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IN VIVO BIOCOMPATIBILITY OF β TRI-CALICUM PHOSPHATE VERSUS WHITE PORTLAND CEMENT IN MANDIBULAR BONE SURGICAL DEFECT IN DOGS

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ABSTRACT

Back ground: Synthetic bone substitutes have been advanced, and researches are developed to more effective biomaterials with promising ability to reconstruct lost bone structure. Tricalcium phosphate biomaterials and white Portland cement are among them. The aim of the current study is to assess the effect of β-tricalcium phosphate versus white Portland cement in surgically mandibular bone defect dogs.

Material and methods: Six adult dogs were used. The anaesthesia was conducted by iv injection of thiopental sodium 20mg/kg.b.wt. and maintained using (atropine 0.04 mg/kg b.wt., xylazine 1mg/kg.b.wt. and diazepam 1mg/kg.b.wt.in saline iv drop by drop infusion), three critical size defects were designed at the buccal side of the mandible. The defects were 5 cm apart from each other. One defects filled with sterile white Portland cement, the middle defect filled with β-tricalcium phosphate and the later one was left empty. The dogs were euthanized at the end of 1 and 8 week postoperatively respectively. The specimens were prepared for light microscope using H&E and Masson Tri-chrome staining.

Results: The experimental sides of all periods revealed increasing the amount of new bone trabeculae extended from original bone towards the center of the defect with both white Portland cement & β-tricalcium phosphate. While, the control sides displayed few new bone trabeculae at central area and the lateral wall of the bony defects. The amount of new bone trabeculae confirmed the histological results.

Conclusion: White Portland cement is a biocompatible osteoconductive economically affordable graft material and it id more efficient than β-tricalcium phosphate.

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INTRODUCTION

Abbrivation: β-tricalcium phosphate (β-TCP), white Portland cement (WPC)**,** Portland cement (PC)**,** Mineral trioxide aggregate (MTA), Masson-trichrome staining (MT).

Alveolar bone is a specialized part of the mandible and maxilla that forms the primary support structure for teeth. Although it compared to other bone tissues in the body, alveolar bone is exposed to constant and rapid remodeling associated with tooth eruption and consequently the functional demands of mastication. The anatomy of alveolar bone, which is relatively complex, has been described in detail [1].

Alveolar bone is composed of bundle bone, which is fashioned in layers in a parallel alignment to the coronal-apical direction of the tooth. Sharpey's fibers boost obliquely from the thin lamella of bone that outlines the socket wall and are continued with fibers of the periodontal ligament. A thicker outer layer of bone formed of cortical plates covers the jaw bone and modes the lingual and labial surfaces of the alveolar process and is built up mainly of spongy cancellous bone. In the cancellous bone numerous marrow spaces, with smaller endosteal spaces exist in the cortical bone. Some of the small endosteal spaces broaden into, and are attached with, the periodontal ligament [2].

Effective repair of bone deficiencies in the cranio-facial skeleton, in case of trauma, tumor resection, or congenital disorders, continues to be a major concern to reconstructive surgeons. Bone grafting is a surgical replacement the missed bone with substantial from the patient's own body [3].

The benefit of autologous bone grafts is considered to be the excellent standard for repair and reconstruction of bone. It has major impediment, inadequate amount, donor site morbidity, and unfortunate form [4].

Researches in alternative bone grafts such as allografts (tissue from other person of the clone species) and xenografts (animal tissues). They are attractive sources, there are several problems encountered in using them including hazard of disease transmission, immunogenic response, and variable quality associated with allografts, impress the necessity for alternative approaches [5].

Significant efforts are being made to develop ideal synthetic bone graft substitutes. The ultimate circumstances of a bone graft are determined by three parallel phenomena, namely osteoconduction, osteoinduction, and osteogenesis [6]. In osteoconduction, the graft material furnishes a scaffold for osteoblasts to grow on its surface. Osteoinduction stimulates differentiation of primitive, undifferentiated, and pluripotent cells to osteoblasts. Osteogenesis develops when graft material has viable osteoblasts adept in repair of the bony defect [7].

The remodeling process for osteoconductive materials is well described. Different osteoconductive materials lead to discrepancy rates of remodeling with secondary inflammation and fibrosis at the remodeling sites [8]. Osteoconductive materials when placed in well vascularized location with influence of medullary elements have copious access to growth factors and proteins important to bone production and remodeling as well as osteoprogenitor cells [9]. There have been many studies explored the aptitude of osteogenetic biomaterials. However, the difference in reparative response of bone affected by osteoconductive properties of particular synthetic and natural materials has not been thoroughly described.

Calcium phosphate biomaterials such as hydroxyapatite and tricalcium phosphate are frequently applied biomaterials because they are significantly biocompatible, osteoconductive and gradually resorbed and replaced with bone. Two major distinct phases of anhydrous TCP crystals exist: α-TCP and β-TCP phases [10].

Kihara et al [11] investigated biodegradation process of TCP particles and new bone formation in a rabbit cranial defect model and established that TCP particles are osteoconductive and space-maintaining.

β-TCP is osteoconductive material acting as space maintainer for bone formation. This biomaterials would be considered auspicious and cost-adequate in maxillofacial bone regeneration, in periapical, periodontal and peri-implant bone defects as well as reduced alveolar bone volume for prosthetic rehabilitations [12].

MTA, a Portland cement-based material, has various endodontic applications. MTA's propriety in the treatment of non-vital immature teeth has been investigated [13]. The principal ingredients and the total of arsenic in PC as well as its bio-compatibility and physicochemical behavior, are similar to those of MTA. The setting time of PC can be shorted by eliminating gypsum from manufacturing process without affecting other properties [14].

Abdullah et al [15] & Bidar et al,[16] prepared PC with they investigated the biocompatibility of PC in vitro by noticing the cytomorphology of SaOS2 (human osteosarcoma cells) in the presence of PC and its effect on the expression of markers of bone remodeling. They ensued that APC is non-toxic and may have prospective to enhance bone healing.

In the present study, we analyzed the histological and cellular events in bone regeneration in response to implantation of TCP, PC into experimentally designed intra-bony defects in dog mandibles. Moreover, we aimed to compare these materials with each other in terms of osteogenesis.

MATERIALS AND METHODS

Animals

Six 2-year-old mongrel dogs with intact dentition and good periodontium were selected (about 20 kg in weight). The dogs were evaluated thoroughly in teaching veterinary clinic observed for 14 days preoperatively for free of any diseases. The dogs were housed in separate cages in the animal house (Faculty of Veterinary Medicine, Department of surgery, anaesthesiology and radiology, South Valley University) and maintained according to guide for care and practice of laboratory animals. The protocol of the study was performed and carried out according to the guidelines of ethical committee of Faculty of Medicine South Valley University.

The animals were starved for 12 hours before anaesthesia. The anaesthesia was induced by iv injection of thiopental sodium 20mg/kg.b.wt. and maintained using (atropine 0.04 mg/kg b.wt, xylazine 1mg/kg.b.wt. and diazepam 1mg/kg.b.wt. in saline iv drop by drop infusion) through cannula.

Materials

1. Sterile powder of WPC (Oasis White Portaland Cement 11/B-L42.Sn Helwan Cement Ital Cement Group). WPC powder was sterilized by autoclaving according to Ravi et al[17] temperatur 134° for 15 minutes in air tight closed bottle.
2. Iceber β-TCP (GMI iler implant group, Espania) is a porous β-TCP synthetic graft in a granular form with a particle size of size 300 - 500 microns.

Experimental design

The operative site was prepared using povidone iodine and infiltration anesthesia was fulfilled using 2% mepivacaine with adrenaline for hemostasis, using scalpel blade No 15 vestibular incision was designed 2 cm in length and the mucoperiostium was reflected and three critical size defects were designed at the buccal surface of the mandible 4 mm away from the lower border.

The defect is 6 mm in diameter and 6m in depth. It is created by trephination bur under copious saline irrigation. The defects were 5 cm apart from each other[18] (Fig.1A).

Each dog had 3defects on the buccal surface of the mandible that are designed as follow:

Group I

Defects at left side were left empty.

Group II

Defects at the middle were filled with β-TCP

Group III

Defects at right side were filled with sterile WPC

The wounds were closed using 3-0 vicryl. Penicillin and streptomycin veterinary preparation was injected twice for 3 days for prophylaxis against infection. Also voltarin was used as analgesic as it is injected twice daily for 3 days.

The dogs were kept on soft diet in assemble of bread and milk for a week and dry foods were introduced freely to the dogs, the dogs were cared, and the wound was examined for any swelling, infection and dehiscence.

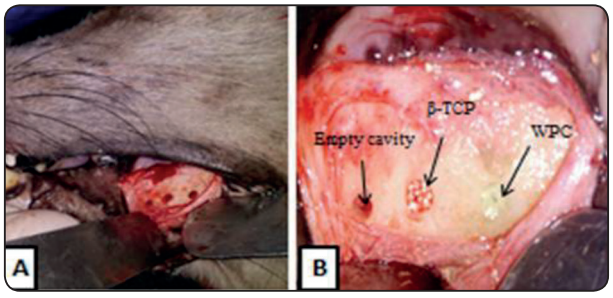

Fig. (1): A. Three equal defects at the buccal surface of the mandible near lower border B. The defects filled with test materials

Three dogs were sacrificed after 1st and 8th week postoperatively respectively. On scarification of the dogs, ketamine was injected and the mandibular bone segments were osteotomized for histologic examination.

Light microscopic study

Biopsies were fixed in 10% buffered formalin for 24 h. Specimens were decalcified in 10 % EDTA. The Specimens washed in tap water over night and then dried out in ascending grades of alcohol, cleared in xylene and then implanted in low melting point (56˚C) paraffin. Serial sections of 5 um thickness were cut down with rotary microtome (lyca) and then processed for H&E and MT staining, then were observed under the light microscope [19].

Statistical histomorphometric analysis of newly formed bone filling the defect:

After processing and staining of MT staining, images were analyzed for quantitative measurement of newly formed using the ImageJ analysis system. All statistical analyses were performed using the (ANOVA) followed by t-test. All values were expressed as means and standard deviations. All statistical analyses were done on an IBM PC using the statistical software "SPSS 20". Results were expressed in the form probability value (p-value) that was differentiated into: non-significant when p-value > 0.05, significant when p-value ≤ 0.05, highly significant when p-value ≤ 0.01, very highly significant when p-value ≤ 0.001.

THE RESULTS

The postoperative periods passed smoothly, the post-operative oedema was slight and gradually disappeared, and the healing of the wound was eventful (Fig.2).

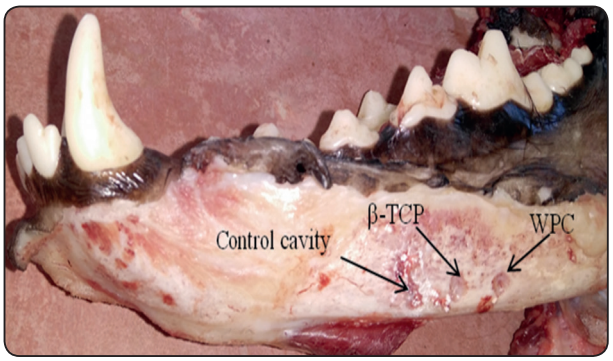

Fig. (2): Healing of the test materials

Light microscopic results

1. H &E Staining:

1.1. Control group:

At the end of 1st week, a fibrous tissue occurred around at the edges of the defect, granulation tissue was appeared filling the bony defect. A combination of spindle cells with elongated nuclei and small rounded cells with large nuclei were detected in the connective tissue. There was evident osteoclastic activity (Fig. 3 A,B).

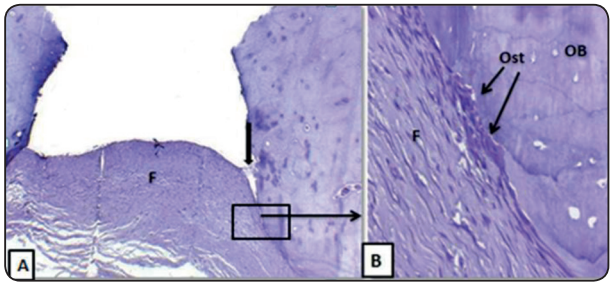

Fig. (3): Photomicrograph of a group I (control gp.) taken at the end of 1st week F: Fibrotic tissue growth, moderate Inflammation (arrow). A. Defect occupied with fibrous tissue (F) along with a clear edge of native bone. B. High magnification of figure A, F: Fibrotic tissue growth, and Ost: Osteoclastic activity, OB: Old bone. A combination of spindle cells, elongated nuclei and small rounded cells with large nuclei were detected in the connective tissue. Osteoclastic activity was evident. (H&E A ×10, B×40).

At the end of 8th week, few new bone trabeculae were observed at central area and the lateral wall of the bony defect, they were lined by active osteoblast with areas of osteoclastic activity. Large areas of CT were noticed (Fig. 6 A,B).

1.2. β-TCP group:

At the end of 1st week, moderate inflamed high cellular CT with collagen fibers and woven bone that is molded at the boundaries of the defect areas in contact with old bone, numerous blood vessels colonizing the granulation tissue, reversal line separating old bone from new bone (Fig. 4 A,B).

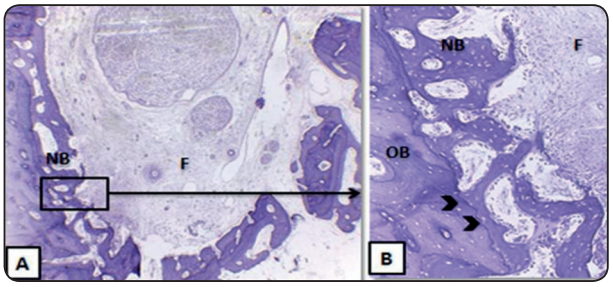

Fig. (4): Photomicrographs of a group II (β-TCP gp.) taken at the end of 1st week F: Fibrotic tissue growth, New bone (NB). A. Woven bone observed at the boundaries of the defects. The defect was full with fibrous tissue. B. High magnification of figure A. Woven bone assembled at the boundaries of the defect areas in contact with old bone, numerous blood vessels colonizing the granulation tissue, reversal line (arrow head) separating old bone (OB) from new bone (NB) (H&E A ×10, B×40).

At the end of 8th week, new woven bone were observed at the boundaries of the defects in contact with old bone, filling large areas of the bony defect, with variable sizes of bone marrow cavities. New bone trabecula have mature osteocytes in their lacunae (Fig. 7 A,B).

1.3. WPC group:

At the end of 1st week, abundant woven bone assembled at the boundaries of the defect areas. New bone trabecula surrounded by CT and large blood vessels. There were active osteoblasts lining the new bone (Fig. 5 A,B).

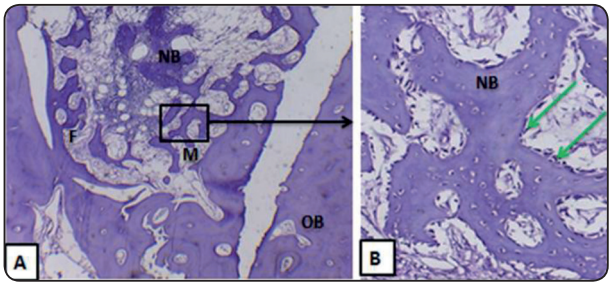

Fig. (5): Photomicrographs of a group III (WPC gp.) taken at the end of 1st week F: Fibrotic tissue growth, New bone (NB), Old bone (OB), Bone marrow (M). A. Abundant woven bone assembled at the boundaries of the defect areas. B. High magnification of figure A. NB trabecula surrounded by CT and large blood vessels, active osteoblast (green arrow) lining the NB. (H&E, A ×10, B×40).

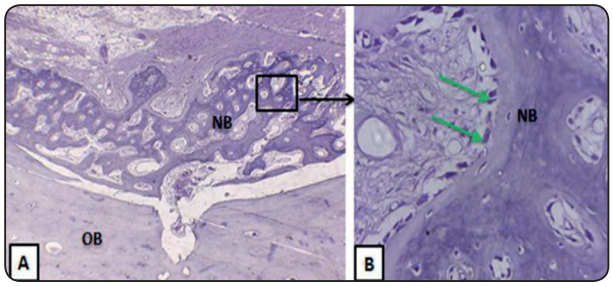

Fig. (6): Photomicrograph of a group I (control gp.) taken at the end of 8thweek A. shows Few bone trabecula (NB) at the center of bone defect. Old bone at extermity of the wound (OB). B. High magnification of figure A, NB surrounded is formed, active osteoblast (green arrows) lining the NB. (H&E, A ×10 ,B×40).

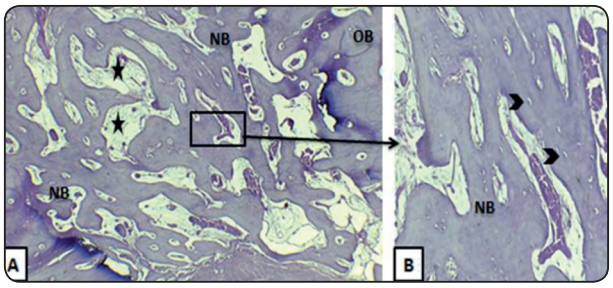

Fig. (7): Photomicrographs of a group II (β-TCP gp.) taken at the end of 8thweek A. shows new woven bone were observed at the boundaries of the defects (NB) in contact with old bone, filling large areas of the bony defect, with variable sizes of bone marrow cavities (stars). B. High magnification of figure A. New bone trabecula (NB) with mature osteocytes in their lacunae (arrow head). (H&E A ×10, B×40).

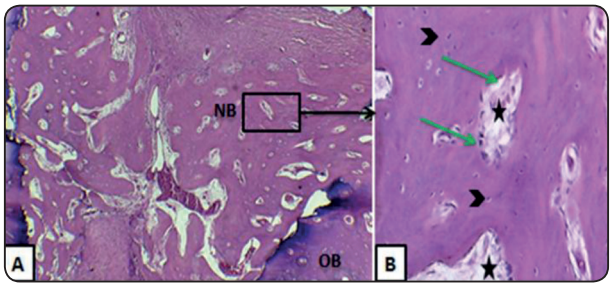

Fig. (8): Photomicrographs of a group III (WPC gp.) taken at the end of 8thweek shows A. well organized large bone trabecula (NB) filling large areas of the bony defect, the most mature one near the old bone (OB). B. Higher magnification of figure A. Mature bone trabecula with large Harversian canal (star), New bone trabecula lined by active osteoblast (green arrows). Mature osteocytes in their lacunae (arrow head). (H&E orig. mag., A×10,B×40).

At the end of 8th week, well organized bone trabecula were observed filling large areas of the bony defect, they were lined by active osteoblasts. Large bone marrows were noticed filled with C.T and large blood vessels. New bone trabecula lined by active osteoblast (Fig. 8 A,B).

2. Masson's Trichrome staining

Masson trichrome staining of all the samples presented a red color for cytoplasm, scattered with bits of blue for the bone, which verified wide range of bone tissue in the form of collagen. Collagen was distributed around the bone lacunae.

2.1. ***At the end of 1st week,*** (control group) we found lots of blue-staining fibrous tissues, chronic inflammatory cells were detected to infiltrate all defect areas, coarse-fibered CT. There were few bone spicules (Fig 9A). In β-TCP group, collagen fibers randomly arranged representing an embryonic bone. There was initial bone neoformation (Fig. 9B). In WPC group, the surgical cavity was partially repaired, with incomplete formation of new cortical bone (Fig. 9C).

2.2. ***At the end of 8th week,*** (control group) the surgical cavity was repaired with the reorganization of the bone marrow (Fig. 9D). In β-TCP group, there were bone trabeculae organized (Fig. 9E). In WPC group, the cortical bone was completely repaired, with thick trabeculae are observed and the small connective tissue area (Fig. 9F).

Statistical quantitative evaluation of the newly formed bone:

At 8th week, experimental side reported the highest mean value of quantitative measurement of newly formed bone with the lowest value in control side at 1st week. Analysis of variance (ANOVA) test revealed a highly significant difference between control & experimental side (p-value < 0.001). In addition to, experimental side in the two periods was showed higher mean value than their corresponding in control side (tab. 1) & (fig. 10).

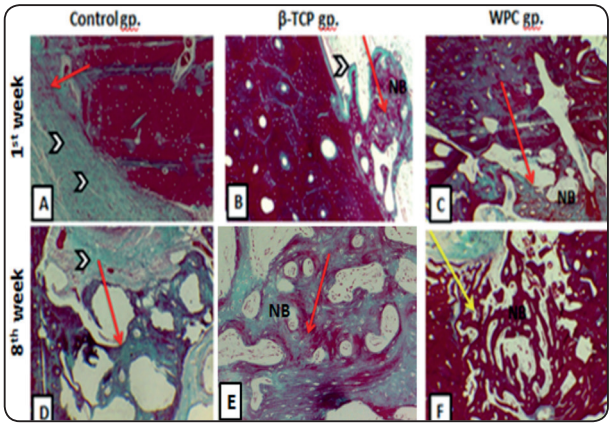

Fig. (9): (A-C), (D-F) representative histological sections of the end of bone defect with Masson's Trichrome staining in (control group, β-TCP group, WPC group after 1st, 8th week Chronic inflammatory cells were detected to infiltrate all defect areas (arrow head). Red arrows indicate the immature bone tissue (NB), and yellow arrows indicate the mature bone tissue. (MT.×20).

TABLE (1): Statistical quantitative analyses of newly formed bone in control and experimental side (βTCP, WPC) at the 1st & 8th week.

		Range	Mean ± S. D	F. test	p. value		
1st week	**Control group**	115 – 133	123.89 ± 5.88	190.427	0.001*	P1	0.001*
	β -TCP group	152 – 176	161.00 ± 7.40			P2	0.001*
	WPC group	310 – 332	321.89 ± 8.22			P3	0.001*
8th week	**Control group**	187 – 206	196.22 ± 5.54	205.864	0.001*	P1	0.001*
	β -TCP group	237 – 266	251.33 ± 9.17			P2	0.001*
	WPC group	381 – 415	399.67 ± 11.53			P3	0.001*

**** Significant***

P1: Control group & β -TCP group

P2: Control group & WPC group

P3: β -TCP group & WPC group

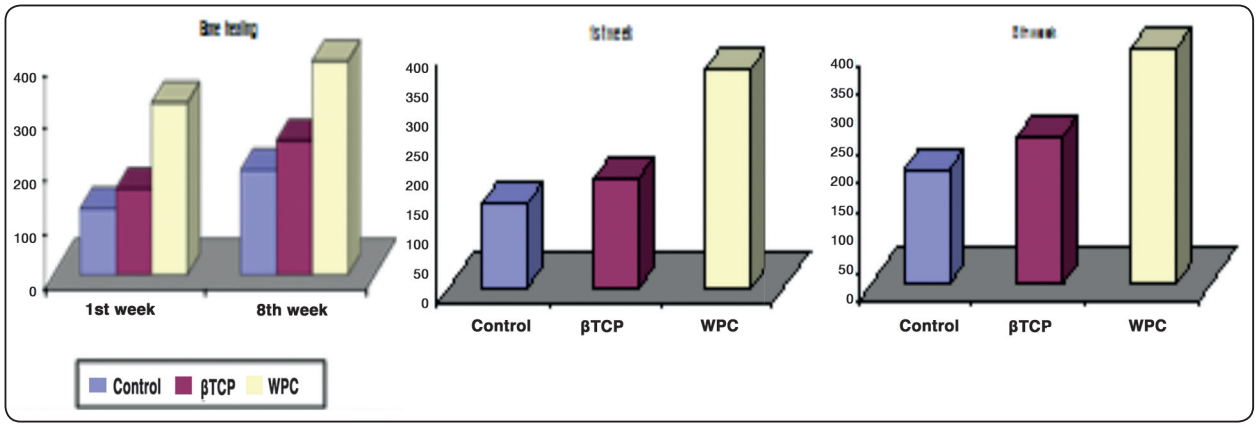

Fig. (10): Column chart representing mean of the quantitative measurement of newly formed bone in control and experimental side (βTCP, WPC) at the 1st, & 8th week.

DISCUSSION

The present study evaluated the biocompatibility and the bone healing effectiveness following the WPC graft material versus βTCP in experimentally created intra bony defects in dog's mandible. In this study the animals were euthanized for evaluation at 1 and 8 weeks after surgery, this research model was selected to be safe for the preservation and quality of animal's life up to the end of the evaluation period. This type of model was used by different experimental research [20]. Lower border of the mandible was selected due to ease accessibility and the reasonable size[21].

With the purpose of improving the osteogenic potential of β-TCP porous ceramics, we evaluated bone enhancement of porous scaffold in bone defect models. Absorptive scaffolds have been studied for decades, the uniform pore architecture of scaffolds looks a significant challenge in inducing the growth of new tissue in the way of natural bone acts [22,23]. The architectonic pattern of natural bone can be described as having a stiff, dense external layer of cortical bone, transitioning to a delicate and spongy cancellous bone internally.

There have been establishing a fabrication method to create a kind of scaffold that is both graded and highly interconnected. Graded porous scaffolds have been verified to be more efficient compared with uniform pore construction in osteogeneses[24, 25].

White Portland cement (WPC) was tested in the last years in different researches. De Deus G et.al.,[26] investigated the Cytotoxicity of PC on human endothelial cells. Limited studies have been born out in vivo. Saidon J. et. Al[27], examined the cell and tissue attitude to PC, while Fabiano S. et. al,[28] evaluate two type of PC in the skull of rats. Therefore, further research should concentrate on the in vivo situation to test the biocompatibility and the possibility of bone healing ability of WPC.

This research examined the histological process of healing and the quantitative measurement of new bone and cell counting of in vivo biocompatibility testing of graft biomaterials. The histology is the most powerful method to evaluate the healing of bone defects, and considered a direct method for testing the biocompatibility[29] .

After one week the control defect, histologically showed defect area occupied by fibrous tissue with a clear edge of native bone. Combination of spindle cells, elongated nuclei and small rounded cells with large nuclei were detected in the connective tissue. Osteoclastic activity was evident. MT staining revealed blue-staining fibrous tissues, chronic inflammatory cells were also detected to infiltrate all defect areas, coarse-fibered CT. There were few bone spicules. The amount and size of bone trabecula were increased at the 8th weeks at the center of the bone defect. The rate of bone formation in control defect was delayed, as only few new bone trabeculae were observed at central area and the lateral wall of the bone defect. The surgical cavity was repaired with the reorganization of the bone marrow (MT staining).

These bone trabeculae were limitedly increased in the 8th week. This delaying in bone healing in control side was confirmed by other studies [30,31] .

Our histological analysis of β-TCP (at the end of 1st week) revealed moderate inflamed high cellular CT with collagen fibers and woven bone that is formed at the boundaries of the defect areas in contact with old bone, numerous blood vessels colonizing the granulation tissue, reversal line separating old bone from new bone. At 8th week, new bone trabecular (filling large areas of the bony defect) have mature osteocytes in their lacunae. MT staining in 1st week showed collagen fibers randomly arranged representing an embryonic bone. There was initial bone neoformation. Bone formation became well organized at end of 8th week.

It is proven that inflammation is a significant element of the host response to biocompatible materials in both acute and chronic periods. Osteoclasts and macrophages are two cell types comprising the major components of the cellular host response that cause the formation of fibrous tissues in place of viable bone tissue[32]. Earlier investigations have revealed that β-TCP granules induce controlled levels of membrane lysis through dissolution of their surfaces. This effect results in high phosphate and low calcium levels, increasing macrophage adhesion behind viable osteoblast adhesion[33] . Previous studies have also recognized that purified β-TCP gives early bone conduction, followed by its resorption and replacement with the newly-formed bone[34].

Although bone tissue in scaffolds provided an uneven distribution and different steps of bone maturity, the structure of the mature part was accustomed with the natural bone in the control group, such as typical lamellar bone blended with woven bone, osteocytes, and Haversian canals.

The histopathological observation of WPC (at the end of 1st week) indicated new bone trabecula surrounded by CT and large blood vessels, active osteoblasts lining the new bone. At end of 8th week, well organized large bone trabeculas were filling large areas of the bone defect. Mature osteocytes in their lacunae. MT staining revealed, the surgical cavity was partially repaired, with incomplete formation of new cortical bone at end of 1st week. In 8th week, thick bone trabeculae are observed.

This finding is in favor of new bone growth in instant contact with the residual space of APC graft material. These results are in agreement with the results obtained by Saidon et al[35] who reported that in more than 50% of cases, there were new bone construction in direct contact with Portland cement.

All result of this study showed a pattern of bone formation in WPC similar to the result of other studies testing many grafts[36,37]. This pattern of bone formation which start from the wall of the bone defect toward the center, suggests that the WPC material is biocompatible and osteoconductive. In osteoconduction the material promotes an alkaline pH and releasing of calcium and phosphate elements that stimulate the calcification process, with absence of foreign body reaction and account for the basic mechanisms of physic mchemical healing of hard tissue[38]. At present several researches establish that WPC is similar to some commercially available graft material in its basic composition, physical, chemical characteristics, and in biocompatibility[39].

Histomorphometric analysis used MT staining to verify the light microscopic findings. All the experimental phases analyzed in this study showed new bone formation, There were a statistically significant differences between the experimental and the control sides in the amount of new bone formation. At the 8th week of WPC, the highest mean value of quantitative measurement of newly formed bone was reported in the experimental group with the lowest value on the control sides at the 1st week. Shayegan et al,[40] had the similar result of pulpal tissue repair with hard tissue formation when they used white MTA, WPC and Beta tricalcium phosphate on pulpotomized primary teeth of pigs.

The histopathologic evaluation of the regenerates confirmed comparative results in case of applying both biomaterials β-TCP & WPC. Thus it is reasonable to assume that under the present study parameters, the healing of defects is largely driven by the release of porous materials which is able to stimulate osteoblast bone-forming behavior. Attention of living organism is such a complex system that a graded porous proposal is far from biomimicking, more investigations on scaffold construction about scaffold degradation, blood supply improvement, and growth factor release, should be considered.

CONCLUSIONS

- With the limitations of this study, it can be concluded that the β-TCP is a novel biomimetic composite with osteoconductive properties. In this study, we showed that β-TCP is a viable bone substitute material in the horizontal dimension, both structurally and functionally.
- It seems that the positive histopathological results of this study are promising for the use of WPC as a bone graft substitute.
- More comprehensive studies are necessary before warranting unlimited clinical use.

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