Evaluation of coral wedge and composite as bone graft substitutes to induce new bone formation in a dog tibial defect.

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Abstract: This study aimed to evaluate and compare morphological and histological changes due to using of natural coral wedge (C.W) as well as coral composite(C.co) to serve as bone graft substitutes, filling gap defects in canine tibia. It was designed to examine the ability of natural coral wedge (C.W) and coral composite (C.co), to induce regeneration of the bone in the created defects and detect their osteoinductive and osteoconductive effect . Twenty four adult apparently healthy Mongrel dogs of both sexes and weighing 15 to 25 kg. were used as recipient for (C.W) and (C.co). Three defects were created in the tibia of the recipient dogs. The first was filled with (C.co), the second left empty to act as control, while the third defect filled with (C.W). The dogs were divided into 8 groups (3 dogs each) according to the observation periods (one, two, three, four, six, eight, twelve and sixteen weeks). At the end of each period, the operated tibia were harvested after euthanasia of the animals, for morphological and histological studies and assessment of graft incorporation. The histological results revealed the regeneration of the created defects which confirmed the clinical evaluation.

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Key words: bone grafts, coral wedge and composite.

1. Introduction

Replacement of extensive local bone loss is a significant clinical challenge to the orthopaedic surgeons in veterinary and human fields**.** So that use of bone graft substitutes are of paramount importance **(Laurencin *et al.,* 2006**; **Nandi *et al.,* 2010).** Numerous synthetic and biologically derived materials have been evaluated for use in the preservation or augmentation of bone defect **(Burchardt,1983;** and **Schepers** and **Ducheyne,1997)**. The synthetic bone graft substitutes not only offer a part solution to the management of localized bone loss, but also it posses some of the desired mechanical qualities of bone, as well as, osteointegrative / osteoconductive properties **(Williams *et al.,* 1992. Ramakrishna *et al.,* 2001).** Bone graft and their substitutes can be divided into osteoinductive, osteoconductive and osteogenic agents **(Perry, 1999; Albrektsson & Johansson, 2001** and **Laurencin *et al.,* 2006)**.Coral as marine invertebrates has been the most widely used for orthopaedics **(White; 1997** and **Clarke *et al.,* 2011)**. Fourteen coral types of more than 2000 coral species have been studied as possible bone substitutes **(Bouchon *et al.,* 1995**).Histological examination is needed to study the healing pattern of bone-grafting or graft substitutes. Histological sections permit characterization of the stages of osseous repair and features including osseous cellular activity and organization of spongiosa and cortex **(Burchardt, 1987)**.The aim of this study is to evaluate and compare morphological and histological changes due to using of processed coral wedge (C.W) and composite containing chitosan, gelatin and coral powder (C.co) as bone substitutes, in repair of artificially induced bone defects in canine tibia, to find new bone substitutes.

2-Material and Methods:

Twenty four adult, apparently healthy, Mongrel dogs of both sexes weighing 15 to 25 kg. were used in this study. The animals were randomly divided into eight groups; (3 dogs each) according to the observation periods at 1^{st} , 2^{nd} , 3^{rd} , 4^{th} ,6^{th} , 8^{th} , 12^{th} and 16^{th} weeks of the end of operation.

For the operation, the following materials were prepared :

- **Coral composite(C.co) :** Natural coral reef **(genus *Porites astreoides***) was powdered alone and added to chitosan (**chitosan®: Yaizu, Suisankagku Co., Japan**) and gelatin (**Gelatin powder®: ADWIC, El Nasr.** The prepared coral composite was sterilized.
- **Coral wedge (C.W):** Natural coral reef **(genus *Porites astreoides***) was used and the prepared coral wedges sterilized then stored at room temperature. The preparation and sterilization of both (C.co) and (C.W) were according to **Arnaud *et al.*, (1999)** and **Saraswathy *et al.*, (2004)**.

2.1-Anaesthesia and Preoperative technique:

All dogs were pre-medicated with I/V injection of mixture of **atropine sulfate 0.05 mg/kg (Atropine sulfate®: 1mg/ml Med. Co., A.R.E.)** and **diazepam 1**

mg/kg (Neuril®: 0.5% sol. Memphis Co. for Pharm. &Animal Ind. Cairo A.R.E.). Anaesthesia was induced immediately through I/V injection of a mixture of **Ketamin 10 mg/kg (Ketalar®: 5% sol. Amoun Co. A.R.E.) and Xylazine 1 mg/kg (Xylaject®: 2% sol. ADWIA Co., A.R.E.).** The anaesthetic depth was maintained with **2.5 % thiopental sodium (Thiopental®: EPICO Co., A.R.E.)** administrated by I/V rout (**Schmidt *et al.*, 1995**).The lower region of the hind limb (tibia) was prepared for aseptic surgery followed by routine orthopedic operative draping and gowning procedures. Aprophylactic course of Cefotaxim sodium (***Cefotax®: EPICO, A.R.E***) at dose of 4.5 mg/kg b.w. intravenously every 8 hours was administered for five successive days preoperatively and continued every 12 **hours, for 3 successive days post-operatively.**

2.2-Surgical procedure:-

A 10 cm skin incision was made at the medial surface of the right tibia. The incision extended through the periosteum, which was reflected to expose the bone (**MacNeill *et al.*, 1999**). Three 10 mm diameter holes and 1cm apart were created at the proximal third using a sterile 10 mm Ø drill bit under contenous sterile saline irrigation**.** Each defect extended through only one cortex. The drilled holes were packed using sterile gauze to control hemorrhage from the medullary cavity**.** The first hole was packed with coral composite (C.co) while, the second one was left empty to serve as negative control. The third hole was packed with coral wedge (C.W) **(Figs. a&b)**. The implantation sites were flushed with sterile saline solution. The surgical wound was closed using polyglactin 910 (Vicryl®) for close approximation of the adjacent muscles to the implanted bone substitutes. The subcutaneous tissue was sutured with Vicryl No. 0 in simple continuous manner and the skin incision was closed as usual.

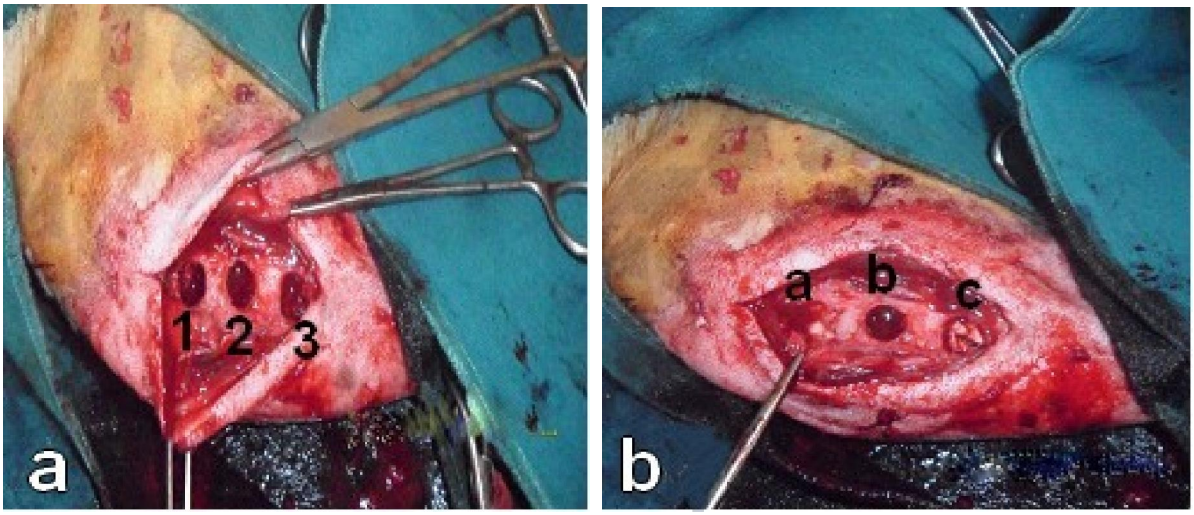


Fig. a: Three induced defects (1,2,3) in the proximal third of the tibia..

Fig. **b:** The defects after filling with the graft materials: (a) filled with coral composite, (b) left empty as control negative while, (c) filled with coral wedge.

2.3-Morphological studies:

At the end of each observation period (1st , 2nd, 3rd, 4th, 6th, 8th, 12th, 16th week). The dogs of each group were euthanised and the operated tibia were harvested and examined grossly. The operated tibia were harvested and examined grossly. The defects were inspected for complete or partial filling in relation to the adjacent cortex.

2.4-Histological studies:

Small bone samples were taken from each operated tibia where each one containing defect hole with its surrounding tibial bone and immediately fixed in 10% formalin for one week. The samples were decalcified using10% EDTA di-sodium solution. P.93®: El Nasr pharmaceutical chemical, Egypt) for one month. **(Shibata *et al.*, 2000)**. Then washed in running tap water for 24 hours, dehydrated in ethyl alcohol ,cleared in xylene and embedded in soft paraffin then blocked in hard paraffin wax. Sections of 5-7 µm thick were cut and stained with **Harri's haematoxylin** and **eosin (Bancroft *et al.*, 1996).** Photomicrographs were taken using binocular digital microscope **(Leica DMLB ®)** with 3.2 mega pixels digital camera (Leica EC3) and program software (**LASEZ. 1.6**).

3- Results

Morphological and Histological results:-

The results of morphological and histological studies were illustrated in tables (1-2-3-4-5-6-7 and 8) , moreover figures in the related plates showing the photomicrographs at one week, two weeks, three weeks, four weeks, six weeks, eight weeks, twelve weeks and sixteen weeks observation period.

Table (1): Morphological and Histological assessments of bone grafts in experimental dogs at one week observation period(Group I).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	Tibial gap defects was empty.	Showed dilated blood vessels and extravasated red blood cells. Fibroblasts could be easily detected lined the bone marrow cavity. (Plate 2,Fig.a)
C.W *	Implanted materials could be easily recognized.	As control, besides presence of small particles of C.W (scanty in amount) inside the bone marrow. (Plate 2,Fig.b)
C.co **	Partial resorption of implanted materials.	As control, besides presence of small particles of C.co (abundant in amount) inside the bone marrow. (Plate 2,Figs.c&d)

* C.W = coral wedge ** C.co = coral composite

Table (2): Morphological and Histological assessments of bone grafts in experimental dogs at two weeks observation period(Group II).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	Tibial gap defects was empty.	The bony spaces were filled with dense fibrocellular tissue. The fibrocellular tissue consisted of abundant number of the fibroblasts arranged in epithelioid manner and dilated blood vessels (Plate 3, Fig a).
C.W *	Implanted materials could be easily recognized and differentiated..	Scanty amount of fibrocellular tissues were detected. Thin layers of woven bone trabeculae were detected in between the fibrocellular tissues. The newly formed bony trabeculae lined by osteoblasts (Plate 3, Fig b).
C.co **	Complete resorption of the implanted material.	As coral wedge defect except the newly formed woven bone trabeculae was denser in nature (Plate 3, Fig c).

* C.W = coral wedge ** C.co = coral composite

Table (3): Morphological and Histological assessments of bone grafts in experimental dogs at three weeks observation period(Group III).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	Tibial gap defects was empty but narrower. (Plate 1,Fig.a)	As previous observation period with increased amount of the fibrocellular tissue (Plate 4, Fig a).
C.W *	Implanted materials could be easily recognized and differentiated.. (Plate 1,Fig.a)	The fibrocellular tissue was fewer than the control defects. The osteoblasts arranged in an epithelioid manner around the woven bone. Localized areas of osteoclastic activities were detected at the surface of the newly formed woven bone indicating bone resorption (Plate 4, Fig b).
C.co **	Completely filled with granulation tissue. (Plate 1,Fig.a)	Increasing the thickness of the formed woven bone trabeculae than the previous period. The newly formed fibrocellular tissue was fewer than the control defect. Localized areas of osteoclastic activities were detected at the surface of the newly formed woven bone (Plate 4, Fig c).

* C.W = coral wedge ** C.co = coral composite

Table (4): Morphological and Histological assessments of bone grafts in experimental dogs at four weeks observation period(Group IV).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	Presence of periosteal reaction at the implantation site manifested by callus formation. (Plate 1,Fig.b)	Thin woven bone trabeculae enclosing fibrocellular tissue in between (Plate 5, Fig. a).
C.W *	Presence of periosteal reaction at the implantation site manifested by callus formation. (Plate 1,Fig.b)	The newly formed woven bone trabeculae were denser than the previous period and enclosing scanty amount of fibrocellular tissue (Plate 5, Fig. b).
C.co **	Presence of periosteal reaction at the implantation site manifested by callus formation. (Plate 1,Fig.b)	More dens layers of woven bone trabeculae. The osteoclastic activities at the surface of the woven bone were accompanied with formation of thin layer of bone matrix (collagen fibers) which, secreted by the newly formed osteocytes (Plate 5, Figs. c& d).

* C.W = coral wedge ** C.co = coral composite

Table (5): Morphological and Histological assessments of bone grafts in experimental dogs at six weeks observation period(Group V).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	The periosteal callus was abundant than the previous stage. (Plate 1,Fig.c)	Denser woven bone trabeculae than that of the previous period enclosing fibrocellular tissue in between (Plate 6, Fig. a).
C.W *	The periosteal callus was abundant than the previous stage.. (Plate 1,Fig.c)	Fibrous tissue containing numerous dilated blood vessels was observed. Beginning formation of bone matrix (collagen fibers) secreted by newly formed osteocytes at the surface of the woven bone trabeculae. The osteoclastic activities at the surface of the woven bone indicating its resorption (Plate 6,Fig. b).
C.co **	The periosteal callus was abundant than the previous stage. (Plate 1,Fig.c)	The newly formed bone matrix was denser and thicker than that of both the previous period and that of the C.W. defect (Plate 6, Fig. c).

* C.W = coral wedge ** C.co = coral composite

Table (6): Morphological and Histological assessments of bone grafts in experimental dogs at eight weeks observation period(Group VI).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	All the induced holes were nearly disappeared. Signs of remodeling could be detected at this period manifested by beginning of callus resorption. (Plate 1,Fig.d)	As the previous period besides, an increasing in the thickness of the formed fibrocellular tissue was observed (Plate 7, Fig. a).
C.W *	All the induced holes were nearly disappeared. Signs of remodeling could be detected at this period manifested by beginning of callus resorption (Plate 1,Fig.d)	As the previous stage with marked increase in the thickness of the formed bone matrix (Plate 7, Fig. b).
C.co **	All the induced holes were nearly disappeared. Signs of remodeling could be detected at this period manifested by beginning of callus resorption. (Plate 1,Fig.d)	The newly formed lamellar bone was denser than that of the C.W defect (Plate 7, Figs.c&d).

* C.W = coral wedge ** C.co = coral composite

Table (7): Morphological and Histological assessments of bone grafts in experimental dogs at twelve weeks observation period(Group VII).

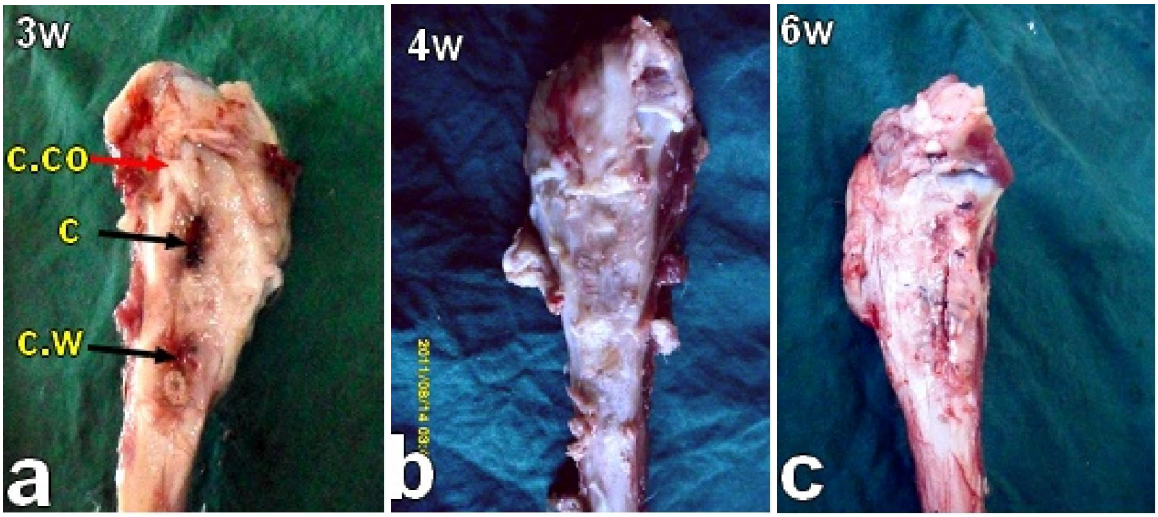
Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	All the holes were completely disappeared. Remodeling of the callus was nearly completed. (Plate 1,Fig.e)	Thick layer of woven bone trabeculae and denser layer of fibrocellular tissue. Localized areas of osteoclastic activities were detected at the surface of the woven bone (Plate 8, Fig. a).
C.W *	All the holes were completely disappeared. Remodeling of the callus was nearly completed. (Plate 1,Fig.e)	The same histological picture of the previous period was detected (Plate 8, Figs. b &c).
C.co **	All the holes were completely disappeared. Remodeling of the callus was nearly completed. (Plate 1,Fig.e)	Thick interconnected bone lamellae which, forming Haversian systems. The small Haversian systems were enclosed Haversian canals. The newly formed lamellar bone was surrounded with remnants of woven bone. Osteoclastic activities were detected (Plate 8, Figs. d &e).

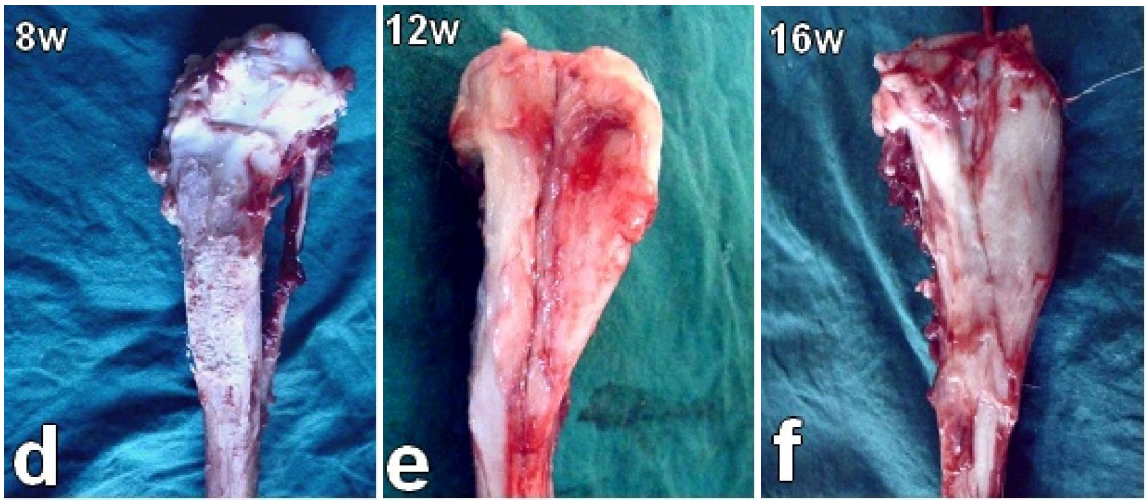
* C.W = coral wedge ** C.co = coral composite

Table (8): Morphological and Histological assessments of bone grafts in experimental dogs at sixteen weeks observation period(Group VIII).

Method of assessments / Type of bone grafts	Morphological	Histological
Control "empty"	The remodeling was completed and the operated tibia returned to the normal anatomical features as compared with the tibia of the contra-lateral limb. (Plate 1,Fig.f)	As previous period (Plate 9, Fig. a).
C.W *	The remodeling was completed and the operated tibia returned to the normal anatomical features as compared with the tibia of the contra-lateral limb. (Plate 1,Fig.f)	Thin trabeculae of bone lamellae forming Haversian systems was detected on the surface of the old woven bone. The newly formed Haversian systems were thinner than that of the C.co defect and they were surrounded by remnants of woven bone and loose fibrocellular tissues (Plate 9, Fig. b).
C.co **	The remodeling was completed and the operated tibia returned to the normal anatomical features as compared with the tibia of the contra-lateral limb. (Plate 1,Fig.f)	Increasing thickness of the lamellar bone of the Haversian systems when, compared to the previous period and to the C.W defects. Also, interstitial lamellar bone could be detected in between the formed Haversian systems. The bone marrow returned to its normal structure (Plate 9, Figs. c& d).

* C.W = coral wedge ** C.co = coral composite





Plate(1)

Fig. a: The harvested tibia at three weeks post operation showing, complete disappearance of C.co defect (red arrow) while the control (c) and C.W defects could be easily recognized. (black arrow)

Fig b: The harvested tibia at four weeks post operation showing periosteal reaction which completely masking the implantation site.

Fig. c; The harvested tibia at 6 weeks post operation showing thick callus covering the implantation site.

Fig. d: The harvested tibia at 8 weeks post operation showing the beginning of callus remodeling.

Fig. e: The harvested tibia at 12 weeks post operation showing, complete remodeling of the periosteal callus

Fig. f: The tibia at 16 weeks post operation showing, complete remodeling of the periosteal callus and the tibia returned to its normal appearance.

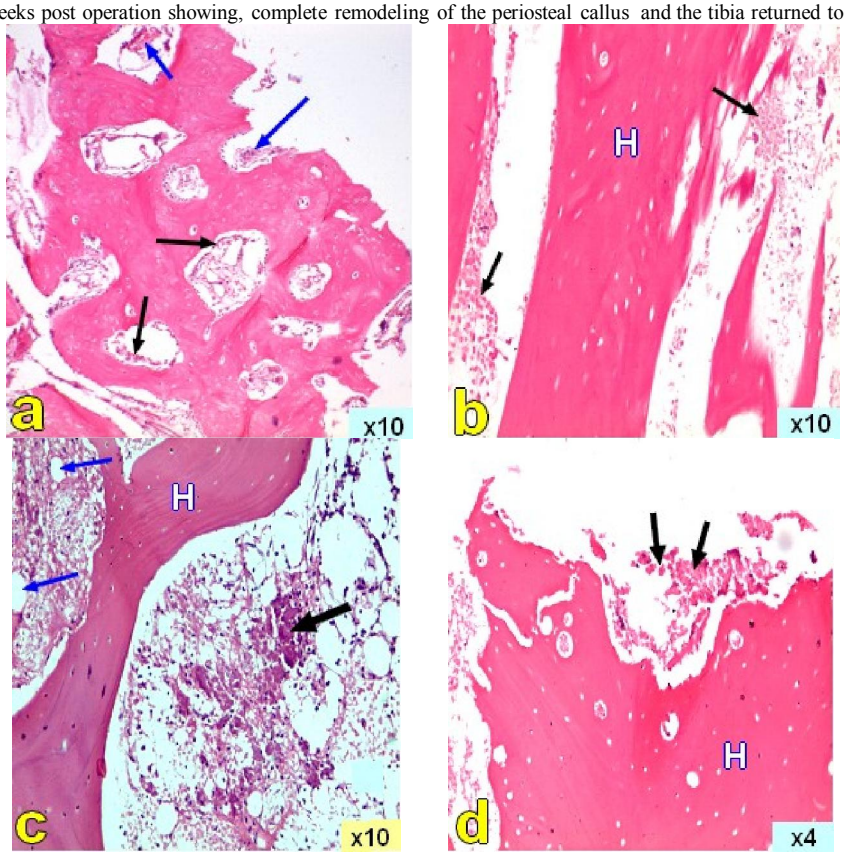


Plate (2):

Fig. a: Control defect at one week post operation showing, red blood cells (red arrow), fibroblast (black arrow) lined the cavity, H&E ×10.

Fig. b: C.W defect at one week post operation showing, C.W particles (black arrow) in the defect cavity adjacent to the host bone (H), H&E ×10.

Fig. c & d: C.co defect at one week postoperation showing; C.co particles (black arrow) in the defect cavity (black arrow) adjacent to the host bone (H) and newly formed dilated blood vessels (blue arrow), H&E ×10 & ×4.

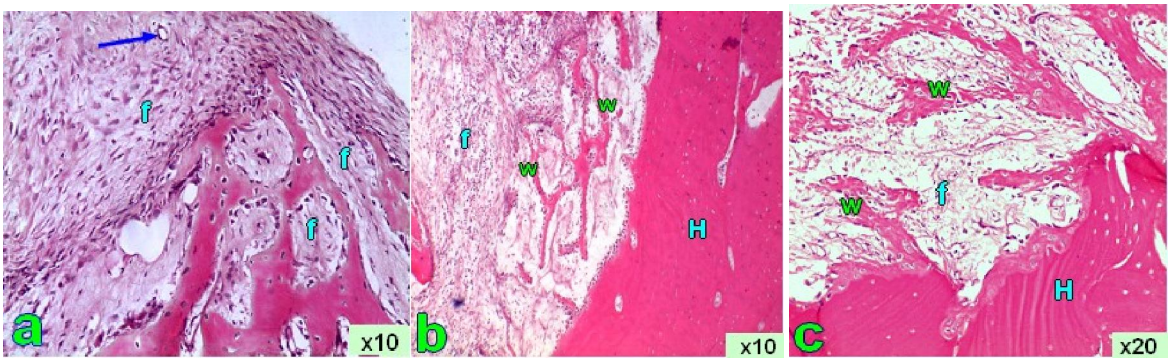


Plate (3):

Fig a: : Control defect at two weeks post operations showing, fibrocellular tissue (f) filled the bony spaces, and dilated blood vessels (blue arrow), H&E. ×10.

Fig. b: C.W defect at two weeks post operation showing; fibrocellular tissue (f), thin trabeculae of woven bone (w) adjacent to the host bone (H) H&E. ×10.

Fig. c: C.co defect at two weeks post operation showing; fibrocellular tissues (f), thin trabeculae of woven bone (w) adjacent to the host bone (H), H&E. ×10.

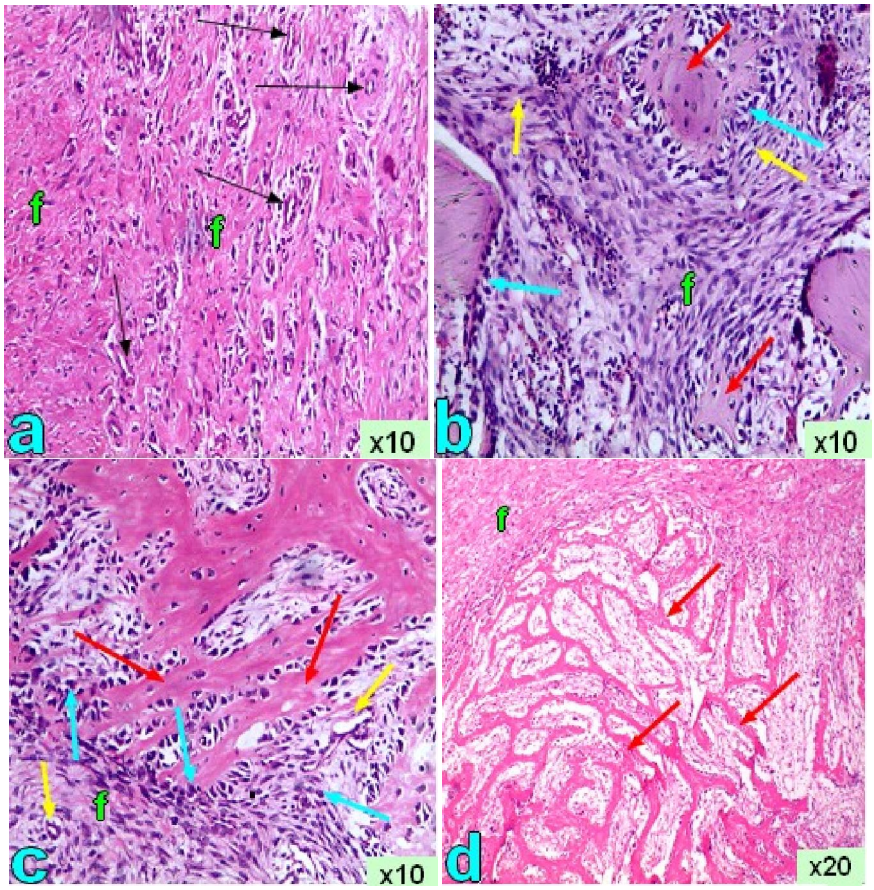


Plate (4):

Fig. a: Control defect at three weeks postoperation showing, increased amount of the fibrocellular tissue (f), containing fibroblasts and dilated blood vessels (arrows), H&E. ×20.

Fig. b: C.W defect at three weeks post operation showing, fibrocellular tissue (f) containing osteoblasts arranged in epithelioid manner (yellow arrows) around the woven bone trabeculae (red arrow) and osteoclasts with osteoclastic activities (blue arrow). H&E. ×10.

Fig. c & d: C.co defect at three weeks postoperation showing, fibrocellular tissues (f) containing dilated blood vessels (yellow arrow), thick woven bone trabeculae (red arrow) and localized areas of osteoclastic activities (blue arrow), H&E. × 10.

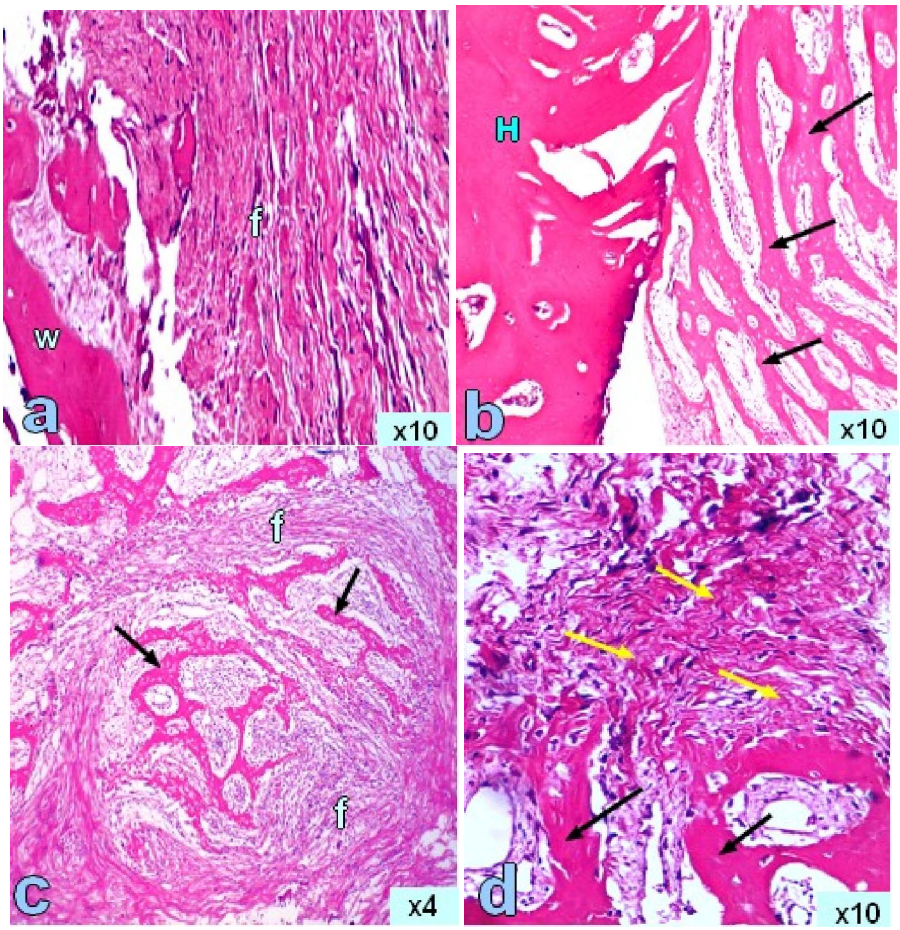


Plate (5):

Fig. a: Control defect at four weeks postoperation showing; fibrocellular tissues (f) are containing thin trabeculae of woven bone (W) H & E ×10.

Fig. b: C.W defect at four weeks postoperation showing; dense woven bone trabeculae (black arrow) and the host bone (H), H & E ×10.

Fig. c & d: C.co defect at four weeks postoperation showing; fibrocellular tissue (f) containing dense trabeculae of woven bone (black arrow) and newly formed thin trabeculae of bone matrix (collagen bundles) (yellow arrow) at the surface of the formed woven bone H & E ×10.

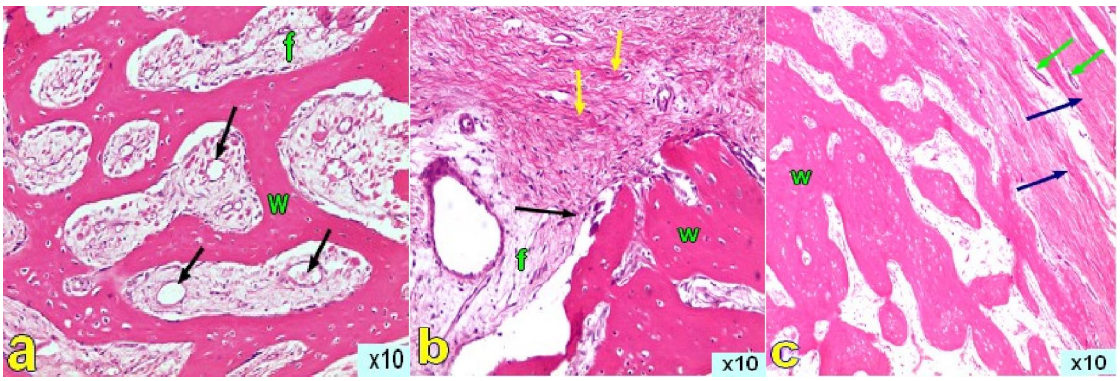


Plate (6):

Fig. a: Control defect at six weeks postoperation showing, fibrocellular tissue (f) containing dilated blood vessels (arrows), red blood cells, and thick trabeculae of woven bone (w), H&E. X 20.

Fig. b: C.W defect at six weeks post operation showing: fibrocellular tissue (f) containing newly formed bone matrix (yellow arrows) at the surface of the formed woven bone trabeculae (w), and osteoclastic activities (black arrows) at the surface of the woven bone H&E. X 20.

Fig. c: C.co defect at six weeks post operation showing, fibrocellular tissue containing dilated blood vessels (green arrow), newly formed bone matrix (blue arrow) and woven bone trabeculae (w), H&E.. X 10.

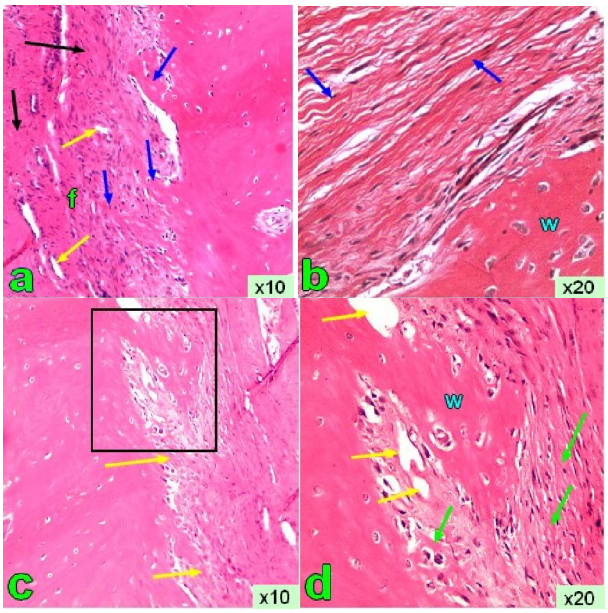


Plate (7):

Fig. a: Control defect at 8 weeks postoperation showing: thick amount of fibrocellular tissue (f) enclosing dilated blood vessels (yellow arrows), fibroblasts (black arrows) and woven bone trabeculae (blue arrows) H&E. X 10.

Fig. b: C.W defect at 8 weeks postoperation showing, newly formed lamellar bone (blue arrow) at the surface of the formed woven bone (w), H&E. X 20.

Fig. c &d: C.co defect at 8 weeks postoperation showing; newly formed lamellar bone (green arrows) at the surface of the formed woven bone trabeculae (w), resorption spaces (yellow arrows) H&E X 10 & X 20.

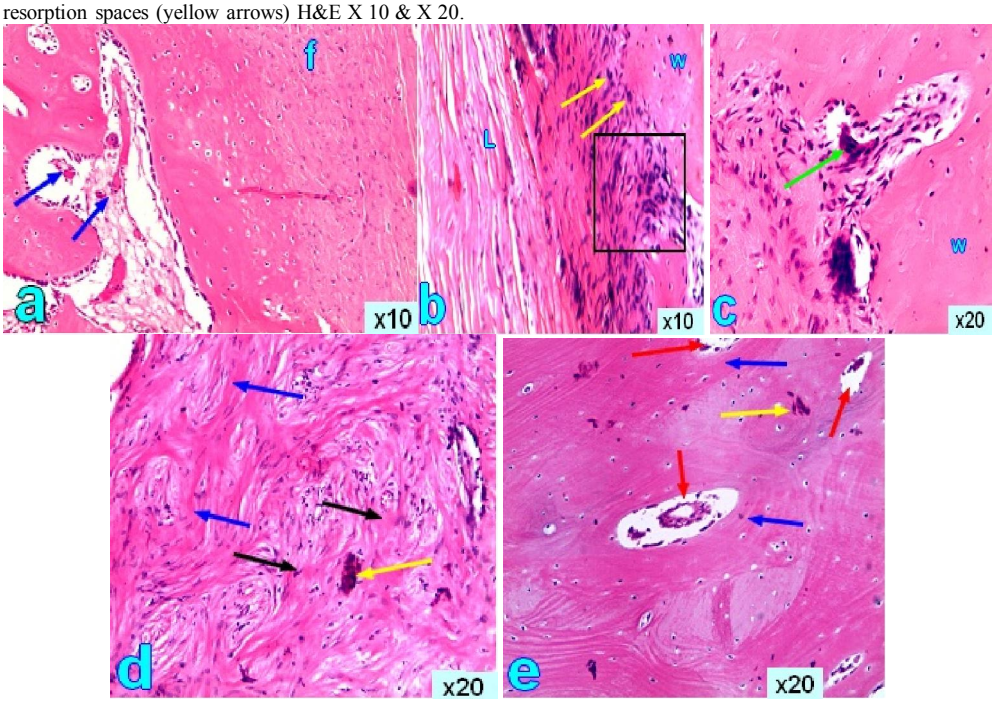


Plate (8):

Fig. a: Control defect at12 weeks postoperation showing: fibrocellular tissues (f) enclosing in between woven bone trabeculae. Osteoclastic activities at the surface of the formed woven bone (blue arrows), H&E. X10.

Fig. b &c: C.W defect at 12 weeks postoperation showing; newly formed bone matrix (L) at the surface of the formed woven bone trabeculae (black arrows), also, osteoclastic activities (yellow arrows), H&E. X10 & X20.

Fig. d &e: C.co defect at 12 weeks postoperation showing; newly formed lamellar bone forming Haversian system (blue arrows) and Haversian canal (red arrow). remnant of woven bone (black arrows) and osteoclasts (yellow arrow) in between the newly formed Haversian systems, H&E. X20

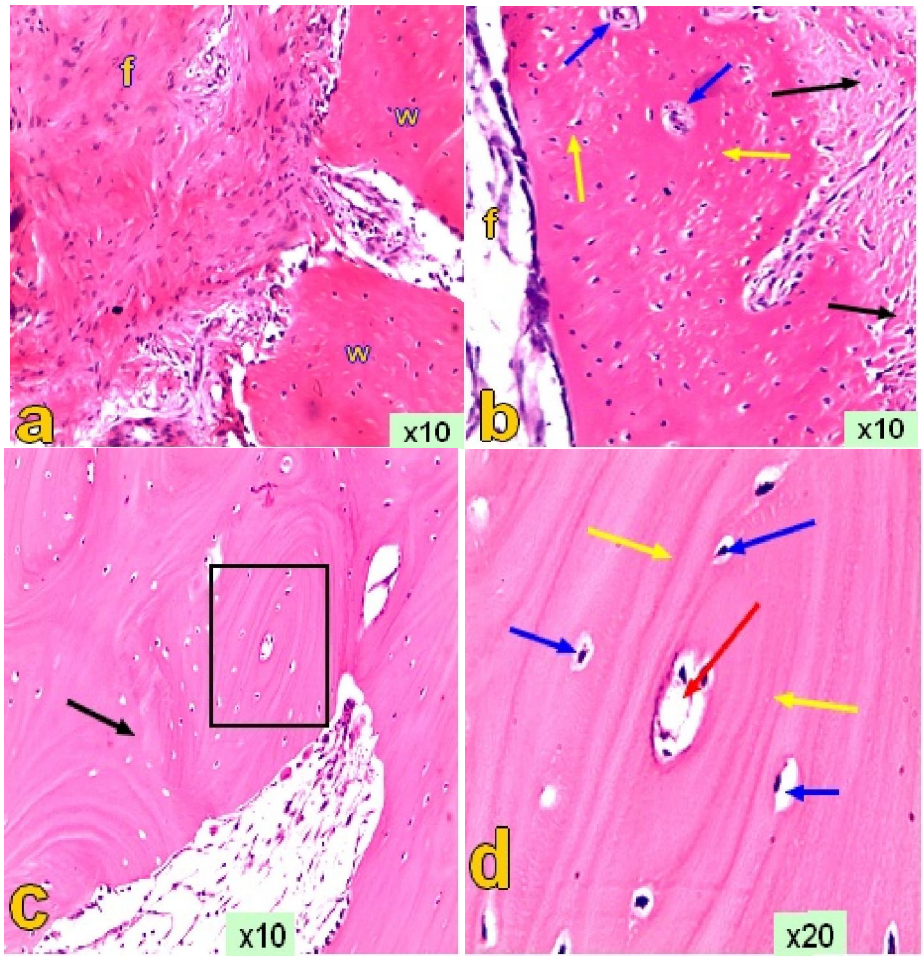


Plate (9):
Fig. a: The control defect at 16 weeks postoperation showing, fibrous tissues (f) enclosing woven bone trabeculae (w) H&E. X10.
Fig. b: The C.W defect at 16 weeks postoperation showing, lamellar bone trabeculae (yellow arrows) forming Haversian systems and enclosing Haversian canal (blue arrow). Remnants of woven bone trabeculae (black arrows) at the surface of the lamellar bone and surrounded by loose layer of fibrocellular tissue (f), H&E. X10.
Fig. c &d: The C.co defect at 16 weeks postoperation showing, Haversian system, composed of lamellar bone (yellow arrows), Haversian canal (red arrow) and osteocytes (blue arrows), interstitial lamellar bone (black arrow) H&E X10 & X20.

4-Discussion

Today different categories of bone graft materials and graft substitutes are available but, each of them has its own advantages and disadvantages **(Bauer and Muschler, 2000**; and **Nandi *et al.*, 2010)**. In the present study two types of bone graft substitutes were selected (including natural coral wedge (C.W) and composite (C.co) containing chitosan, and gelatin), and compared between them clinically, and morphohistologically in reconstruction of an experimentally induced bone defects in canine model.

Adult mongrel dogs of both sexes were used in this study because the biological repair process of mature dogs is similar to those of human **(Burchardt, 1987).**

Concerning the gross and microscopic examination of the natural coral reef (genus ***Porites*)**, the present study revealed that it has skeleton with a structure similar to both cortical and cancellous bone. This skeleton, combined with an open, highly interconnected porous structure, similar findings were reported by **Chiroff *et al.*, 1975 ;Ripamonti, 1991** and **Geiger *et al.*, 2007).**

The histological study revealed that the complete regeneration of bone at the gap defects appeared at the end of sixteen week of observation periods. This was in agreement with radiological and morphohistological results that recorded by **Johnson *et al.*, (1996).** In the same respect **Naglaa Abdel Wahed (2003)** mentioned that 24 weeks were necessary for complete incorporation of the grafted materials in the host bed.

The present results showed that the center of the implanted material (coral wedge) begin to be resorped by the end of the fourth week, while, the reminder part of the C.W completely resorped and replaced by host bone tissues by the end of the sixteen weeks. **Petite *et***

***al.*, (2000),** mentioned that the resorption pattern proceeded in a centripetal manner which begins from the center and directed toward the periphery. On the contrary, **Vuola (2001)** mentioned that the resorption pattern of the coral begins from the periphery and proceeded toward the center. He added that the resorption of the coral exceeded six months and extended up to one year postoperation. He also stated that the resorption appears to proceed more rapidly in animals than in humans.

The histological picture of the defects at fourth week observation period showed that the defect cavity of (C.W) filed with fibrocellular tissue and the coral granules showed resorption pattern characterized by presence of multinucleated phagocytic cells which engulf the coral granules while at eighth week the defect cavity was filed with woven bone which showed resorption and osteoclastic activities, these results correlated with that reported by **Arnaud *et al.*, (1999).**

The histological findings of this study at six weeks post-implantation showed delayed new bone growth in C.W defect compared to C.co defects. **Vuola (2001)** attributed this fact to the rapid resorption of the coral leading to rapid loses of its internal porous structure, and after that the bone does not actually invade the pores but replaces the matrix. He also, observed that when coral implants are placed into bone defects, the amount of new bone formation without addition of inducing factor is scarce. From the present histological results we suggested that the coral has limited osteoinductive capacity but has good osteoconductive properties. These findings are similar to that reported by **Guillemin *et al.*, (1987)**; **Chen *et al.*, (2007)** and **Zhang *et al.*, (2007).** While, **Hutmacher (2000)** recorded that Osteoconductive materials must have a three dimensional structure and a large number of interconnected pores that permit cellular and vascular proliferation, and the formation of granulation tissue within them. **Williams (1986)** added that an ideal biomaterial should be osteoconductive, resorbable and susceptible to osteoclastic action so as to permit bone replacement in the grafted area.

Comparing the amount of the newly formed fibrocellular tissues at the control defects with that formed at the implantation site filled with C.co, it was greater in amount in case of control defect rather than C.co. This fact attributed to the inhibitory effect of chitosan upon fibroblasts proliferation while, stimulating osteoblastic activities **(Lee *et al.*, 2002),** while **(Martino, et al., 2005**) added that chitosan is well known for its biocompatibility, biodegradability and bioactivity. It enhances the functions of inflammatory cells**.**

The morphohistological observations of the present study appeared without any complication and confirmed the clinical evaluation of the post-operative animals. The regeneration of the defects of C.co was occurred faster than that of C.W.

5-Conclusion:

we can concluded that the natural coral wedge has good osteoconductive properties, but, it has limited osteoinductive properties.

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