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# Histological Evaluation of Pulp Response after Local Application of Fibronectin in Direct Pulp Capping with Mineral Trioxide Aggregate

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**Abstract**

**Introduction.** Pulp regeneration therapy is important to overcome the limitation of conventional therapy to induce dentinogensis. Mineral trioxide aggregate (MTA) was used in endodontics to seal off all the pathways of communication between the root canal system and the external surface of the teeth. It has antibacterial and anticompatibilty properties high PH, radio-opacity and has ability to aid in release of bioactive dentine material proteins.
**Purpose.** Evaluation of pulp response to local application of MTA and fibronectin separately in direct pulp capping by means of histological analysis.
**Materials and methods.** Experimental study of thirty-six rat teeth were chosen for the experimental technique that involved exposing the pulp and removing the coronal pulp tissue as an attempt to induce pulp regeneration – was proceeded. The rats were aged (3–6) months, males, and weighted between (300–400 g). In accordance with the applied agent during the healing periods (1 and 4 weeks).
**Results.** Histological finding showed variable response of pulp tissue to applied materials throughout the following healing period as compared to control groups. The combination groups showed a high significant value in number of inflammatory cells pulp tissue cells and blood vessels than followed by fibronectin group and control group with MTA is the last in result.
**Conclusion.** According to the obtained results it was recorded that pulp tissue was more reaction to MTA with fibronectin as a combination material in term of acceleration of healing process.
**Keywords:** fibronectin, MTA, direct pulp capping, pulp response, histological evaluation

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# Гистологическая оценка реакции тканей пульпы на местное применение фибронектина при прямом покрытии пульпы минеральным триоксидным заполнителем

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**Резюме**

**Введение.** Восстановительная терапия пульпы важна для устранения свойственных традиционной терапии ограничений в плане индуцирования дентиногенеза. Для герметизации патологического сообщения между корневым каналом и наружной поверхностью зуба в эндодонтии используют минеральный триоксидный агрегат (MTA), поскольку он обладает антибактериальными свойствами, высокой биосовместимостью (вследствие характерных для него значений pH), рентгеноконтрастностью, а также способствует высвобождению биоактивных белков дентинного материала.
**Цель.** Гистологическая оценка реакции пульпы зуба как на сочетанное местное применение MTA и фибронектина, так и на применение каждого препарата в отдельности при прямом покрытии пульпы.
**Материалы и методы.** Для экспериментального исследования были отобраны тридцать шесть зубов крыс, на которых была применена экспериментальная методика, включающая обнажение тканей пульпы и удаление коронковой части пульпы с целью индуцирования регенерации пульпы. Возраст крыс составлял 3–6 месяцев, это были самцы весом 300–400 г. В зависимости от использованного материала периоды заживления составили 1 и 4 недели.
**Результаты.** Гистологическое исследование показало различную реакцию тканей пульпы на использованные материалы в течение всего последующего периода заживления по сравнению с контрольными группами. В комбинированных группах отмечен высокий достоверно значимый уровень воспалительных клеток в тканях пульпы и кровеносных сосудах, за ними следовала группа фибронектина, контрольная группа с использованием MTA была последней по полученным показателям.
**Заключение.** На основании полученных результатов установлено, что ткань пульпы лучше реагирует на комбинированное использование MTA с фибронектином, которое к тому же в большей мере ускоряет процесс заживления.
**Ключевые слова:** фибронектин, MTA, прямое покрытие пульпы, реакция пульпы, гистологическая оценка

## ■ INTRODUCTION

Dental pulp is a loose, stiff chamber filled with connective tissue comprises of cementum, dentine, and enamel. These distinct cells, which include fibroblasts, odontoblasts, resident immunocompetent cells, and undifferentiated mesenchymal cells, make up this tissue. Additionally, there are numerous sensory neurons and capillary networks in the dental pulp [1]. The interstitial fibroblasts and odontoblasts in the pulp's cell-rich zone in its core make up the majority of the pulp dental, separate, loose connective tissue which position the dentin surface's perimeter in alignment. Stem progenitor cells are resided in inter fibroblasts and may be in blood vessels. The primary objective of regenerate endodontic to revive a vitality or functionality of the d-pulp-complex opposed for filling roots canal with bioinrt materials [2]. The most appropriate method for direct pulp capping is when mechanical agents or trauma have exposed the pulp. In order to encourage the differentiation of new odontoblasts from pulp stem cells, a biomaterial is applied directly over the exposed pulp. This then permits the development of tertiary reparative dentin, preserving the pulp's vitality and its typical functions [3]. The biomaterials employed in vital pulp therapy should have the ability to sustain pulp vitality, promote the growth of reparative dentin, work effectively to bactericidally and/or bacteriostatically affect the pulp, and seal the pulp [4]. Strategies for regeneration are based on the dental pulp's condition, including the degree of inflammation and the quantity of harmed and infected tissues. It is possible to execute an indirect or direct capping on the pulp with biomaterials for less serious lesions [5]. Molecularly heavy (500–600 kDa) A glycoprotein called fibronectin that interacts with membrane-spanning integrins is present in the extracellular matrix. protein receptors. Fibronectin also interacts with other extracellular matrix proteins, including as proteoglycans made of heparan sulfate, collagen, fibrin and syndecans. A protein dimer called fibronectin is composed of two nearly identical monomers that are joined together by two disulfide links. While many isoforms of the fibronectin protein can be generated from single is gene by alternative of splicing of its premRNA. In vertebrates, there are two forms of fibronectin: soluble plasma Hepatocytes in the liver create fibronectin, formerly known as CIg, or cold globulin, is major was protein present in plasma at a concentration of 300 g/ml. The extracellular matrix mostly consists of insoluble cellular a fibronectin. It is formed into insoluble matrix through complicated cell-mediated op process after being secreted by a variety of cells, principally fibroblasts, soluble protein dimer. Fibronectin serves a variety of purposes that guarantee vertebrate animals function normally [6]. It affects cell proliferation, migration, differentiation, and adhesion. An insoluble network known as the extracellular matrix separate and support the tissues and organs of an as organism, is built up from cellular fibronectin. In order for wounds to heal, fibronectin is essential [7].

## ■ PURPOSE OF THE STUDY

Evaluation of pulp response to local application of MTA and fibronectin separately in direct pulp capping by means of histological analysis.

## ■ MATERIALS AND METHODS

A total of 36 rat tooth samples were chosen for the experimental technique in an effort to stimulate pulp regeneration after the coronal pulp tissue was removed and the pulp

exposed. The rats were male, between 300 g and 400 g in weight, and between (3–6) months old. According to the administered agent during the healing periods (1 and 4 weeks) the collected teeth were separated into groups as follows:

- 1 – Control (MTA) group the exposed pulp capped with 1 mg MTA then closed with readymade temporary filling;
- 2 – Group II the exposed pulp capped with 1 mg of fibronectin protein then closed with ready-made temporary filling;
- 3 – Group III the exposed pulp capped with 0.5 mg of fibronectin and 0.5 mg of MTA then closed with readymade temporary filling.

The rats will be sacrificed at 1 and 4 week healing period so it will be 12 rats for each group, 6 rats for each period. The surgical process is carried out using a gentle operating approach and in a well-sterilized environment. Each animal's weight is used to determine how much general anesthetic should be administered. The tiny engine is ready for use with its hand piece and 2 mm surgical bur. Ketamine HCL 50 mg and 2% xylazine were injected intramuscularly to induce general anesthesia. For 10 minutes, cotton soaked in a solution of 75% alcohol and 25% hydrogen peroxide should be placed over freshly cleaned teeth. Under aseptic settings, the pulp chamber was opened using a round bur and intermittent the drilling while being irrigated. Hemorrhage is controlled with sterile cotton pellets and saline solution. A guiding hole was produced till the pulp was reached, and when the pulp had been exposed, the coronal section was removed for a 3 mm depth. After performing standard saline irrigation until hemostasis is achieved, the cavity is gently dried with cotton [8].

**Application of the materials**

1. Mineral Trioxide Aggregate material was applied by using dycal applicator and a small burnisher – for groups.
2. By using a tweezer, and a small spoon excavator a fibronectin material was applied to fill the coronal pulp space – for groups.
3. To seal the cavity, the ready-made filler was placed using an ash tool.

The tooth extracted and prepared for histological and histomorphometrical evaluation of inflammatory cell, pulp organized tissue include odontoblast cell layer, fibroblast and blood vessels to see the effected of fibronectin protein on pulp cell response. The analysis was accomplished by a single standardized operator in a blinded method under light microscope. Inflammatory cells accounting it were done by measuring the number of inflammatory cells in each pulp chamber's histological slice, in five microscopic fields at X40 magnification. Pulp cells accounting it was performed by counting odontoblast cell layer (odontoblast cells) and fibroblast in histological section for each pulp chamber, in five microscopic fields at X40 magnification. Blood vessels accounting were performed by counting blood vessels in histological section for each pulp chamber, in five microscopic fields at X40 magnification. Data were analyzed using descriptive statistic that estimated the accounting the inflammatory cells, pulp cells and blood vessels in each group and each period and inferential statistic that include Kolmogorov-Smirnov, Shapiro-Wilk, T-test and ANOVA test.

## RESULTS

### Histological findings

**Control group**

Microphotograph view of control group after one week of pulp capping with MTA material shows a layer of reparative dentin, inflammatory cells, and other view shows predentin. Histological examination of other section shows inflammatory infiltration, alongside the odontogenic layer, odontoblast like cells (fig. 1).

**Fibronectin group**

View of cavity site after one week of application fibronectin shows thin layer of predentin deposited by odontoblasts which appear disoriented at some sites, collagen fibers and fibroblasts, and with newly formed blood vessels and other view shows inflammatory cells, odontoblast-cell observed, and odontoblast like cells (fig. 2).

**Combination group**

After one week of MTA and fibronectin applications the histological examination shows inflammatory reaction invading pulp tissue, zone of predentin deposited by odontoblasts like cells differentiated from pulp stem cells, collagen fibers and fibroblasts (fig. 3).

**Control group**

Microphotograph view of tooth section of control group after four weeks shows increase number of odontoblast cells, and extensive fibroblast and shows number of blood vessels in pulp tissue (fig. 4).

**Fibronectin group**

View of cavity site after 4 weeks of application of fibronectin shows increase fibroblast and collager fibers and shows organized odontoblasts, and no inflammatory cells (fig. 5).

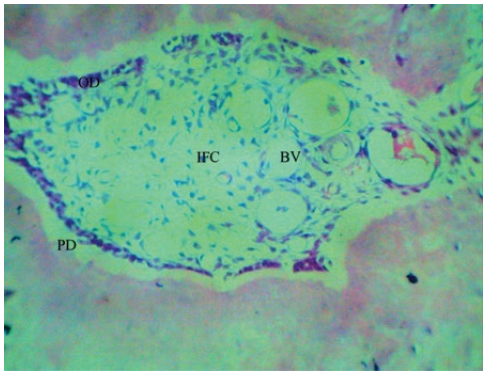


**Fig. 1. Histological view of control group for 1 week duration shows the presence of predentin and odontoblast cells (OD), with abundant inflammatory cell (IFC) and blood vessels (BV) H&E, X20**

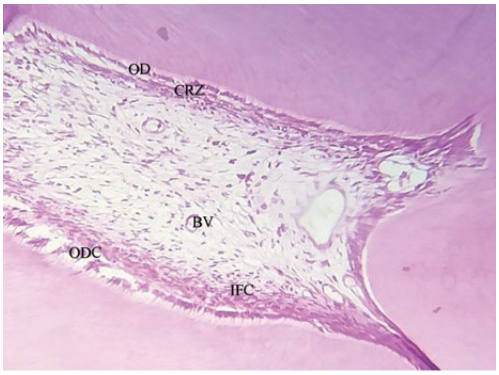


**Fig. 2. Histological view of fibronectin group for 1 week duration shows presence of predentin, cell rich zone (CRZ), inflammatory cells odontoblast and blood vessels (BV) odontoblast like cell (ODC) H&E, X20**

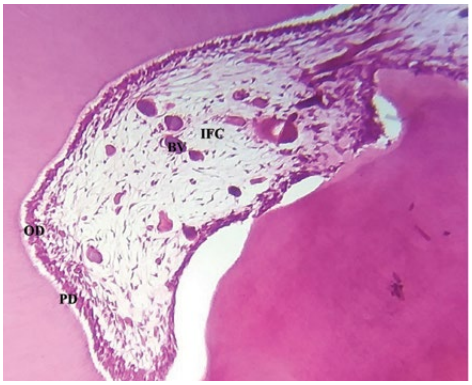


**Fig. 3. Histological view of combination group for 1 week duration shows the presence of predentin and odontoblast cells (OD), blood vessels a with inflammatory cell (BV) H&E, X10**

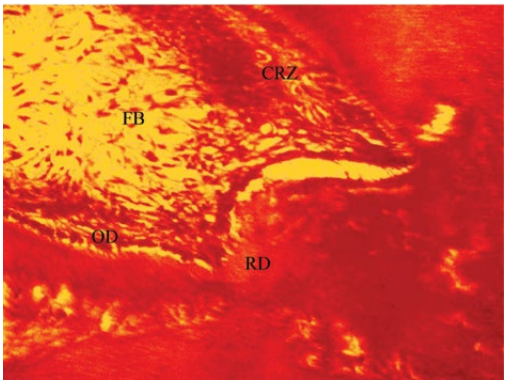


**Fig. 4. Histological view of control group for 4 week duration shows the presence of odontoblast cells (OD), reparative dentin with abundant of fibroblast cell rich zone (CRZ), and blood vessels (BV) H&E, X20**

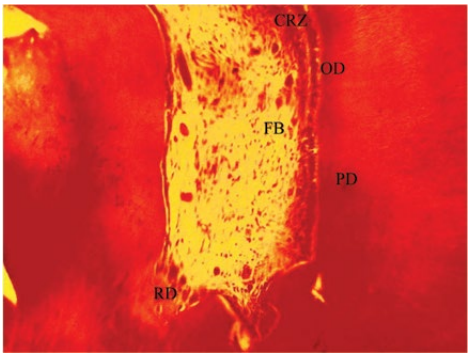


**Fig. 5. Histological view of fibronectin group for 4 week duration shows the presence of odontoblast cells (OD) blood vessels with abundant of fibroblast (BV) H&E, X10**

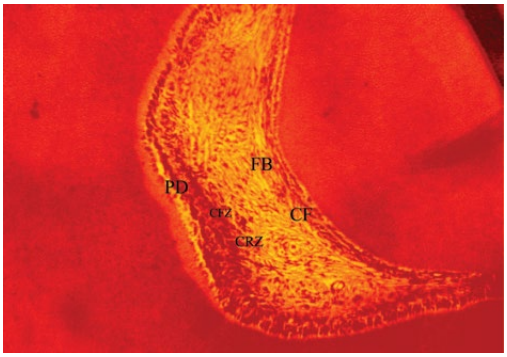


**Fig. 6. Histological view of combination group for 4 week duration shows the presence of odontoblast cells (OD), predentin, cell rich zone with abundant of fibroblast, collagen fibers and blood vessels (BV) H&E, X10**

### Combination groups

After four weeks of MTA and fibronectin applications the histological examination of tooth sections shows highly vascular regenerating pulp tissue, prominent fibroblasts with remodeling collagen fibers, congested blood vessel increase odontoblasts oriented alongside the newly formed predentin, shows, and huge number of active fibroblasts (fig. 6).

### Statistical analysis for histological findings H&E

The ANOVA test shows the P-value is highly significant for all parameters among all groups at 1 week duration (table 1).

**Table 1**
**ANOVA test of inflammatory cells, pulp cells and blood vessels in control and experimental groups at 1 week duration**

| 1 week | | Odontoblast | Fibroblast | Blood vessels | Inflammatory cell |
|---|---|---|---|---|---|
| MTA | Mean | 11.67 | 13.33 | 10.58 | 15.83 |
| | | | | | |
| | SD | 1.506 | 1.538 | 1.021 | 1.633 |
| FIBRONECTIN | Mean | 15.67 | 15.92 | 13.50 | 10.00 |
| | | | | | |
| | SD | 1.080 | 1.429 | 1.871 | 1.304 |
| COMBINATION | Mean | 18.67 | 19.25 | 17.92 | 7.67 |
| | | | | | |
| | SD | 1.080 | .935 | 1.201 | 1.080 |
| Total | Mean | 15.33 | 16.17 | 14.00 | 11.17 |
| | | | | | |
| | SD | 3.172 | 2.787 | 3.374 | 3.757 |
| p-value | | 0.00 | 0.00 | 0.00 | 0.00 |

ANOVA test shows the P-value is highly significant for all parameters among all groups at 4 week duration (table 2).

**Table 2**
**ANOVA test of pulp cells and blood vessels in control and experimental groups at 4 week duration**

| 4 week | | Odontoblast | Fibroblast | Blood vessels |
|---|---|---|---|---|
| MTA | Mean | 15.92 | 16.75 | 9.83 |
| | | | | |
| | SD | 1.744 | 1.541 | 0.876 |
| FIBRONECTIN | Mean | 17.75 | 16.50 | 3.92 |
| | | | | |
| | SD | 1.440 | 1.183 | 0.801 |
| COMBINATION | Mean | 27.67 | 21.67 | 7.25 |
| | | | | |
| | SD | 1.780 | 1.080 | .935 |
| Total | Mean | 20.44 | 18.31 | 7.00 |
| | | | | |
| | SD | 5.536 | 2.729 | 2.623 |
| p-value | | 0.00 | 0.00 | 0.00 |

## ■ DISCUSSION

One of the fundamental objectives of endodontics is the preservation of pulp life. In the past, applying a dressing material directly to the curiously afflicted pulp has always been viewed as a contentious operation; as a result, in these situations, traditional endodontic therapy has frequently been advised [9]. Rat incisors differ significantly from human incisors in terms of their composition and form because they have a wide-open apex, permanent development, and cannot be compared to human teeth [10]. One week duration-control group [11], showed the animal model using dogs, to evaluate the outcomes of DPC performed using four materials, at two time periods, after 1 week to represent the acute and chronic phase, respectively.

The specimens of both groups MTA and Biodentin (BD) demonstrated mild to no inflammatory cell infiltration in both time periods, which disagree with our result in MTA group which shows extensive inflammatory cells confirming early and delayed biocompatibility of this class of materials. Furthermore, the exposure site was lined by a calcific bridge even in the early period of follow-up (1 week) in pulps capped with MTA and BD, reflecting the strong bioactive nature of these cements. This result agreed with previous study done by [12]. After one week of pulp capping with MTA material shows pulp canal filled with necrotic pulp and inflammatory cells, and a layer of reparative dentin, other view shows predentin. This result agreed with previous study done by MTA is an effective material against microorganisms including E. faecalis [13]. One week duration-Fibronectin group a common extracellular matrix (ECM) glycoprotein called fibronectin (FN) is a biomaterial that is essential for tissue repair.

The plasma form of FN circulates in the blood and, in response to tissue damage; it is absorbed into fibrin clots to influence the platelet function and mediates hemostasis [12]. This result agreed with previous study done by [13], at 5 days durations of logical application of fibronectin the histological view of facial skin section in the dermis shows numerous blood capillaries, surrounded by number of inflammatory cells, fibroblasts and remodeling collagen fiber.

Which conform that the fibronectin differentiated the stem cells to fibroblast for formation of collagen fibers of the connective tissue? Combination group after one week of MTA and fibronectin applications shows inflammatory reaction invading pulp tissue, zone of predentin deposited by odontoblasts like cells differentiated from pulp stem cells. This result agreed with previous study done by [14], in 7-day samples, the pulps capped with MTA, Propolis, and Platelet-rich plasma (PRP) showed fibrous tissue formation. In MTA and Propolis samples, certain diffused calcified areas were also observed. Four week duration-control group the goal of direct pulp capping is to seal away the injured pulp by stimulating reparative dentine formation in response to pulp capping material placed at the site of pulpal exposure [15]. This result agreed with previous study done by [16], that the rate of formation of reparative dentine was highest initially in the 27 day interval (3.5 lm per day). When MTA was used as a pulp capping agent it induces cytologic and functional changes within pulpal cells, resulting in formation of fibro dentine and reparative dentin at the surface of mechanically exposed dental pulp, its causes proliferation, migration and differentiation of odontoblast-like cells that produce a collagen matrix [17]. In other studies, dentine bridge formation has been observed after a 2-week period in response to capping with MTA. The teeth were extracted at 30 days following the pulp capping procedure in this study. Calcific bridge formation was observed sandwiched between the capping material and dental pulp in all the samples. Fibronectin Group, the cavity site after 4 weeks of application of fibronectin shows increases fibroblast and collager fibers. This result agreed with previous study done [18]. The Fibronectin has been demonstrated to play a crucial role in odontoblast differentiation during tooth development. This study tested the hypothesis that fibronectin is involved in the initial stages of replacement odontoblast differentiation and reparative dentin formation. Combination Group after four weeks of MTA and fibronectin applications. This result agreed with previous study done by [19], the Fibroblast-like cells exhibiting intense immunoreaction only at 21 days were mainly associated with the crystalline precipitates on the cement surfaces or within the surrounding pulp.

## ■ CONCLUSION

According to the obtained results it was recorded that pulp tissue was more reaction to MTA with fibronectin as a combination material in term of acceleration of healing process. MTA with fibronectin accelerate pulp healing in direct pulp capping.

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