Localization of transforming growth factor-beta expression in the peri-implant tissues of dental implants coated with placental collagen

Athraa Y. Alhijazi, B.D.S., M.Sc., Ph.D. [(1)]
Basim Mohammed Khashman B.Sc., M.Sc. [(2)]
Ban Abdul Ghani, B.D.S., M.Sc., Ph.D. [(3)]
Eman Isaa, B.D.S., M.Sc., Ph.D. [(4)]

ABSTRACT

Back ground : The transforming growth factor beta (TGFB) signaling pathway is involved in many cellular processes in both the adult organism and the developing embryo including cell growth, cell differentiation, apoptosis. The interaction between implant material and surrounding tissues is believed to play a fundamental role in implant success and illustrates different expression of growth factors by different cells that involved in the formation of peri-implant tissue.

The aim of this study was to localize expression of TGF B by newly formed bone tissue around surface-conditioned implants with placental collagen at different time intervals: 3 ,7,14,28, and 56 days .

Materials and Methods: Commercially pure Titanium (CPTi) implants coated with collagen protein were placed in the tibia of 20 new Zealand rabbits . Immunohistochemical study for localization of TGF B in peri –implant tissue for interval periods 3 ,7,14,28, and 56 days was performed under light microscope..

Results: Positive expression of Transforming growth factor B can be detected in osteoblast, osteocyte, newly deposited matrix includes collagenous tissue and non mineralized osteoid tissue. Endothelial cells line blood vessel showed positivity too. Minerlized bone trabeculae and mature bone illustrate negative expression.

Conclusion: The present study suggests that placental collagen, coated Ti implant illustrates positive expression of transforming growth factor B by osteoblast and endothelial cell that enhanced bone formation.

Key words: Transforming growth factor, dental implant, bone. (J Bagh Coll Dentistry 2013; 25(2):66-69).

INTRODUCTION

Transforming growth factor-beta (TGF-beta) is a multifunctional cytokine, whose numerous cell and tissue activities include cell-cycle control, the regulation of early development, differentiation, extracellular matrix formation, hematopoesis, angiogenesis, chemotaxis, immune functions, and the induction of apoptosis.[(1)]

Titanium (Ti) surface modifications aiming to increase implant osseointegration is one of the most active research areas in dental implantology [(2)]. Many studies concerned with Surface-conditioned dental implants [(3)], include implants coated with collagen increases bone formation and implant stability, compared with uncoated controls. Data analysis suggests that collagen has a positive influence on bone formation of endosseous heal [(4)]. Recent researches studied the role and the expression of growth factors in peri-implant tissues include TGF B [(5)], VEGF (angiogenesis) during early bone formation [(6,7)].

(1)Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(2) Assistant Lecturer, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(3) Assistant Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.
(4) Assistant Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad.

MATERIALS AND METHODS

Materials

- Commercially pure titanium (CPTi) implant from Friatec AG company in diameter 3.5 mm and 8mm in length (5mm threaded and 3 mm flat).
- Placental Collagen protein (N0. C-7521, SIGMA P).
- Rabbit polyclonal to Transforming Growth Factor (TGF) beta 3 from Abcam company UK (ab15537). Rabbit Specific HRP/DAB Detection kit from Abcam company (England) (ab80436).

Methods

Twenty New Zealand rabbits, age (10-12 months) were used to insert CPTi implant coated with collagen in their tibea. Each 4 animals were sacrificed at interval periods 3 ,7, 14, 28, and 56 days.

Immunohistochemical investigation

Sections of 5µm thickness of paraffin embedded specimens for all study animals were carried for immunohistochemical localization of TGF B, examined under light microscope and in accordance with manufacturer instruction. Positive reading was indicated when the cells display a brown cytoplasmic pigmentation staining, while negative reading was indicated for absence of immunostaining.

RESULTS

Expression of TGF B in peri-implant tissue of dental implant coated with collagen shows positive chromogen DAB by collagen mesh and endothelial cells of blood vessel after 3 days post operation period, figures [1, 2].

At 7 day duration, positive expression of TGF B localized in newly deposit collagen fibrous tissue and in osteoblast cells in woven bone, figure [3].

Figures [4, 5] illustrate positive expression of TGF B by osteoblast and osteocyte ,while bone trabeculae and basal bone showed negative DAB stain .This findings related to dental implant coated with collagen for 14 days duration.

At 28 day post operation period, positive expression of TGF B identified in osteoid tissue while negative result illustrated in bone trabeculae and basal bone by staining with hematoxylin counter stain, figures [6, 7].

At 56 day post operation period , osteoblast cells rimming bone surface,osteocyte and reticular connective tissue showed positive expression for TGF B .While basal bone and thread illustrate negative DAB stain.

Figure 1: Immunohistochemical view for positive identification of expression of TGF B in threads (arrow) of Ti implant coated with collagen, 3 days duration post operation.DAB chromogen with hematoxylin counter stain ×20.

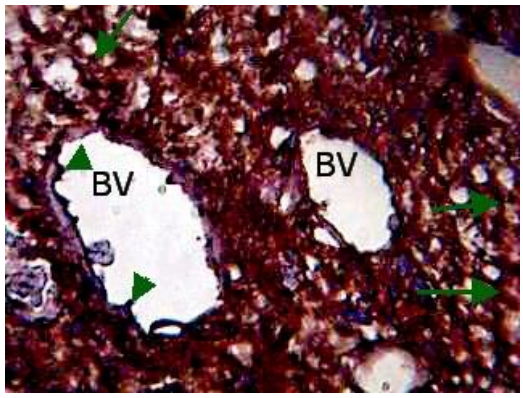

Figure 2: Magnifying view for previous figure (1) shows positive expression of TGF B in endothelial cell(arrow head) in blood vessel(BV), and in collagen mesh(arrow). DAB chromogen with hematoxylin counter stain ×40 .

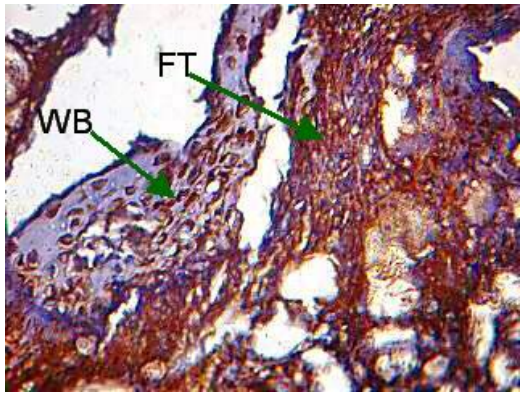

Figure 3: Positive expression of TGF B by proliferative osteoblast in woven bone(WB) and fibrous tissue(FT) of Ti implant coated with collagen, 7 days duration post operation.DAB chromogen with hematoxylin counter stain ×10.

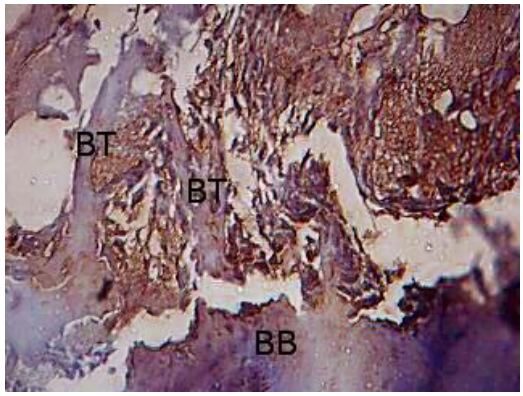

Figure 4: Immunohistochemical view illustrates negative expression of TGF B by bone trabeculae(TB), and basal bone(BB).While osteoid tissue around shows positivity. Ti implant coated with collagen,14 days duration post operation. DAB chromogen with hematoxylin counter stain ×10 .

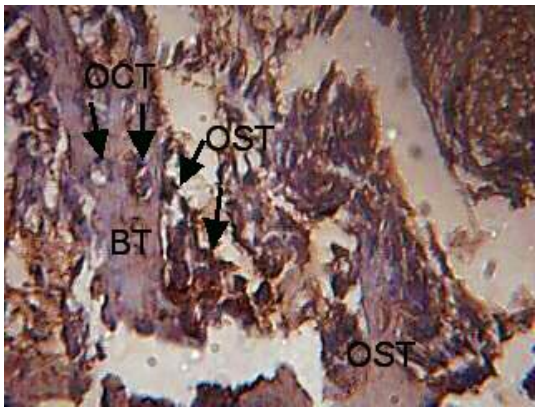

Figure 5: Magnifying view for previous figure (4) shows positive expression of TGF B by prolifrative osteoblast (OST) and osteocyte(OCT). DAB chromogen with hematoxylin counter stain ×20 .

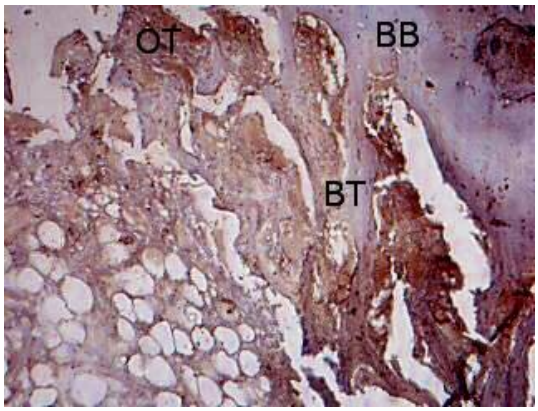

Figure 6: Negative expression of TGF B in bone trabeculae (BT) and basal bone (BB) ,while osteoid tissue (OT) shows positivity. Ti implant coated with collagen, 28 days duration post operation.DAB chromogen with hematoxylin counter stain ×10 .

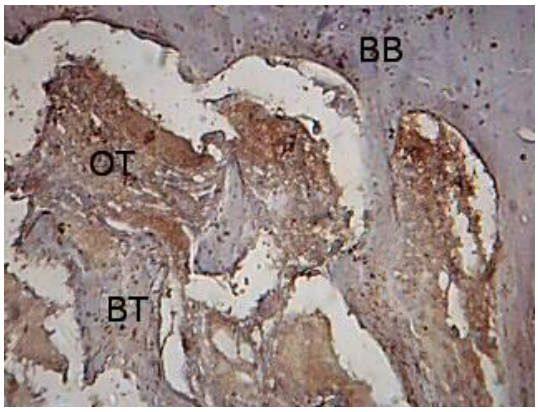

Figure 7: Magnifying view for previous figure (6) shows negative expression of TGF B in bone trabeculae (BT) and basal bone (BB) ,while osteoid tissue (OT) shows positivity.DAB chromogen with hematoxylin counter stain ×20

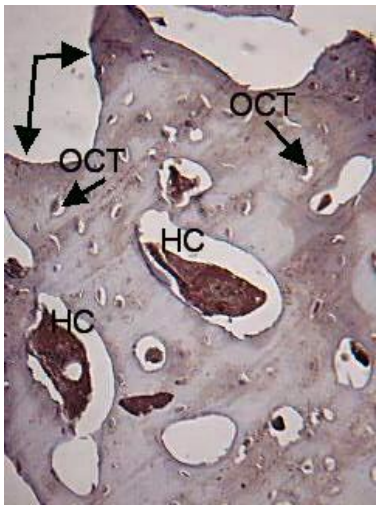

Figure 8: Mature bone of Ti implant coated with collagen, 56 days duration post operation ,shows well developed threads (arrow), positive DAB for TGF B by osteocyte (OCT) and content of haversian canal (HC) . DAB chromogen with hematoxylin counter stain ×20.

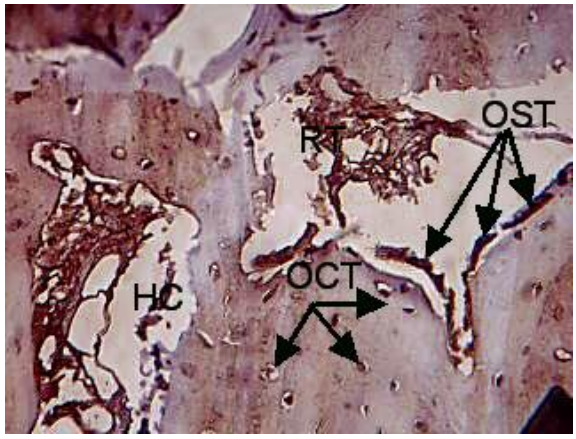

Figure 9: Magnifying view ,illustrates haversian canal (HC), positive DAB stain for expression of TGF B by osteoblast (OST) , reticular tissue(RT), and osteocyte (OCT) within mature new bone. DAB chromogen with hematoxylin counter stain ×40.

DISCUSSION

The distribution patterns of the cells were examined on surface of the titanium-dental implant with studied of adaptive cellular responses to the implant material include alterations in the cytoskeleton, integrin expression, synthesis of extracellular matrix proteins and cytokines like TGF B [8] .

Successful attachment on artificial surface is prerequisite for inducing new bone formation locally at the site of implantation. Protein-coated surfaces may influence the biocompatibility of

implant materials by initiating and supporting osteogenesis [9].

Collagen, fibronectin, vitronectin or mixtures of natural extracellular matrix proteins are the mostly investigated proteins for this purpose [10,11].

The present findings illustrate a positive expression of TGF B by collagen mesh at 3 day post-operative duration, which coincide with results of Holmes et al [12] and Verrecchia and Mauviel [13] ,who reported that TGF-β has been characterized as a cytokine that plays a vital role in driving fibrosis via promoting induction of matrix proteins, including type I collagen,and as a key player in fibrogenesis

O`Toole et al.[14] demonstrated that osteoinductive protein such as bone sailoprotein, fibronectin and collagen that coated surfaces of implant may stimulate adherence of osteoblas to its surface. These results coincide with present results which show positive expression of TGF B by osteoblast in different osseointegretion periods ,and this may attributed that, osteoblasts is up-regulated by hormones and cytokines that promote bone formation as demonstrated by transforming growth factor-beta .In addition, Our results presented a set of evidences that coating CPTi with collagen may stimulate bone formation at the cellular and molecular levels, include positive expression of TGF B by endothelial cells and by unminerlized osteoid tissue .While mineralized bone shows negative DAB stain ,and this may be related to TGF-β role as one of the most important factors in the regulation of the production of newly formed tissue during early healing periods represented by it's positive expression by endothelial cells and haversian canal content of established, well developed bone.

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