (29) who investigate the interactions between white Portland cement(WOPC) paste and simulated body fluid (SBF) invitro and conclude that exposure to SBF has been found to promote the precipitation of a layer of ‘bone-like’ hydroxyapatite on the surface of WOPC paste these results showed that WOPC was a bioactive material which when came in contact with tissue fluid, it would release an abundance of Ca$^{4+}$ and OH$^{-1}$ which were react with PO$_4$$^{-3}$ that present in tissue fluid leading to the formation of hydroxyapatite crystals [Ca$_{10}$(PO$_4$)$_6$(OH)$_2$]; this layer develops strong direct chemical bond with bone, therefore the osteogenic activity of OPC was attributed to this dissolution-precipitation reaction. Also the direct bone contact with Portland cement supported by (30) who thought that the deposition of hydroxyapatite layer onto the Portland cement paste surface is attributed to both the dissolution of calcium hydroxide and to the high proportion of preexisting Si-OH nucleation sites presented by the nanoporous calcium silicate hydrate gel structure of WOPC and indicate that the likely mechanism of bonding between WOPC paste and viable bone tissue is the spontaneous formation of an intermediate layer of hydroxyapatite on contact with human plasma.

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


