**Republic of Iraq**
**Ministry of Higher Education**
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**University of Baghdad**
**College of Dentistry**

# *EVALUATION OF LOCAL APPLICATION OF VITAMIN D3/ BETA-TRICALCIUM PHOSPHATE ON HEALING PROCESS OF INDUCED BONE DEFECT IN RABBITS BY mRNA RUNX2 AND VITAMIN D3 RECEPTOR EXPRESSION*

**( HISTOLOGICAL AND HISTOMORPHOMETRICAL STUDY)**

A Thesis submitted to the council of the College of Dentistry/ University of Baghdad in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Oral Histology

By

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# ABSTRACT

**Background:** Defects of osseous tissues have become a global health care problem. Beta-tricalcium phosphate (β -TCP), a ceramic alloplastic, has interconnected system of micropores, world widely utilized as a biologically safe osteoconductive bone substitute. Vitamin D3 (Vit.D3) is a fat-soluble, as it is biosynthesized from cholesterol and controls gene expression by binding to nuclear Vitamin D3 receptors (VDRs) therefore, it is considered a hormone. Active form of Vit.D3 (calcitriol) can affect human osteoblast growth and differentiation stimulating bone formation and mineralization. The reverse transcription real-time quantitative PCR (RT-qPCR) is probably the most straight forward and essential measurement technique available for RNA quantification and is widely used in many branches. Formalin-fixed paraffin embedded (FFPE) tissue regards a treasury of samples undiscovered for biomedical research. The chemical RNA–protein crosslinking and RNA fragmentation challenging possibility to extraction of RNA from FFPE tissue due to both of which heavily impact on RNA quantity and quality for downstream analysis.

**Objectives of the study**

**Primary objective**- Investigation of Runx2 and Vitamin D3 Receptor expressions in relation to cellular proliferative activity during early phase of bone healing after local application of *β* -TCP and vitamin D separately and in combination .

**Secondary objective**- Study the effect of local application of *β* -TCP and vitamin D separately and in combination on bone deposition in rabbits by histological and histomorphometric analysis.

**Materials and methods:** Twenty-four adult male New Zealand rabbits weighting an average of (1.5 – 2 kg) were used in this study and 24 rabbits

divide into two healing periods 7 and 21 days (12 for each healing period). Four intra bony holes (3) mm in depth and (4mm) diameter were created in both tibias (2 holes in each one) for each animal and divided as follows:

1- Group A: Bone defect left to heal spontaneously as control (C group).

2- Group B: Bone defect filled with *β*-TCP (TCP group).

3- Group C: Bone defect filled with (calcitriol) (VitD3 group).

4-Group D: Bone defect filled with combination of *β* -TCP and (calcitriol) Vit.D3. in a ratio of 1:1(TCPD group).

Animals sacrificed at (7) and (21) days done by over dose of general anesthesia. Histological and histomorphometric analysis of bone microarchitectures (bone trabeculae number, trabecular area and bone marrow space) and bone cells count (osteoblasts, osteocytes and osteoclasts), Analysis of mRNA of both Runx2 and Vitamin D3 Receptor expression of the formalin fixed paraffin embedded(FFPE) in the early phase of induced bone defect healing by RT-PCR technique was done on all groups for both healing periods.

**Results:** Histological findings indicated that bone defects in (TCPD), (TCP) and (Vit.D3) groups showed early bone matrix formation at 7 days and signs of early mineralization in comparison to healing of (Control) group at 21 days. Histomorphometric analysis for all bone parameters examined in this study, showed variation in significance among all groups in both durations.

The expression of mRNA of RUNX2 fold change revealed high significant differences at 7 days among the studied groups except between TCPD and TCP groups there was no significant difference with highest mean value appeared in TCP groups, while at 21 days highest mean of expression change was recorded in TCPD group.

At both healing periods 7 and 21 days of mRNA VDR gene expression showed high significant difference among groups and highest

mean value recorded in TCPD followed by Vit.D3 groups in both durations.

**Conclusion:** The study revealed that combined application of vit.D3\ β-TCP (TCPD group) was more effective in enhancement of bone regeneration and in acceleration of bone healing process. The measurements of mRNA of RUNX2 by RT-PCR revealed possible osteoinduction activity of β-TCP at 7 days duration. Moreover, the direct local application of active form of Vit.D3 showed osteoinductive effect, as indicated by mRNA VDR expression.

جمهورية العراق
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جامعة بغداد
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# *تقييم التطبيق الموضعي لفيتامين د وبيتا ترايكالسيوم فوسفات على عملية شفاء العظام المستحث في الأرانب بواسطة التعبير الجيني للحامض النووي الرايبي لعامل النسخ المرتبط الثاني ومستقبلات فيتامين د*
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