Original Article

Implantation of Octacalcium Phosphate Stimulates both Chondrogenesis and Osteogenesis in the Tibia, but Only Osteogenesis in the Rat Mandible

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Abstract:

Statement of problem: It is not known whether endochondral and intramembranous bones have distinct biological characteristics. Octacalcium Phosphate (OCP), a hydroxyapatite precursor, has been reported to stimulate bone formation after being implanted in parietal bone defects of rats.

Purpose: The present study was designed to investigate the response of endochondral and intramembranous bones to OCP implantation and to compare their biological characteristics

Materials and Methods: Full-thickness standardized trephine defects were made in rat tibiae and mandibles and synthetic OCP was implanted into the defects. The biologic response was examined histologically to identify bone and cartilage formation.

Results: Both chondrogenesis and osteogenesis were initiated in the tibia, 1 week after implantation of OCP and most of the cartilage was replaced by bone at week 2. However, the mandible only showed osteogenesis in response to OCP implantation at week 2, and no cartilage formation was associated with the osteogenesis.

Conclusions: According to the results obtained in the present study, endochondral and intramembranous bones exhibit different biological responses to OCP implantation in rats.

Key Words: Octacalcium phosphate; Endochondral bone; Intramembranous bone; Osteogenesis; Chondrogenesis; Rat

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INTRODUCTION

Autogenous bone grafting remains the most predictable and proven method for augmenttation and repair of oral bony defects or deficiencies. Recent advances in biomaterials have improved the predictability of synthetic bone substitutes [1]. Synthetic hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$ is one of the most commonly used bone graft substitutes [2]. It is not known whether endochondral and intramembranous bones have distinct biological characteristics in responding to exogenous bone growth factors such as transforming growth factor-beta (TGF-beta) and bone morphogenetic protein (BMP) [3,4]. Joyce et al [5] reported that injection of exogenous TGF-beta into the subperiosteal region of murine long bones can cause both intramembranous and endochondral bone formation. However, others showed that TGF-beta induces intra-

membranous bone formation without prior formation of cartilage in murine calvaria [6,7]. Cellular responses to the stimulating effects of TGF-beta on bone formation with or without chondrogenesis, appears related to the comm.-itted phenotype at the site of TGF-beta administration [8,9]. Previous investigations have not compared the biological response of endochondral and intramembranous bone forming cells to TGF-beta Hydroxyapatite is the prototype for mineral in bone and teeth [10,11]. The biological apatite has been suggested to be formed via precursor phases, such as Octacalcium phosphate [Ca_8H_2 $(PO_4)_6$ $5H_2O$] [12]. Synthetic octacalcium phosphate (OCP) granules have been shown to stimulate osteogenesis, when implanted into the sub-periosteum of mice [13]. Former studies indicated that implantation of synthetic granules of OCP cause new bone regeneration and could eventually be replaced by newly formed bone [14,15]. Furthermore, the implanted OCP can serve as a core for initiating bone formation and if implanted in the critical-sized calvarial defects of rats, can show an osteoinductive and osteoconduction ability [16,17]. OCP unlike TGF-beta is an inorganic compound. How OCP stimulates the biological activity of skeletal tissue is yet unknown.

The present study was designed to investigate the response of endochondral (tibia) and intramembranous (mandible) bones to OCP implantation and compare their biological characteristics.

MATERIALS AND METHODS

Animals

The sample consisted of forty 5- to 6-week-old male Sprague Dawley rats weighing from 125 to 150 gm. All animals were obtained from the Animal Research Center of Pasteur Institute (Tehran, Iran) and kept under a standard light-dark schedule and relative humidity. Stock diet and tap water were available *ad libitum*. All procedures were approved by the Animal Research Committee of Tehran University of Medical Sciences.

Preparation of Implants

OCP was prepared according to the method described by Legeros [18]. In brief, 250 ml of 0.04 M calcium acetate solution [Ca $(CH3CO_2)_2$ H2O] was slowly added to 250 ml of 0.04 M sodium acid phosphate solution [$NaH_2PO_4.2H_2O$] over a period of one hour, while being stirred at 400 rpm at 67.5°C. Ground granules of OCP between 32 and 48 mesh (particle size: 300–500 μm) were used for implantation. The sieved granules were sterilized by being heated at 120°C for 2 hrs and were subsequently implanted. Previous studies have shown that heat does not affect physical properties such as crystalline struc-ture or the specific surface area of the OCP granules [13].

Experimental Periods

The rats were divided into two groups of 20 animals each: tibia bone group and mandibular bone group. Five rats from each group were sacrificed and tissues were fixed at 7, 14, 21 and 28 days after the OCP implantation or sham operations.

Implantation Procedure

The animals were anesthetized with an intraperitoneal injection of ketamine chlorhyd-rate (Ketholar™) at a dose of 60-mg/kg-body weight, supplemented by ether inhalation. In the mandibular bone group, a 15-mm long horizontal incision was made aseptically in the submental triangle just posterior to the symphysis. This was followed by reflection of skin, musculature and periosteum in order to expose the mandible. A full-thickness trephine defect, 3 mm in diameter, was prepared bilate-rally near the superior ridge of the mandible between the incisor and first molar. In the tibia bone group, a 15-mm long vertical incision was created bilaterally, in the medial surface of the tibia, under aseptic conditions. The skin, musculature, and periosteum were then

reflected, exposing the tibia. Full-thickness trephine defects, 3 mm in diameter, were made in the mid-diaphyseal portion of the tibia. A 3-mm trephine bur was used to create the defects under constant irrigation with sterile normal saline to prevent overheating of the bone edges. For practical reasons and for the sake of systematization, the right defect was used to test the osteopromotive substance and implanted with 6 mg of OCP granules and surgically covered. A similar procedure was followed in the left defect without implantation. The periosteum and muscles were sutured in place using catgut 4-0 (Ethicon), and the skin was sutured with silk 4-0 (Ethicon). Post-operative antibiotics (Terramycin, Tokyo, Japan) were administered intraperitoneally at the time of surgery. The rats were reinstated in their individual cages and observed until full recovery.

Tissue Preparation

All animals were anesthetized by intraperitoneal injection of ketamin chlorhydrate (60 mg/kg body weight). They were then fixed with 4% paraformaldehyde in 0.1 M phosphate-buffer saline (PBS, pH 7.4) by perfusion through the heart. The implants were resected together with the surrounding bone and tissues and kept in the same fixative overnight at 4°C. All specimens were decalcified in a mixture of 10% formic acid, 2.9% citric acid and 1.8% trisodium citrate dehydrate. The samples were dehydrated in a graded series of ethanol and embedded in paraffin according to previous investigations [12]. Serial sections with a thickness of 5-µm were cut and stained with hematoxyline/eosin (H&E) and alcian blue. Sections were studied under a light microscope (Olympus AX-800).

RESULTS

Tibia

Both chondrogenesis and osteogenesis were initiated around the OCP particles, near the defect margin of the tibia, 1 week after implantation (Fig.1). Cartilage matrix was identified by alcian blue staining (Fig.2). New bone formation was observed at the margin of the defect and between the implanted OCP. No cartilage was seen in the defect at week 2. An inflammatory cell infiltration was observed at the implantation sites and the OCP implant was surrounded by multinucleated giant cells (MNGCs). In week 3, newly formed bone filled the defects and directly surrounded the implanted OCP. Less inflammatory cell infiltration was observed as compared to week 2. Osteoblasts were seen on newly formed bone in the defect and some of the implanted OCP was surrounded by MNGCs (Fig.3). In week 4, the bone matrix became more compact compared to week 3 and was barely distinguishable from the host bone, whereas

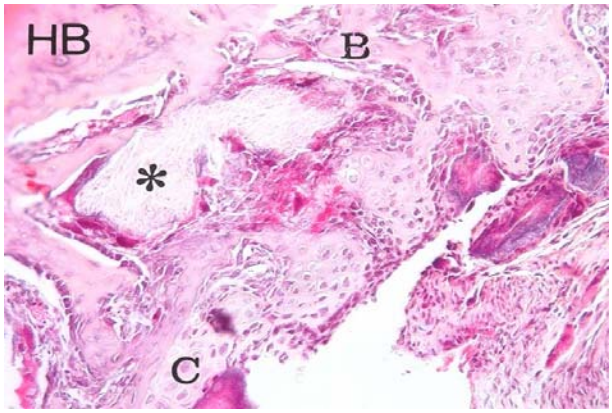

Fig. 1: H&E stained Photomicrograph (×200) of treated tibia on day 7, Both chondrogenesis (C) and osteogenesis (B) were seen around the OCP particles (*).

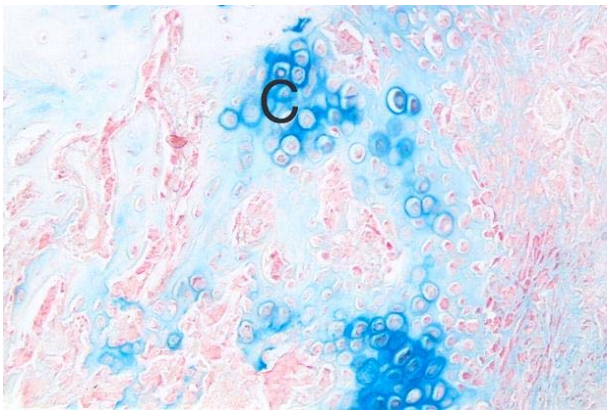

Fig. 2: Photomicrograph of treated tibia on day 7; Cartilage matrix (C) identified by alcian blue staining (×200).

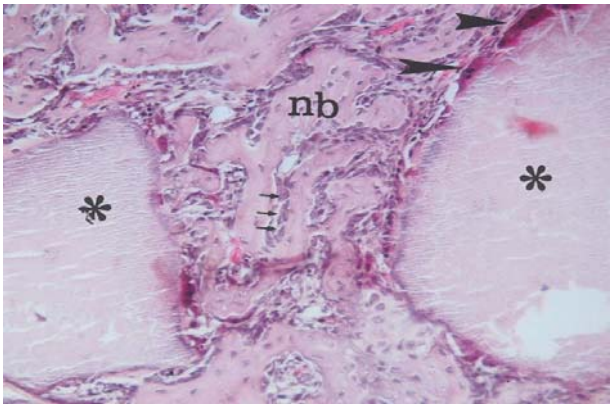

Fig. 3: H&E stained Photomicrograph (×200) of treated tibia on day 21; newly formed bone (nb) was observed between the OCP particles (*). Osteoblasts (short arrows) were seen on newly formed bone. OCP particles were surrounded by multinucleated giant cells (arrow heads).

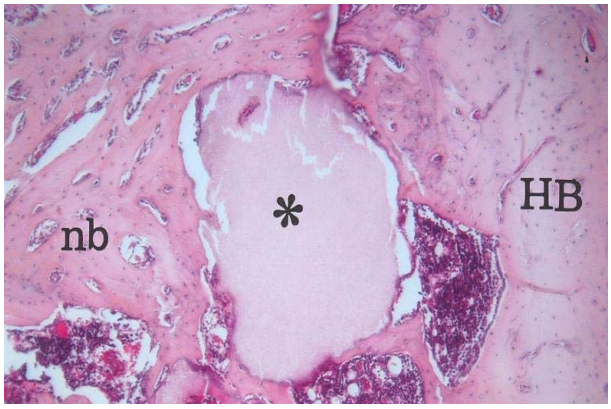

Fig. 4: Photomicrograph of treated tibia on day 28; the OCP particle (*) is enclosed in the newly formed bone (nb). The newly formed bone is indistinguishable from the host bone (HB), due to maturation (H&E stain; original magnification, ×200).

the remaining OCP in the defect seemed to be decreased (Fig.4). In the control groups, the defects were mostly filled with fibrous connective tissue, and bone formation was observed near the margin of the bone defects. Inflammatory cell infiltration was observed at week 2 and had declined at week 4 (Fig.5).

Mandible

Implantation of OCP showed inflammatory cell infiltration. The space occupied by the OCP implants was recognized by the organic matrices accumulated on OCP, which remained after decalcification of the specimen. Neither osteogenesis nor chondrogenesis was observed in response to OCP implantation at week 1. Osteogenesis was initiated either from the defect margin or on the implanted OCP, away from the margin and no chondrogenesis was identified by week 2. Osteoblasts were seen on newly formed bone around the implanted OCP and the defect margin (Fig.6). An inflammatory cell infiltration was observed and the OCP implant was surrounded by MNGCs as was found the tibia. At week 3, new bone formation was noticed at the margin of defect and on the implanted OCP. Newly formed bone without intervening cellular components surrounded the remaining OCP in the defect but OCP was not found in the

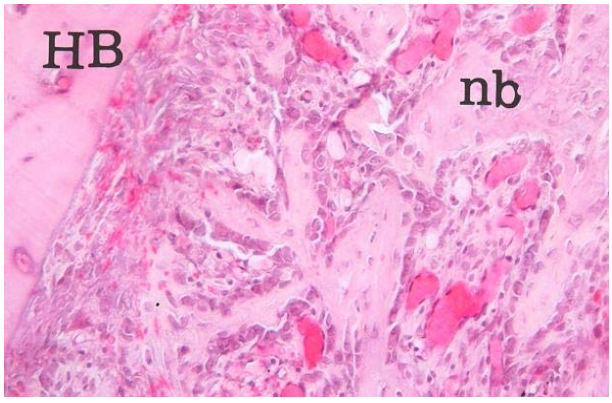

Fig. 5: H&E stained photomicrograph of untreated tibia on day 28; The defects are mostly filled with fibrous connective tissue. New bone formation (nb) is observed near the margins of the bone (×200).

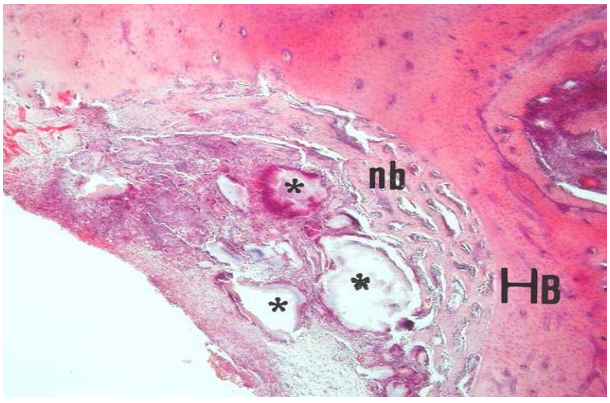

Fig. 6: H&E stained photomicrograph of treated mandible on day 14: New bone (nb) is formed near the defect margin and on the implanted OCP particle (*) away from the margin (×200).

connective tissue. At week 4, the defect was almost filled with newly formed bone along with remaining OCP, and was barely distinguishable from the host bone (Fig.7).

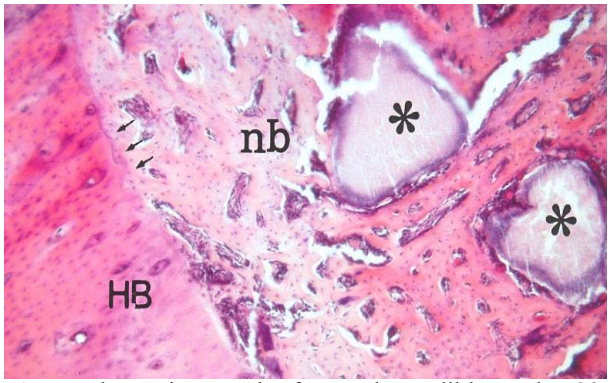

Fig. 7: Photomicrograph of treated mandible on day 28: Implanted OCP particle (*) is surrounded by newly formed bone (nb). Short arrows show the margin of the bone defect and host bone. H&E stain; original magnification ×200.

DISCUSSION

The present study was designed to investigate how endochondral (tibia) and intramembranous bones (mandible) respond to OCP, implanted into a trephine defect of the same recipient (5-6 week old rats). OCP implanttation induced both cartilage and bone formation in the tibia. However, only bone formation was stimulated and no cartilage formation was seen in the mandible. In addition, the tibia responded to OCP implantation to show cartilage and bone formation 1 week after implantation, whereas the mandible did not respond until the second week.

The current investigation demonstrates the distinct characteristics of the biological response to OCP implantation between endochondral and intramembranous bones in terms of stimulation of cartilage and bone formation as well as their approximate timing.

Previous studies suggested that endochondral bone matrix contains osteoinductive factors to induce endochondral ossification. This is in contrast to intramembranous bone matrix, which can induce only intramembranous ossification without chondrogenesis [8,19]. Osteogenic cells from endochondral bones such as the tibia and femur have been suggested to have different properties compared to those from intramembranous bones such as the calvarium and mandible [3,11,20-24].

Distinct osteoinductive factors in bone matrix and/or difference in cell properties may contribute to the different biological responses to OCP observed in endochondral and intramembranous bones. These responses include simultaneous stimulation of osteogenesis and chondrogenesis, and their timing.

Different factors in the microenvironment including oxygen supply from the vasculature [25], or mechanical stimulation exerted by the surrounding muscles [26] could affect the phenotype expression of the osteogenic cells at the implantation sites of endochondral and intramembranous bones.

Taniguchi et al [27] found that injection of TGF-beta into the outer periosteum of rat parietal bones induced only osteogenesis in neonates, but both chondrogenesis and osteogenesis in adults. How osteogenic cells respond to OCP implantation may depend on the age of the recipient.

OCP was shown to be converted to apatitic crystals when implanted in the subperiosteal region of mouse calvaria by 10 days [12,13]. The glycoconjugates accumulated on the converted apatite (recognized by maclura pomifera agglutinin (MPA) lectin), are candidates for bone stimulating factors following OCP implantation [13]. The MPA binding glycoconjugates may also be involved in osteogenesis and/or chondrogenesis, initiated by OCP implantation in rat endochondral and intramembranous bones. The ability of OCP to enhance the activity of skeletal tissues, appears to be comparable to that of TGF-beta or prostaglandin E_1 (PGE_1) [13,28]. Bioactive factors, such as TGF-beta and PGE_1, in surrounding tissues or tissue fluids may accumulate on OCP or the converted apatite,

so that they could be released to work on the osteogenic cells as OCP or apatite are decayed in the tissues. Biochemical analysis of the organic matrices accumulated on OCP or the converted apatite will be required to identify the factor(s) that can activate skeletal tissues.

The fate of the implanted OCP is not known. Some OCP implants were observed to be surrounded directly by newly formed bone. The implants may be resorbed by the MNGCs after being exposed by the remodeling process of the surrounding bone [15]. Previous studies showed that the MNGCs, which surround the implanted OCP, share ultrastructural features of osteoclasts, and the MNGCs could resorb the implanted OCP [29].

In the present study, the implants decreased with time, but still remained up to the 4th week after implantation. It is possible that they might be further resorbed with time.

CONCLUSION

The present study demonstrates that OCP is an inorganic compound that stimulates osteogenesis in both endochondral and intramembranous bones. It could be used as an effective bone substitute for augmentation of the alveolar ridge or for filling bony defects, such as that left after cystectomy or a tooth socket after extraction. Further studies are suggested in order to assess the feasibility of this material for clinical use in various aspects of oral and maxillofacial surgery.

REFERENCES

1- Misch CE, Dietsh F. Bone-grafting materials in implant dentistry. Implant Dent 1993 Fall;2(3): 158-67.

2- Schliephake H, Gruber R, Dard M, Wenz R, Scholz S. Repair of calvarial defects in rats by prefabricated hydroxyapatite cement implants. J Biomed Mater Res A 2004 Jun 1;69(3):382-90.

3- Vuola J, Bohling T, Goransson H, Puolakkainen P. Transforming growth factor beta released from natural coral implant enhances bone growth at calvarium of mature rat. J Biomed Mater Res 2002 Jan;59(1):152-9.

4- Vehof JW, Takita H, Kuboki Y, Spauwen PH, Jansen JA. Histological characterization of the early stages of bone morphogenetic protein-induced osteogenesis. J Biomed Mater Res 2002 Sep 5;61(3):440-9.

5- Joyce ME, Roberts AB, Sporn MB, Bolander ME. Transforming growth factor-beta and the initiation of chondrogenesis and osteogenesis in the rat femur. J Cell Biol 1990 Jun;110(6):2195-207.

6- Noda M, Camilliere JJ. In vivo stimulation of bone formation by transforming growth factor-beta. Endocrinology 1989 Jun;124(6):2991-4.

7- Tanaka T, Taniguchi Y, Gotoh K, Satoh R, Inazu M, Ozawa H. Morphological study of recombinant human transforming growth factor beta 1-induced intramembranous ossification in neonatal rat parietal bone. Bone 1993 Mar-Apr;14(2):117-23.

8- Scott CK, Bain SD, Hightower JA. Intramembranous bone matrix is osteoinductive. Anat Rec 1994 Jan;238(1):23-30.

9- Iwasaki M, Nakata K, Nakahara H, Nakase T, Kimura T, Kimata K, Caplan AI, Ono K. Transforming growth factor-beta 1 stimulates chondrogenesis and inhibits osteogenesis in high density culture of periosteum-derived cells. Endocrinology 1993 Apr;132(4):1603-8.

10- Fujita R, Yokoyama A, Kawasaki T, Kohgo T. Bone augmentation osteogenesis using hydroxyapatite and beta-tricalcium phosphate blocks. J Oral Maxillofac Surg 2003 Sep;61(9):1045-53.

11- Yoshikawa M, Toda T. Reconstruction of alveolar bone defect by calcium phosphate compounds. J Biomed Mater Res 2000;53(4):430-7.

12- Suzuki O, Nakamura M, Miyasaka Y, Kagayama M, Sakurai M. Bone formation on synthetic precursors of hydroxyapatite. Tohoku J Exp Med 1991 May;164(1):37-50.

13- Suzuki O, Nakamura M, Miyasaka Y, Kagayama M, Sakurai M. Maclura pomifera agglutinin-binding glycoconjugates on converted

apatite from synthetic octacalcium phosphate implanted into subperiosteal region of mouse calvaria. Bone Miner 1993 Feb;20(2):151-66.
14- Kamakura S, Sasano Y, Nakamura M, Suzuki O, Ohki H, Kagayama M, Motegi K. Initiation of alveolar ridge augmentation in the rat mandible by subperiosteal implantation of octacalcium phosphate. Arch Oral Biol 1996 Nov;41(11):1029-38.
15- Kamakura S, Sasano Y, Homma H, Suzuki O, Kagayama M, Motegi K. Implantation of octacalcium phosphate (OCP) in rat skull defects enhances bone repair. J Dent Res 1999 Nov; 78(11):1682-7.
16- Kamakura S, Sasano Y, Nakajo S, Shimizu T, Suzuki O, Katou F, Kagayama M, Motegi K. Implantation of octacalcium phosphate combined with transforming growth factor-beta1 enhances bone repair as well as resorption of the implant in rat skull defects. J Biomed Mater Res 2001 Nov;57(2):175-82.
17- Sargolzaei-aval F, Sobhani A, Arab MR, Sarani SA, Heydari MH. The efficacy of implant of octacalcium phosphate in combination with bone matrix gelatin (BMG) on bone regeneration in skull defects in rat: Iran J Med Sci 2004;29(3): 124-129.
18- LeGeros RZ. Preparation of octacalcium phosphate (OCP): a direct fast method. Calcif Tissue Int 1985 Mar;37(2):194-7.
19- Scott CK, Hightower JA.The matrix of endochondral bone differs from the matrix of intramembranous bone. Calcif Tissue Int 1991 Nov;49(5):349-54.
20- Srouji S, Rachmiel A, Blumenfeld I, Livne E. Mandibular defect repair by TGF-beta and IGF-1 released from a biodegradable osteoconductive hydrogel. J Craniomaxillofac Surg 2005 Apr;33(2):79-84.
21- Saadeh PB, Khosla RK, Mehrara BJ, Steinbrech DS, McCormick SA, DeVore DP, Longaker MT. Repair of a critical size defect in the rat mandible using allogenic type I collagen. J Craniofac Surg 2001 Nov;12(6):573-9.
22- Siqueira JT, Cavalher-Machado SC, Arana-Chavez VE, Sannomiya P. Bone formation around titanium implants in the rat tibia: role of insulin. Implant Dent 2003;12(3):242-51.
23- Tielinen L, Manninen M, Puolakkainen P, Kellomaki M, Tormala P, Rich J, Seppala J, Rokkanen P. Inability of transforming growth factor-beta 1, combined with a bioabsorbable polymer paste, to promote healing of bone defects in the rat distal femur. Arch Orthop Trauma Surg 2001;121(4):191-6.
24- Sasano Y, Li HC, Zhu JX, Imanaka-Yoshida K, Mizoguchi I, Kagayama M. Immuno-histochemical localization of type I collagen, fibronectin and tenascin C during embryonic osteogenesis in the dentary of mandibles and tibias in rats. Histochem J 2000 Oct;32(10):591-8
25- Barou O, Mekraldi S, Vico L, Boivin G, Alexandre C, Lafage-Proust MH. Relationships between trabecular bone remodeling and bone vascularization: a quantitative study. Bone 2002 Apr;30(4):604-12.
26- Burr DB, Robling AG, Turner CH. Effects of biomechanical stress on bones in animals. Bone 2002 May;30(5):781-6.
27- Taniguchi Y, Tanaka T, Gotoh K, Satoh R, Inazu M. Transforming growth factor beta 1-induced cellular heterogeneity in the periosteum of rat parietal bones. Calcif Tissue Int 1993 Aug; 53(2):122-6.
28- Marks SC Jr, Miller S. Local infusion of prostaglandin E1 stimulates mandibular bone formation in vivo. J Oral Pathol 1988 Nov;17(9-10):500-5.
29- Kamakura S, Sasano Y, Homma-Ohki H, Nakamura M, Suzuki O, Kagayama M, Motegi K. Multinucleated giant cells recruited by implantation of octacalcium phosphate (OCP) in rat bone marrow share ultrastructural characteristics with osteoclasts. J Electron Microsc (Tokyo) 1997;46(5):397-403.