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Expression of TGFβ1 by Pulp Tissue of Human Permanent and Primary Teeth Capped by Biodentine™

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Abstract
Biodentine™,represented a novel bioactive tricalcium silicate cement ,that is introduced into dentistry. It was suggested to be biocompatible, having optimal working and setting time, excellent workability with superior adhesion to tooth structure. Biodentine™ was proved to maintain pulp as a vital tissue and enhance dentinal bridging, as alternative to Mineral trioxide aggregate that can be provided with a better handling characteristics and shorter working time. Moreover, dental pulp cells have the potential to differentiate into odontoblast-like cells and enhanced reparative dentine.In the present study "Biodentine™" was directly applied on the dental pulp of cultured tooth of (36) human maxillary first premolars and (36) human maxillary first primary molar tooth . After various culture periods (2,14 and 28) days, the interaction of the material with dental pulp tissue was analyzed on tissue cultures. The effect of this material on TGFB1 expression on pulp tissue was studied by immunohistochemical investigation. The results illustrate that Biodentine™" induced mineralized foci formation early after its application as direct pulp capping material in permanent and primary teeth. The mineralization appeared beneath the reparative dentine. Biodentine™ shows a highl significant increment of TGF- β1 expression by pulp cells (P< 0.01) in both permanent and primary teeth.**Conclusions**: When "Biodentine™" was applied as direct pulp capping material for permanent and primary teeth, it induced an early form of reparative dentine synthesis, probably due to a modulation of pulp cell for expression TGF-β1.
Keywords: Transforming growth factor(TGF), Biodentine, pulp cells

1.INTRODUCTION

The conservative therapy of vital pulp for the temporary tooth seems to be important to preserve the mesiodistal space and the vertical dimension that guide the physiological position of normal eruption of succor teeth [1]". Therefore the preservation of vitality in the primary dentition is important to avoid all risks of periapical diseases that could compromise the fate of immature permanent tooth [2]". When the tooth is mature, the therapeutic aims will also be directed towards preserving pulp vitality, especially if the patient is young [3,4]". A new experimental calcium silicate based restorative cement has been developed, named as "Biodentine™ [5]".

Many studies have demonstrated that "Biodentine™" significantly increased TGFβ1 secretion level by injured cells after 14 days and induced odontoblast differentiation from pulp progenitor cells [6,7]".

Transforming growth factor beta family proteins have a potential role in regulation of a variety of cellular function, matrix synthesis, and tissue repair of the dental pulp ·And in the human tooth, odontoblasts express TGFβ1 that becomes sequestrated within the matrix and induced pulp repair by including cell proliferation , cell migration, and type I collagen synthesis [8,9]"

The present study was designed to evaluate pulp response for the direct application of "Biodentine™" with the expression of TGFβ1.

2.MATERIALS AND METHODS

2.1 Study samples

Thirty six healthy human maxillary permanent first premolars freshly extracted from 9-10 years old patients and thirty six human maxillary first primary molar extracted for orthodontic purposes were collected according to National Research Council's guide by informed patients and parents 'consent and institutional review board approval of the protocol used.

2.2 Materials

- polyclonal antibodies (TGF-b1, Abcam UK).
- BiodentineTM (Septodont, Saint-Maur-des-Fosses, France).

2.3 Methods

2.3.1 Direct pulp capping with Biodentine™ using a human entire tooth culture model

All teeth samples were stored for 2 h at 4 C° in a Dulbecco's Modified Eagle medium (DMEM) supplemented with 500 UI mg/1 penicillin, 500 mg/1 streptomycin and 0.75 mg/1 amphotericin. Then a cavity Class I was done on each tooth at the occlusal aspect using air turbine carbide # 301 bur with copious water cooling, (bur for

every cavity) the cavity was 3mm in width and 2mm in depth by used orthodontic ruler, and the depth was limited to reach shiny pulp, then pulp exposure were induced using dental explorer in the middle point of the cavity. "Biodentine™" was prepared by squeezing out the liquid of a single-dose container into the powder-containing capsule. The capsule was then placed in a mixing device The cavity was dried with a sterile cotton pellet, and Biodentine™ was applied as a direct pulp capping material without any conditioning treatment of enamel/dentine. For the untreated group (without medication) the exposure were left without medication and cavities were sealed with promedica photo polymerized resin (Composan LCM,*PROMEDICA*, Germany) .Then sterile metallic wire was sealed on the crown with a little drop of photopolymerized resin. The roots of the teeth were suspended into DMEM supplemented with 20% fetal bovine serum, 100 mg/l penicillin, 100 mg/l streptomycin and 0.062mg/l amphotericin B in 12-well cell culture plates. The culture medium was changed every day. The cultured teeth were incubated in 37oc for 2 days (n = 24), 14 days (n =24), 28 days (n = 24) .

2.3.2 Histological evaluation

At the end of each culture period, the teeth were fixed in 10% formalin solution, decalcified, paraffin embedded and routinely processed as described previously [10]". Ten slides per tooth were stained with haematoxylin and eosin.

2.3.3 Immunohistochemical evaluation

TGF β1 was evaluated in odontoblastic layer and in subodontoblastic layer include stromal cell (fibroblast, mesenchymal cell, inflammatory cell) [11]". For each specimen, the number of positive cells that expressed of TGFβ1 was determined under x40power field by counting positive cells in100 cells for each slide, and then the mean of count for eight slides .

2.4 Statistical analysis

The statistical methods which was done using spss software program version 19 and including:

A- Descriptive statistics

1. Means, Standard deviations, Standard errors, Frequency, Percentage, Statistical tables and figures.

B- Inferential statistics

1. Mann-Whitney U test 2. Chi square

3.RESULTS

Histological findings for inflammatory response showed a significant difference at 2 and 28 days in permanent teeth treated and untreated with Biodentine™" .while high significant difference was found in 14 days, as presented in table (1) .For primary teeth ,they showed significant difference at 14 days and highly significant at 28(table 2).

Table (3) represent the result of inflammatory response in permanent and primary teeth treated with Biodentine™ ,that showed no significant differences in all time intervals (P> 0.05).

The **histological results** demonstrated that all experimental permanent teeth capped with Biodentine™" illustrated deposition of reparative dentine underneath the physiologic dentine. Odontoblasts showed normal appearance with their odontoblastic process included along the pulpal walls where the dentinal tubules had been cut underneath cavity preparation, and in all experimental primary teeth capped with Biodentine™" illustrated deposition of reparative dentine underneath the physiologic dentine, while the untreated primary teeth showed internal resorption of dentin with vacuolization in pulp, and necrotic tissue as shown in figure (1,a,b,c,d).

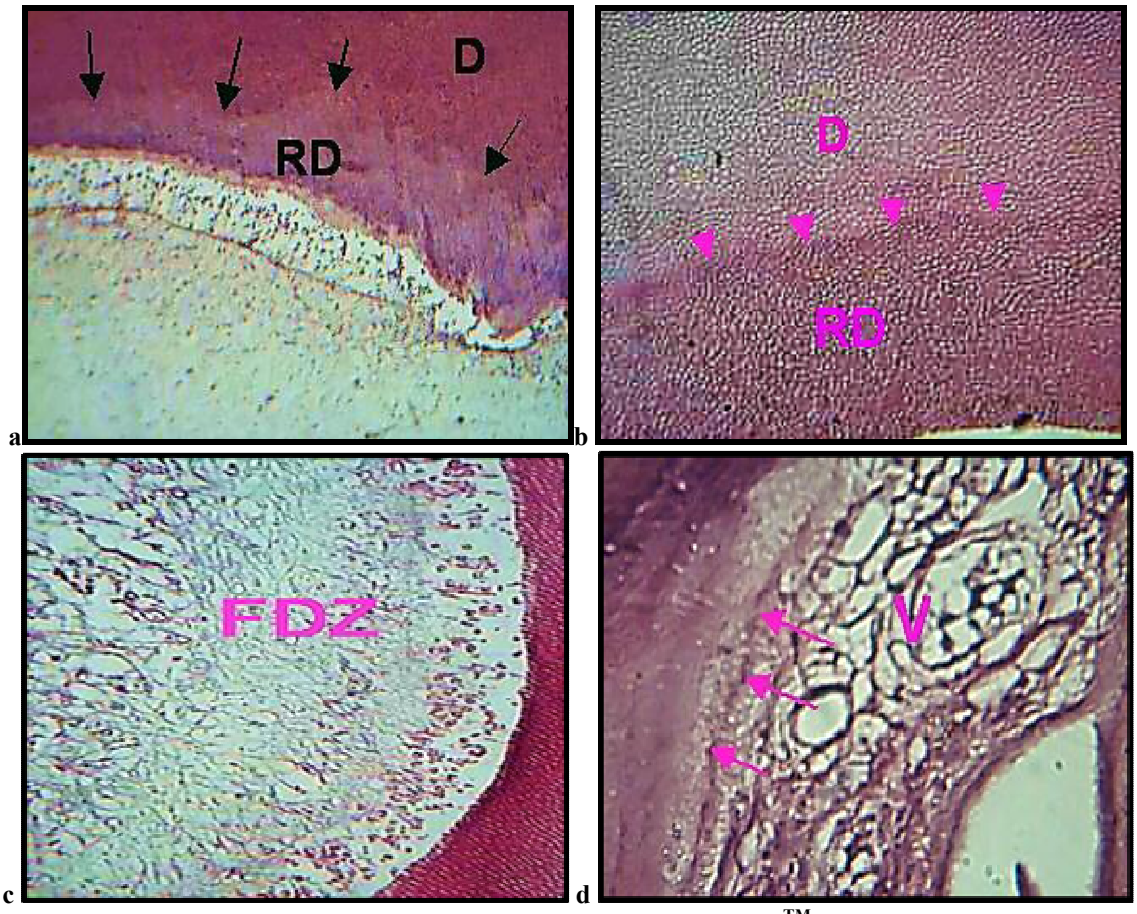

Figure 1: a-Pulp repair of permanent teeth treated with Biodentine™" , 28 day duration, shows reparative dentin (RD) underneath the physiologic dentin (D), separated demarked line b-Demarked line (arrow heads)in primary teeth treated with Biodentine™" , 28days duration separated reparative dentin (RD) from the old physiologic dentin (D). c-Pulp tissue of permanent teeth untreated with Biodentine™", 28 day duration shows demarked fibrous degenerative zone (FDZ) observed in sub-odontoblast area, lack of dentin bridge formation. d- Vacuolization (V) in pulp of primary teeth untreated with Biodentine™", 28 day duration, with internal resorption of dentin (arrows).

For **immunohistochemical findings**, pulp tissue of permanent teeth treated with Biodentine™ exhibits positive stain of TGFB1by odontoblast like cell proliferation and newly formed reparative dentine figure (2,a), whereas pulp tissue of untreated permanent teeth presented negative reactivity of TGFB1 with a faint stain of fibrosis degenerative zone figure (2,b).

Pulp tissue of primary teeth treated with Biodentine™" ,it showed positive immune reactivity to TGFB1 by hyaline blood vessels figure (2,c), while in untreated primary teeth exhibited negative immune reactivity to TGFB1 by necrotic tissue figure (2,d).

Immunohistochemistry study for the expression of TGFB1,indicated that Biodentine™ significantly increased TGFB1 secretion from pulp cells ($P < 0.05$) in both permanent and primary teeth. TGFB1 expression in 2nd days in primary teeth treated and untreated with BiodentineTM", illustrated a high significant difference by odontoblast cells and a significant difference in permanent teeth (treated and untreated) for the TGFB1positive expression by stromal cells,as presented in table (4) .

Table(5) demonstrated a non significant difference at ($P> 0.05$) in expression of TGFB1 between the permanent and primary teeth treated with Biodentine™ at 2 days period.

While untreated permanent and primary teeth showed a high significant difference at ($P< 0.01$) by odontoblast cells, and a non significant difference to stromal cells.

Table (6)showed a high significant difference at ($P< 0.01$) in TGFB1expression to odontoblast cells in permanent and primary teeth treated with Biodentine™" in 28 days, and a non significant difference to stromal cells that appears too in both untreated teeth for odontoblast and stromal cells.

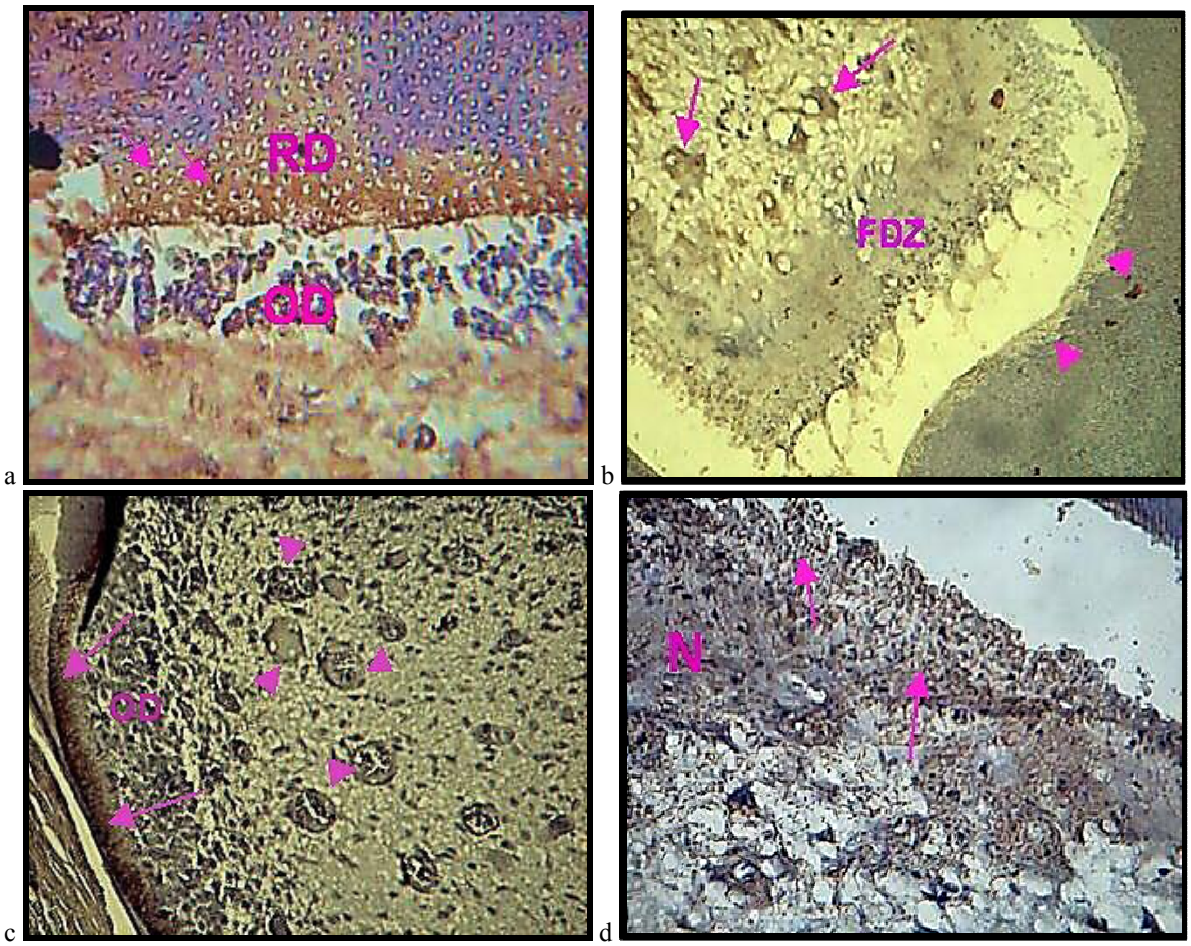

Figure 2: a- Permanent teeth treated with Biodentine (28 days),show reparative dentin (RD) ,odontoblast (OD) , odontoblast process (arrows) with intense TGFB1 immune-labeled b- Negative immunoreactivity TGFB1 in pulp tissue of permanent teeth untreated with Biodentine™",28 days duration show intense stain by hayalinized blood vessel (arrows), faint stain for fibro degenerative zone (FDZ), and reparative dentin (head arrows). c- ImmunoreactiveTGFB1 in pulp tissue of primary teeth treated with Biodentine™" 28 days duration shows intense stain by reparative dentin (arrows), odontoblast (OD), early hyalinized blood vessel (arrows heads). d- Necrotic tissue (N) in primary teeth untreated with Biodentine™" 2 days duration shows inflammatory cells (arrows).

4. DISCUSSION

The prognosis of direct pulp capping treatment is reported to be poor for primary teeth,and may related to high cellular content of primary pulp tissue that may be responsible for failures of direct pulp capping in primary teeth .It believes that undifferentiated mesenchymal cells may differentiate into odontoclastic cells in response to either the caries process or the pulp capping material, which could lead to internal resorption[12,13]". in the present study, however, no failure was observed in the primary and permanent teeth treated with Biodentine™ as pulp capping materials.

The results illustrated that using of Biodentine™ could induced TGFB1release from stromal pulp cells that stimulated odontoblasts and increased their secretory activity and reparative dentinogenesis.

The hard tissue formation beneath the Biodentine™ in the pulp wound area of permanent and primary teeth revealed that Biodentine™ is a biocompatible material for pulp capping, in which the odontoblast like cells have the ability to form an extracellular matrix and then apposition of the minerals to form reparative dentin and to complete dentin bridge to protect exposed dental pulp[14,15]".

This early form of mineralization has already been observed after Biodentine™ application to permanent teeth using the same entire tooth culture model [16]". Mineralization foci were also observed in vivo after pulp capping with Biodentine™ of primary pig teeth [17]".

The reparative dentine synthesis is directly related to a disruption of the odontoblast cell, and the subsequent pulp healing requires the recruitment and differentiation of pulp progenitor cells to protect pulp tissue [18]".

The results illustrated that non-medicated permanent, primary teeth showed more inflammatory pulp reaction, less reparative dentin formation, pulp fibrosis, internal resorption of dentin ,vacuolization in pulp, and necrotic pulp of primary teeth. These findings suggested that photo polymerized resin may not contribute to pulp healing and the material is unable to cause stimulation to the pre-existing odontoblast for formation of reparative dentin.

This work provides further evidence that, in entire human tooth cultures, these cells can differentiate into odontoblast-like cells and secrete a form of reparative dentine after capping with Biodentine™. This form of mineralization was not the typical tubular one usually observed after longer delays. This is because of the fact that the ex vivo model used here has some drawbacks such as a culture period limited to 1 month, absence of noxious components clearance, absence of circulation and a limited inflammatory reaction. However, it allows investigating the early steps of dentine regeneration in a whole-tooth environment. It also allows prediction of dental pulp cells behavior after application of restorative materials, thus reducing the use of animal experiments before studies on human beings.

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Table (1): The inflammatory score in permanent teeth treated and untreated with Biodentine™

Permanent teeth	2nd days				14th days				28th days			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Treated	4 (33.3%)	1 (8.3%)	1 (8.3%)	0 (0%)	4 (33.3%)	2 (16.7%)	0 (0%)	0 (0%)	5 (41.7%)	1 (8.3%)	0 (0%)	0 (0%)
Un-treated	0 (0%)	1 (8.3%)	5 (41.7%)	0 (0%)	0 (0%)	1 (8.3%)	3 (25%)	2 (16.7%)	1 (8.3%)	1 (8.3%)	2 (16.7%)	2 (16.7%)
X^2	6.67				9.33				6.67			
Likelihood ratio	8.46				12.82				8.46			
d.f.	2				3				3			
p-value	*0.015 (S)				**0.005 (HS)				*0.037 (S)			

***P<0.01High significant (HS); **P<0.05 significant (S)

Table (2): The inflammatory response in primary teeth treated and untreated with Biodentine™

Primary teeth	2nd days				14th days				28th days			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Treated	3 (25%)	2 (16.7%)	1 (8.3%)	0 (0%)	4 (33.3%)	2 (16.7%)	0 (0%)	0 (0%)	5 (41.7%)	1 (8.3%)	0 (0%)	0 (0%)
Un-treated	0 (0%)	3 (25%)	3 (25%)	0 (0%)	0 (0%)	2 (16.7%)	2 (16.2%)	2 (16.7%)	0 (0%)	1 (8.3%)	3 (25%)	2 (16.7%)
X^2	4.2				8				10			
Likelihood ratio	5.41				11.09				13.86			
d.f.	2				3				3			
p-value	*0.067 (NS)				**0.011 (S)				***0.003 (HS)			

***P<0.01High significant (HS); **P<0.05 significant (S) ;*P> 0.05 Non significant (NS)

Table (3): The inflammatory response in permanent and primary teeth treated with Biodentine™ at different time intervals.

Treated	2nd days				14th days				28th days			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Permanent	4 (33.3%)	1 (8.3%)	1 (8.3%)	0 (0%)	4 (33.3%)	2 (16.7%)	0 (0%)	0 (0%)	5 (41.7%)	1 (8.3%)	0 (0%)	0 (0%)
Primary	3 (25%)	2 (16.7%)	1 (8.3%)	0 (0%)	4 (33.3%)	2 (16.7%)	0 (0%)	0 (0%)	5 (41.7%)	1 (8.3%)	0 (0%)	0 (0%)
X^2	0.48				0				0			
Likelihood ratio	0.48				0 *				0 *			
d.f.	2				1				1			
p-value	**0.79 (NS)				**1 (NS)				**1 (NS)			

*Continuity correction test
**P> 0.05 Non significant (NS)

Table (4): TGFB1 expression in 2nd days in permanent and primary teeth treated and untreated with Biodentine™

Types of pulp cells	Teeth samples	Mean±SD	Mann-Whitney U test	P-value
Odontoblast	Treated permanent	21.67±5.32	-1.45	0.15*(NS)
	untreated permanent	17.17±5.08		
	Treated primary	24.17±6.55	-2.9	0.004*** (HS)
	untreated primary	11.17±1.60		
Stromal	Treated permanent	9.5±3.56	-2.1	0.036 ** (S)
	untreated permanent	19.5±8.09		
	Treated primary	8.5±4.28	-1.85	0.07 *(NS)
	untreated primary	15.33±5.99		

**P<0.01High significant (HS); **P<0.05 significant (S) ; *P> 0.05 Non significant (NS)

Table (5): TGF-B1 expression by pulp cells at 2nd day teeth comparison

Types of pulp cells	Teeth samples	Mean±SD	Mann-Whitney U test	P-value
Odontoblast	**Treated permanent**	**21.67±5.32**	**-0.56**	**0.57*(NS)**
	Treated primary	**24.17±6.55**		
	untreated permanent	**17.17±5.08**	**-2.59**	**0.01**(HS)**
	untreated primary	**11.17±1.60**		
Stromal	**Treated permanent**	**9.5±3.56**	**-0.48**	**0.63*(NS)**
	Treated primary	**8.5±4.28**		
	untreated permanent	**19.5±8.09**	**-1.05**	**0.29*(NS)**
	untreated primary	**15.33±5.99**		

****P<0.01High significant (HS); *P> 0.05 Non significant (NS)**

Table (6): TGFB1 expression by pulp cells in 28 day teeth comparison

Types of pulp cells	Teeth samples	Mean±SD	Mann-Whitney U test	**P-value**
Odontoblast	**Treated permanent**	**10.67± 1.75**	**-2.89**	**0.004 ***(HS)**
	Treated primary	**5.17± 3.13**		
	untreated permanent	**5.50± 1.05**	**-1.71**	**0.09*(NS)**
	untreated primary	**2.17± 1.83**		
Stromal	**Treated permanent**	**3.33± 1.03**	**- 1.89**	**0.06*(NS)**
	Treated primary	**5.50± 1.87**		
	untreated permanent	**2.33± 0.52**	**-1.37**	**0.17*(NS)**
	untreated primary	**3.17± 3.25**		

****P<0.01High significant (HS) ;*P> 0.05 Non significant (NS)**

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