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The Effect of Incorporating Nanocalcium Phosphate Particles into Biodentine on Pulpal Tissue Response (In Vitro Study)

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ABSTRACT

Objectives: This study was conducted to evaluate the effect of incorporating Nanocalcium phosphate (NCP) particles into Biodentine™ on pulpal tissue response as a direct pulp capping material (entire tooth culture model). **Materials and methods: -** A total of forty freshly human premolar teeth extracted for orthodontic reasons were used. After cavity preparation and mechanical pulp exposure, the teeth were divided into 2 main groups (20 teeth each) according to the capping material used; where group (**M1)** refers to pulps capped with Biodentine™ , and group (**M2)** refers to pulps capped with Biodentine incorporated with NCP. Each group was further subdivided into two subgroups according to the culture period; where subgroup (**P1)** refers to teeth cultured for 14 days, and subgroup (**P2)** refers to teeth cultured for 28 days. At the end of each culture period, the teeth were histologically examined to assess the dentine bridge thickness of each group after 14 and 28 days. Moreover, elemental analysis of Calcium and Phosphorous ions in the dentine bridge was performed using SEM-EDX. **Results:** Direct pulp capping with Biodentine incorporated with NCP showed thicker dentine bridge when compared to Biodentine™ alone. There is a positive correlation between the weight % of Calcium and Phosphorous ions in the dentine bridge below both capping materials and the dentine bridge thickness. **Conclusion:** Biodentine incorporated with NCP could be a good candidate for pulp capping in the future.

KEYWORDS

Biodentine™ , Calcium Phosphate Nanoparticles, entire tooth culture model.

INTRODUCTION

Maintaining pulp health following carious, traumatic or iatrogenic injuries remains a challenge. Several biomaterials are used in vital pulp therapies, with the prognosis depending on several factors such as its biocompatibility and its ability to prevent bacterial microleakage;

A paper extracted from Doctor's Thesis entitled "The Effect of Incorporating Nanocalcium Phosphate Particles into Biodentine on Pulpal Tissue Response (In Vitro and In Vivo Study)"

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the outcome also depends on the pulp's ability to respond to injury [1].

Calcium hydroxide ($Ca(OH)_2$) has been considered the "gold standard" of direct pulp capping materials for several decades [2]. However, it was reported that $Ca(OH)_2$ has poor sealing ability to dentin, dissolves over time and that the dentin bridge adjacent to the material contains multiple tunnel defects [3]. Portland cements such as ProRoot_ MTA have been used for pulp capping. It stimulates reparative dentine formation faster and thicker than calcium hydroxide cement. However, MTA has delayed setting time, poor handling characteristics and is relatively expensive [4].

Many materials were recently developed to overcome the disadvantages of $Ca(OH)_2$ and MTA. Calcium silicate-based cement (Biodentine™) has been developed in 2010 by Gilles and Olivier [5]. With improved physical properties and reduced setting time to 12 minutes as compared to MTA [6], the new biomaterial can be used as dentine substitute in several clinical indications. Many Studies have proved the biocompatibility of Biodentine™. It has positive effect on vital pulp cells, promotes the formation of reparative dentin and stimulates tertiary dentine formation [7]. Biodentine™ offers certain advantages over calcium hydroxide; it has high compressive strength, decreased porosity, higher density, being less soluble and produces tighter seal to dentin [8]. Biodentine™ was reported to be denser and less porous when compared to MTA [9].

Synthetic calcium phosphate has been considered as ideal biomaterial with excellent biocompatibility due to the similar chemical properties to the inorganic component in calcified tissues [10]. Nanostructured calcium phosphate(NCP) materials play an important role in the formation of hard tissues in nature. It was reported that calcium phosphates materials in nano-size can mimic the dimensions of constituent components of calcified tissues. The highly soluble (NCP) have small particle size and large surface area facilitating the fast release of Ca and PO_4 ions making them good candidates for tooth remineralization [11]. Thus, Nanocalcium phosphate particles was selected in this study to be incorporated into Biodentine™ .

This current study tried to answer the following questions: Does the incorporation of nanostructured calcium phosphate (NCP) into Biodentine™ have a positive effect on pulpal tissue? Could the Biodentine™ incorporated with NCP be a good candidate for pulp capping?

Hence, this study was designed to evaluate the effect of using Biodentine incorporated with nanostructured calcium phosphate (NCP) on pulpal tissue response as a direct pulp capping material in vitro.

MATERIALS AND METHODS

Preparation of β-Tricalcium Phosphate Nanoparticles:

Nanocalcium phosphate particles (NCP) used in this study was β-Tricalcium phosphate nanoparticles. β-Tricalcium phosphate nanoparticles had been prepared by wet chemical method [12].

Sample preparation

A total of forty freshly human premolar teeth extracted for orthodontic reasons were used in this study. The collected teeth were washed carefully. The apical 3 mm of the tooth root was cut perpendicular to the long axis of the tooth. Then the teeth were transferred to 12 -well cell culture plate, each tooth was placed in a separate well containing Dulbecco's Modified Eagle Medium (DMEM) to be stored for 2 hours at 4°C to preserve pulp vitality [13,14].

Cavity preparation with mechanical pulp exposure

Standard coronal openings were prepared using #245 round carbide bur mounted on a high-speed (300,000 rpm) dental hand-piece with water coolant under complete aseptic conditions. Standard mechanical exposures were made directly in the center of the floor of pulp chamber using the same bur.

Samples grouping

After cavity preparation and mechanical pulp exposure, the forty samples were divided into 2 main groups (20 teeth each) according to the capping material used: Group **M1** refers to pulps capped with Biodentine™ , and Group **M2** refers to pulps capped with Biodentine incorporated with NCP. Each group was further subdivided into 2 subgroups (10 teeth each) according to the culture period: subgroup **P1** refers to teeth cultured for 14 days, and subgroup **P2** refers to teeth cultured for 28 days.

Application of Biodentine™:

Five drops of the liquid were added onto the powder-containing capsule. Then the capsule was closed and placed in an amalgamator and mixed for 30 seconds at 4000 rpm. After drying the cavity with a sterile cotton pellet, the Biodentine™ mix was applied as a direct pulp capping material using amalgam carrier and condenser. The material was used for restoring the tooth till the cavosurface margin.

Preparation and application of Biodentine incorporated with nanocalcium phosphate particles:

Nanocalcium phosphate particles powder (NCP) (40% by weight) was mixed with Biodentine™ powder. Since Biodentine™ powder weight is 0.7 gm, so the weight of NCP added to the Biodentine™ powder was calculated using the following equation = (0.7 x 40) / 100 = 0.28gm [15]. The powder-containing capsule of Biodentine™ was opened and the NCP powder (0.28 gm) was added to the Biodentine™ powder then 8 drops of the Biodentine™ Liquid were added to the new mixed powder in the capsule, then the capsule was closed. The capsule was placed in an amalgamator and mixed for 30 seconds at 4000 rpm. Biodentine incorporated with NCP was applied as a direct pulp capping material till the cavosurface margin to restore the whole cavity.

Entire Tooth culture model: (Fig.1)

After cavity preparation and direct pulp capping, a sterile orthodontic metallic wire was sealed on the crown with light-cured resin composite. The roots of the treated tooth were dipped in 4 ml of the culture medium (Dulbecco's Modified Eagle Medium) without touching the bottom of the culture well. The wire was suspended on the two adjacent wells. The culture medium was changed every day. The cultured teeth were incubated for 14 days or 28 days in CO2 incubator [16].

Fig. (1) The apical part of the cultured tooth dipped in DMEM. A metallic orthodontic wire (arrows) was sealed on the crown with light-cured resin composite, then the wire was suspended in the 2 adjacent wells.

Histological examination:

At the end of each culture period, the teeth were fixed in 10% formalin solution, demineralized, paraffin embedded and routinely processed [17]. The slides were stained with haematoxylin and eosin (H&E). The Thickness of calcified tissue formed at the interface of the capping material was measured in microns.

Quantitative elemental analysis of calcium and phosphorous ions in dentine using Scanning Electron Microscope - Energy Dispersive X-ray Analysis (SEM-EDX):

At the end of each culture period (14 days and 28 days) the tooth was sectioned longitudinally into 2 symmetrically halves. Then quantitative elemental analysis of calcium (Ca) and phosphorous (P) ions (weight %) in dentine below the capping materials was carried out using SEM Model Quanta 250 FEG

(Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses).

RESULTS

The histogical results demonstrated that both capping materials (Biodentine™ (M1) and Biodentine incorporated with NCP (M2)) promoted the formation of reparative dentine. A significantly higher mean value of dentine bridge thickness was recorded in teeth capped with Biodentine incorporated with NCP when compared to that capped with Biodentine™ alone after both periods of capping 14 and 28 days (Table 1, Fig 2).

For both elements (Ca and P), a significantly higher mean value was recorded in teeth capped with Biodentine incorporated with NCP when compared to that capped with Biodentine™ alone after 14 and 28 days (Fig. 3).

Table (1) *Dentine bridge thickness in all groups and significance of the difference (ANOVA test)*

	M1P1	M2P1	M1P2	M2P2
Mean	295.29^{c}	525.19^{b}	486.03^{b}	867.07^{a}
SD	62.26	108.90	119.33	101.35
Min	138.63	145.47	256.94	703.72
Max	391.02	1066.12	722.17	1077.82
F	39.157			
P	<0.0001*			

**significant at p<0.05*
Tukey's post hoc test: means with different superscript letter are significantly different

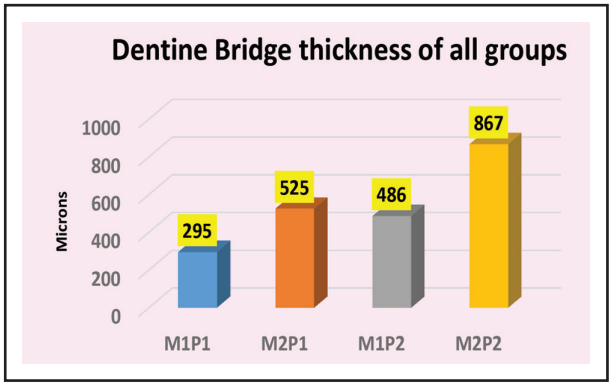

Fig. (2) Column chart showing dentine bridge thickness (μm) after capping with Biodentine™ (M1P1, M1P2) and Biodentine incorporated with NCP (M2P1, M2P2) after 14 and 28 days.

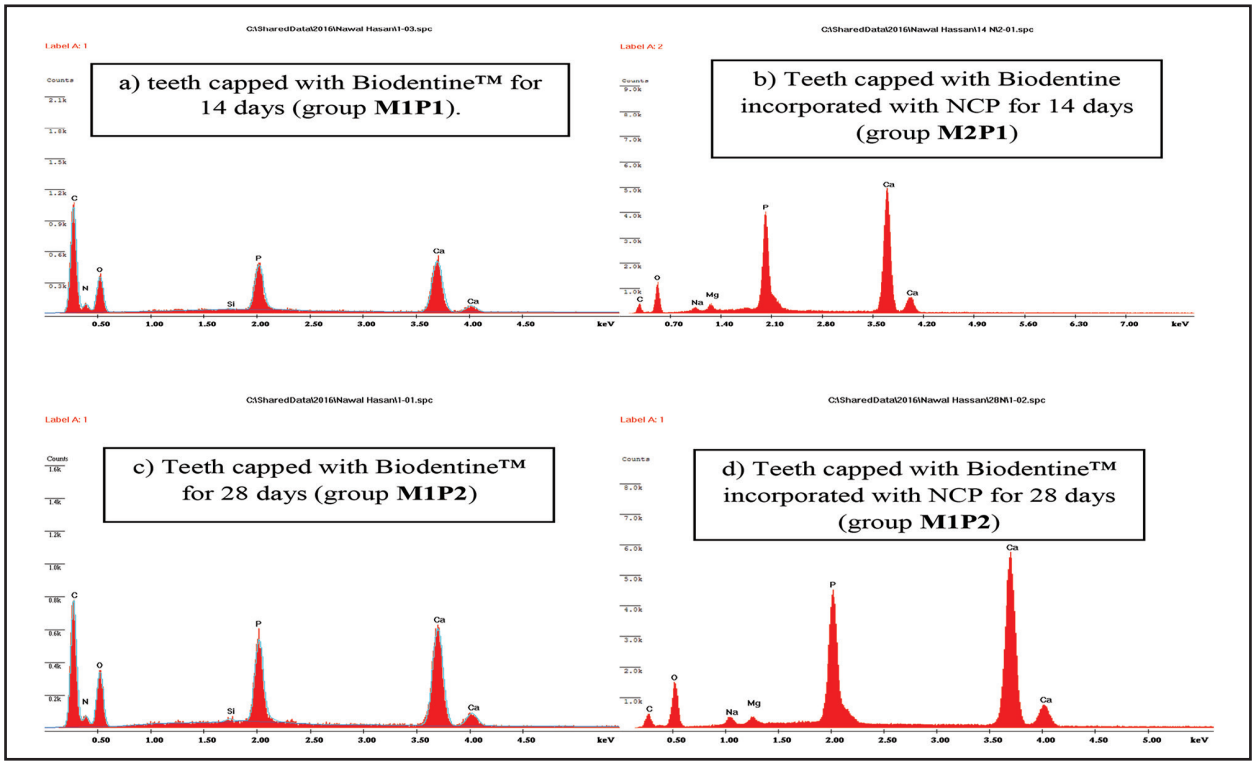

Fig. (3) EDX analysis of teeth capped with Biodentine™ (M1) and Biodentine incorporated with NCP (M2) for 14 and 28 days (P1 and P2).

DISCUSSION

Direct pulp capping is a treatment for exposed vital pulp involving the placement of a capping material over the exposed area to facilitate the formation of protective barrier and to maintain the vitality of the tooth [18]. Calcium hydroxide has long been considered the gold standard for the maintaining of pulp vitality. However, $Ca(OH)_2$ has some drawbacks as poor bonding to dentine, material resorption and mechanical instability. The tunnel defects of the newly formed hard tissue act as a portal of entry for microorganisms which may cause secondary inflammation of the pulp tissue[2]. Biodentine™ offers certain advantages over calcium hydroxide; it has higher compressive strength, higher density, it is less soluble and produces tighter seals to dentin [8]. Biodentine™ was reported to be denser and less porous when compared to MTA.

Nanocalcium phosphates are good candidates for several applications such as tooth remineralization and capping of the dental pulp due to its greater specific surface area which facilitates the fast release of Ca and PO_4 ions [19]. Nanocalcium phosphate materials could potentially solve the problem of microleakage and recurrent carries as well as promote remineralization of demineralized dentine [20].

Nanocalcium phosphate particles (NCP) was selected in the current study to be incorporated into Biodentine™. β-Tricalcium phosphate (β-TCP) nanoparticles was selected in particular among the multiple types of Nanocalcium phosphates as it is biocompatible and represent an excellent substrate on which odontoblast-like cells can attach to produce a hard tissue [21].

In the current study, the effect of incorporating 40% NCP into Biodentine™ on the pulpal response was evaluated after direct pulp capping for mechanically exposed pulp of entire tooth culture model. The entire tooth culture model provides a useful tool to predict the biocompatibility of dental materials. This model allows to investigate the early steps of dentin-pulp regeneration and dental pulp cells behavior after applying pulp capping materials. It reproduces the clinical situation for pulpal exposure in a whole-tooth environment, thus reducing the use of animal experiments before studies on human beings [14].

The histological results demonstrated that both capping materials [Biodentine™ (M1)) and Biodentine incorporated with NCP (M2)] promoted the formation of reparative dentine. This may be related to the fact that Biodentine™ induces odontoblast-like cell differentiation as it increases the transforming growth factor-β1 (TGF- β1) secretion by pulp cells[14,22]. Calcium silicate-based materials share their properties to induce the proliferation and genes activation of dental pulp cells, and to stimulate the reparative hard tissues [23,24].

Direct pulp capping with Biodentine incorporated with NCP led to the formation of thicker dentine bridge when compared to that formed by Biodentine™ alone at the same period of time. This could be due to the fact that NCP promotes Human differentiated pulp cells (HDPC) differentiation [25]. Moreover, β-TCP act as an excellent substrate on which odontoblast-like cells can attach themselves to produce a hard tissue, thus preserving normal histological pulp patterns and pulp vitality [21].

The elemental analysis results of this study showed that the highest mean weight % of Ca and P ions was recorded in dentine of teeth capped with Biodentine incorporated with NCP for 28 days (49.84±4.47), whereas the lowest mean was recorded in teeth capped with Biodentine for 14 days (12.76±0.77). This could be due to the great specific surface area of NCP enhancing the fast release of Ca and PO_4 ions [19]. Moreover, the resorbablility of β-TCP nanoparticles explains the elution of ca and P ions into the adjacent dentine [26].

CONCLUSION

Biodentine incorporated with NCP could be a good candidate for pulp capping in the future.

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