

## The influence of locally delivered vitamin D3 on alveolar ridge preservation after tooth extraction(a clinical, radiographic and histological evaluation)

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### Abstract

**Purpose of the study:** The purpose of this randomized controlled clinical trial study was to evaluate & compare preservation of alveolar ridge dimensions following atraumatic tooth extraction using collagen membrane and xenograft mixed with active vit D<sub>3</sub> gel versus collagen membrane & Xenograft only

**Materials and Methods:** Ten patients were selected. All patients required extraction of two non restorable premolars (or anterior teeth) located in symmetrical quadrants of maxillary or mandibular arches and requested implant restoration. Two surgeries were carried out for each patient; the first was for extraction alveolar ridge preservation while the second was for taking core bone biopsy and implant placement in a routine fashion. In the same patient, one extraction socket (Test site) was treated with collagen membrane and xenograft mixed with active vit D<sub>3</sub> while the second extraction socket (Control site) was treated with collagen membrane and xenograft only. Clinical, radiographic and histological/ histomorphometric evaluations were performed.

**Results:** Clinically and radio graphically through the whole study period, there was no statistically significant difference between mean % changes in BL ridge widths and ridge height in the two groups. Histomorphometric analysis showed statistically

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significant difference between mean area fraction of new bone formation in the two groups.

**Conclusion:** local applied of vitamin d3 with xenograft in alveolar ridge preservation accelerates new bone formation and promotes bone maturation when comparing with xenograft only.

**Keywords:** alveolar ridge preservation, xenograft, vitamin D<sub>3</sub>, bone regeneration.

**Abbreviations:** ARP: alveolar ridge preservation ; CBCT: Cone Beam Computed Tomography; SD: Standard Deviation; BL: Bucco Lingual

## Introduction

Tooth extraction is a complicated outcome that is largely caused by dental caries, periodontitis, and their sequelae, and it reflects an individual's history of dental disease and its treatment by dental services over the life course. Tooth extraction causes loss of several orofacial structures such as bone tissue, nerves, receptors and muscles and most or facial functions are decreased. Studies showed that decrease number of teeth cause less efficient chewing ability[1].

Many studies showed evidence that tooth loss is associated with impairment in oral health-related quality of life[2].

Loss of alveolar bone may be caused by variety of factors, such as endodontic pathology, periodontitis, facial trauma and aggressive maneuvers during extractions[3]. Most extractions are done with no regard for maintaining the alveolar ridge. Whether due to caries, trauma or advanced periodontal disease or tooth extraction and subsequent healing of the socket commonly result in osseous deformities of the alveolar ridge, including reduced height and reduced width of the residual ridge which may make the placement of an implant challenging; and it creates an esthetic problem prosthesis [4,5].

The alveolar process is formed during tooth eruption and undergoes atrophy after the loss of single or multiple teeth[6,7]. In the post-extraction healing phase, the alveolar bone undergoes atrophy as a result of natural remodeling[8,9]

Resorption of the buccal compartment of the ridge is more pronounced than that of the lingual/palatal portion [10].

After tooth extraction, resorption of up to 50% of the alveolar ridge (AR) width can occur within 12 months which creates a residual knife edge ridge [11]. The estimated structural loss is as much as 40% and 60% of the pre-extraction AR height and width, respectively [12].

This loss has a detrimental effect on potential treatment with dental implants or conventional prosthesis. Thus, nowadays alveolar ridge preservation have been widely performed to allow successful prosthetic & implant options after extraction [13].

So alveolar ridge preservation is defined as a procedure performed to limit consequent ridge resorption after tooth extraction . Many techniques are available to improve alveolar ridge collapse after tooth extraction; including connective tissue grafts, a cellular dermal matrix grafts and guided bone regeneration with or without the association of different bone grafts [14].

Recently, dental research has focussed on improving bone substitutes and implant surfaces by morphologic or biochemical modification to achieve faster and better osseointegration [15] These Biochemical modifications can improve the quality and the amount of peri-implant tissue, reducing economic costs and treatment times, and consequently improving long-term outcomes. Among the biological mediators used around implants to improve osseointegration are: Growth Hormone[16] and vitamin D3 [17]. Active Vitamin D3 " calcitriol" exerts its effects on the body by binding to and activating vitamin D receptors (VDRs), which are located in the nuclei of target cells [18]. The major physiological role of vitamin D is to facilitate the intestinal absorption of calcium,

by stimulating the expression of proteins involved in calcium transport. Vitamin D also plays a crucial role in providing the proper balance of minerals necessary for bone growth and function. However, it turns out that VDRs are present in the cells of most organs in the body, suggesting that there is wide diversity in the types of responses that vitamin D3 can promote [19-20].

One of the target cells is **osteoblast**. Osteoblasts are bone-forming cells that play an essential role in the development and maintenance of bone extracellular matrix and they are target cells for vitamin D. Osteoblasts express vitamin D receptors (VDR) and vitamin D3 regulates the expression of osteoblastic specific genes such as osteocalcin and osteopontin [21].

**Satue et al. in 2014** reported that ultraviolet activated 7-dehydrocholesterol (7-DHC) has been successfully used as a biocompatible coating for titanium (Ti) implants producing active vitamin D with positive effect on osteoblast differentiation and a significant decrease of osteoclastogenesis [22]. Moreover, an experimental study by **Satué et al. in 2015** showed that the use of ultraviolet irradiation on 7-dehydrocholesterol-treated titanium implants surfaces generates a bioactive coating that promotes the osteogenic differentiation of human umbilical cord mesenchymal stem cells, with regenerative potential for improving osseointegration in titanium-based bone anchored implants. [23]. A study carried by **Satué et al. in 2016** showed that titanium implants coated with UV-irradiated vitamin D precursor and vitamin E promote in vivo gene expression of bone formation markers and alkaline phosphatase activity, while they keep their osteopromotive potential in vitro and composition when stored up to 12 weeks at 4°C [24]. Finally a study carried by **Gogolewski S et al. in 2007** showed that a biodegradable polyurethane bone graft substitutes with vitamin D3 could enhance bone regeneration of bicortical defects in the iliac crest of oestrogen-deficient sheep [25]. On the basis of these studies, it has been suggested that calcitriol might exert a positive local effect

on alveolar bone regeneration. So this study will be carried to explore the regenerative possibility of locally delivered vitamin D3 in alveolar ridge preservation.

**Purpose of the Study:** The purpose of this randomized controlled clinical trial study was to evaluate & compare preservation of alveolar ridge dimensions following a traumatic tooth extraction using collagen membrane and xenograft mixed with active vit D3 versus collagen membrane & Xenograft only.

## Materials and Methods:

**Patient selection:** Ten patients were selected from the clinic of the oral diagnosis, Faculty of Dentistry, Ain Shams University. All patients required extraction of two non restorable premolars (or anterior teeth) located in symmetrical quadrants of maxillary or mandibular arches and requested implant restoration. All patients had no particular medical history (Medically free). Male or female with age range 20- 40 years were included in the study.

Smokers, patients who showed residual infection in the edentulous area, patients with poor oral hygiene and vulnerable groups as pregnant females and decisionally impaired individuals were excluded from the study. The nature of the study was explained to each patient and a signed, written, informed detailed consent form was obtained from all patients prior to any study-related procedures.

Preoperative analysis included complete medical history, past dental history, clinical examination, clinical photographs and Cone beam computed tomography to evaluate edentulous alveolar ridge morphology and to measure height and width of the alveolar ridge.

**Preparation of vitamin D<sub>3</sub> gel:** Active vitamin D<sub>3</sub> gel was prepared by (pharmaceutical factory, Faculty of Pharmacy, Tanta University). Accurately weighted methyl cellulose was added to required amount of biocompatible solvent to prepare methyl cellulose in situ gel. The mix was heated at 50 oC to 60 oC

and shaken well with a mechanical shake to obtain a clear solution. Weighted amount of aqueous solution of vitamin D<sub>3</sub> (active material manufactured by MUP company (medical union pharmaceuticals)) was added to previous solution and dissolved completely to obtain a homogenous gel then it loaded in sterile plastic syringes and stored in dry cool environment for use.

### **Surgical procedures:**

Two surgeries were carried out for each patient; the first was for alveolar ridge preservation while the second was for taking core bone biopsy and implant placement in a routine fashion.

The first one involved atraumatic extraction under local anesthesia and alveolar ridge preservation (ARP) by xenograft (hypross, Germany) and collagen membrane (hyprosb, Germany) in control site and alveolar ridge preservation (ARP) by xenograft and active vitamin D<sub>3</sub> gel and collagen membrane in test site. In the both sites the barrier membranes cover the graft & its edges were placed beneath the top margins. Mucoperiosteal flaps were always replaced and sutured with 4-0 polypropylene.

**Post-surgical care for both groups:** Oral antibiotic (amoxicillin 875mg/clavulanic acid 125 mg) and oral non-steroidal anti-inflammatory were prescribed for 1 week. Postoperative antibiotics following bone regenerative techniques were recommended by several authors [38,40,41]. Patients were also instructed to use Chlorhexidine mouth wash (*Antiseptol. Cairo pharmaceuticals*) three times daily for 2 weeks starting the second day following the surgery to reduce the risk of infection. The sutures were removed ten days following the surgery.

**Surgical procedures for bone core biopsy and placing implant (Re-entry Procedure):** After 3 months after the grafting surgery, a reentry procedure was performed in order to take bone core biopsy, and to place implants. Paracrestal incision and full thickness

mucoperiosteal flap was elevated labially and palatally. A trephine bur of 2 mm inner diameter (Hu-Friedy, USA) was used to obtain bone biopsy at the implant site before preparation of the implant recipient bed. The implant site preparation was then performed in routine fashion. All implants in both groups were placed with adequate primary stability ( $\geq 35$ ) N.cm. Then the cover screws were placed and the flaps were closed using interrupted sutures for 7-10 days.

### **Clinical and radiographic evaluations**

:The alveolar ridge labiolingual horizontal width was measured before and after the augmentation procedures using the bone caliper (Com Dent, UK) and the Cone Beam Computed Tomography (CBCT). To optimize the vertical measurements, a customized acrylic template was used as a fixed reference guide to introduce the K-Files that were used in order to register the vertical bone height at four sites.

**Histological evaluation:** After obtaining the Bony specimens, they were then quickly removed from the trephine bur and fixed in 10% neutral buffered formalin solution. The specimens were then rinsed with distilled water and then decalcified then they were stained with H&E and Masson's Trichrome for qualitative and quantitative measurements of bone trabeculae and osteoid tissue.

**Statistical analysis:** Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data of horizontal and vertical values and area fraction were normally distributed (parametric data), therefore, unpaired t test was used for intergroup comparisons, while paired t test was used for intragroup comparisons.

## Results:

### **Horizontal measures (width) for alveolar ridge by bone caliper**

**In control side**, In control group: a higher mean value was recorded at baseline. The absolute value of difference between baseline and 3 months was  $1.06 \pm 0.42$ . Paired t test revealed that this difference was statistically significant **and in test side**, a higher mean value was recorded at baseline. The absolute value of difference between baseline and 3 months was  $0.813 \pm 0.53$ . Paired t test revealed that this difference was statistically significant ( $p=0.003$ ). greater percent of bone loss was noted in control group more than test group after 3 months but not statistically significant ( $p=0.171$ ) , **Horizontal measures (width) for alveolar ridge by CBCT**

Showed that a higher mean value was recorded at baseline and decrease after 3 months. The absolute value of difference between baseline and 3 months in both groups this difference was statistically significant and in comparison of percent change after three months there. was greater mean percent decrease was noted in control group but the difference was not statistically significant.

**vertical measures (height) for alveolar ridge by customized acrylic stent. In control side**, a higher mean value was recorded at 3 months. The absolute value of difference between baseline and 3 months was  $2.18 \pm 0.449$  **and in test side**, a higher mean value was recorded at 3 months. The absolute value of difference between baseline and 3 months was  $1.76 \pm 0.29$ .

statistical analysis for **new bone formation** our results showed higher mean value was recorded in the **test side** ( $24.26 \pm 5.66$ ) in comparison to **control** ( $17.91 \pm 5.46$ ) and this was **statistically significant**.

## Discussion:

Many studies mentioned that the presence of a tooth has crucial role in maintaining the dimensions of the alveolar process. During the alveolar wound healing, most changes occur in the first months and a reduction in the vertical ridge height with a horizontal reabsorption in the buccolingual direction must be expected [26-27].

Furthermore, many studies showed that success of Osseo integrated dental implants depends on the presence of a sufficient volume of healthy bone at the recipient efforts site at the time of implant placement. Thus, it seems prudent to prevent alveolar ridge destruction and make to preserve it after extraction procedures [28] .

Regarding to the results of horizontal measures (width) for alveolar ridge by bone caliper in control group, a higher mean value was recorded at baseline. The absolute value of difference between baseline and 3 months was, also in test group higher mean value was recorded at baseline this might due to decrease in bone width after extraction and this in agreement with previous reports that describe a post extraction healing always characterized by osseous reabsorption especially in the horizontal plane of the residual alveolar ridge [29-30]

Regarding to vertical measures (height) for alveolar ridge by customized acrylic stent. **In control side**, a higher mean value was recorded at 3 months. The absolute value of difference between baseline and 3 months was  $2.18 \pm 0.449$  **and in test side**, a higher mean value was recorded at 3 months. The absolute value of difference between baseline and 3 months was  $1.76 \pm 0.29$  and this feature agreement with other systemic reviews showed that a certain degree of ridge height loss should be expected even if ARP is applied. So, ARP may significantly prevent post extraction alveolar bone remodeling, but this effect is variable, likely due to the influence of local and systemic factors [31].

Regarding to our histomorphometric analysis for **new bone formation** our results showed higher mean value was recorded in the **test side** ( $24.26 \pm 5.66$ ) in comparison to **control** ( $17.91 \pm 5.46$ ) and this was **statistically significant** difference ( $p < 0.0001$ ). So our results indicated that **locally applied vit D<sub>3</sub>** exhibited positive effects on bone remodeling by reducing osteoclastogenesis and promoting osteoblast differentiation, leading to accelerated bone regeneration and new bone formation. This supported by **lui et al., 2015** who suggested that local administration of vit d3 accelerates bone formation and promotes bone maturation and regulated collagen maturation, he used same dose of local vit d3 and also use same stain in histological analysis [32] and also **Rajkovi et al., 2015** showed that locally applied vitamin D3 in combination with bovine bone mineral matrix improve the bone formation and strengthen the site of the fracture in ovariectomized rats. Moreover, study by **yoon et al., 2007** showed that local vitamin d3 loaded in Biodegradable PLGA scaffolds provide more new bone formation when applied in bone defect in femur [33].

## Conclusion:

Local applied of vitamin D<sub>3</sub> with xenograft in alveolar ridge preservation accelerates new bone formation and promotes bone maturation when comparing with xenograft only

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