http://dx.doi.org/10.35630/2199-885X/2022/12/2.1

# PULP TISSUE MORPHOLOGY IN EXPERIMENTAL PULPITIS BY USING DIFFERENT MATERIALS FOR DIRECT PULP CAPPING

*Received 18 January 2022;*
*Received in revised form 22 February 2022;*
*Accepted 25 February 2022*

***Anzhela Brago*[1]✉, *Svetlana Razumova*[1], *Vadim Astashov*[3], *Yuliya Kozlova*[1], *Natalia Zolotova*[2]**

[1] *Department of Propaedeutics of Dental Diseases, Medical Institute, Peoples' Friendship University of Russia, Moscow;*
[2] *Research Institute of Human Morphology, Moscow;*
[3] *Department of Human Anatomy, Medical Institute, Peoples' Friendship University of Russia, Moscow, Russia*

✉ *anzhela_bogdan@mail.ru*

**ABSTRACT** — The minimally invasive concept of treatment in clinical medicine and, in particular, in dentistry is associated with the introduction of pulp-saving treatment technologies into clinical practice. The aim of the study was to identify the reaction of the structure of pulp tissues in traumatic pulpitis in the experiment using various means and techniques of direct pulp capping. The experiment was carried out on male minipigs aged 4–5 months with deciduous dentition. Milk molars were selected for trepanation. After teeth preparation under anesthesia, the crowns of the first and second milk premolars were trepanned on the vestibular surface in the cervical region with pulp probing until bleeding. After bleeding has been stopped, the trepanation hole was closed with various preparations. For direct pulp capping we used mineral trioxide aggregate (MTA) — containing silicate material and glass ionomer cement (GIC). All samples of teeth with experimental pulpitis using MTA-containing materials (Rutdent), tricalcium silicate materials (Biodentin) and even GIC (Vitremer) did not reveal inflammatory changes in the pulp tissues when they were directly covered with various materials. Of all the experimental samples of pulpitis, inflammation was detected with a sample of the Rutdent material with fragment damage to the pulp. With traumatic pulpitis and the use of various materials for direct pulp capping, active reparative regeneration of pulp tissue was revealed. All studied materials contribute to the reparation of the pulp after damage and reduction of pulp inflammation.

**KEYWORDS** — pulp morphology, pulpitis, treatment of initial and reversible pulpitis, MTA-containing materials, direct pulp capping

## BACKGROUND

The minimally invasive concept of treatment in clinical medicine and, in particular, in dentistry is associated with the introduction of pulp-saving treatment technologies into clinical practice. For the treatment of reversible forms of pulpitis, along with calcium hydroxide material, calcium salicylates, materials based on the mineral aggregate trioxide (MTA), tricalcium silicate cements are used. In a review of publications on the effectiveness of the use of various materials Paula A.B., Laranjo M. et al. (2018), using a meta-analysis method, found that MTA cements showed a significantly higher success rate in all parameters compared to calcium hydroxide cements. However, when compared with tricalcium silicate cements, there were no statistically significant differences. Adhesive systems showed significantly lower success rates in all respects compared to calcium hydroxide cements. The authors concluded that MTA and tricalcium silicate cements have a higher success rate, with a lower inflammatory response and more predictable formation of a hard dentinal barrier than calcium hydroxide cements [1].

A high success rate of pulp-saving treatment methods, comparable to the success of extirpation methods, will allow avoiding invasive intervention and resource-intensive endodontic procedures. However, different success rates of pulp-saving methods make it necessary to search for new methods of treating pulpitis.

Dhar V., Marghalani A.A., et al. (2017) published an evidence-based review of recommendations for the use of pulp treatments in primary teeth: indirect pulp capping, direct pulp capping, and pulpotomy. The group of authors was unable to recommend the benefit of any particular type of pulp therapy due to the lack of studies directly comparing these interventions. The authors recommended the use of MTA and formocresol for pulpotomy; these recommendations are based on moderate quality evidence over 24 months. The same recommendations apply to direct pulp capping. Researchers considered the use of tricalcium silicate cement, ferrous sulfate, sodium hypochlorite, and a laser as evidence of poor quality [6]. Thus, the issues of minimally invasive interventions on the dental pulp are relevant in modern endodontics.

*The aim of the study* is to detect structural changes in the dental pulp enabling to identify responses of pulp tissues in traumatic pulpitis; and to test various materials and techniques for direct pulp capping.

## MATERIALS AND METHODS

To determine the responses of pulp tissues when using various materials for direct pulp capping, an experiment was carried out on 10 male laboratory pigs (mini-pigs), aged 4–5 months (Kennel LLC "Krolinfo", Russia), weighing not less than 20 kg, deciduous dentition (Decision of the Ethics Committee of MI Peoples' Friendship University of Russia, Protocol No. 1, 20 September 2018). All experiments were carried out in accordance with the principles of humanity set out in the European Community directive (86/609 / EC).

For premedication, Atropine 0.02–0.04 mg/kg subcutaneously was used, Meditin 0.1 — 0.2 ml/5 kg. Then, chloral hydrate anesthesia was performed by intravenous administration. A 10% solution of chloral hydrate in isotonic solution with the addition of 5% glucose at the rate of 1 ml per kg was injected into the large ear vein of the auricle. Induction: Propofol 7 mg/kg, if necessary, maintenance dose 0.2–0.5 mg kg/min. Maintenance of anesthesia: Isoflurane 5% (induction and deepening), with an oxygen flow of 0.1 l/kg/min, Isoflurane 1.5–2% (maintenance of anesthesia), with an oxygen flow of 0.1 l/kg/min. Antimedin 0.1 — 0.2 ml/5 kg was used for induction. The animals were divided into two groups: intact animals (5 individuals) and animals that underwent trepanation of their teeth with further closure of the trepanation hole with various medications (5 individuals). For trepanation milk molars were selected: 5.4, 6.4, 6.5, 7.5, 7.4, 8.5 in each animal. After cleaning the teeth with a toothbrush, the crowns of the first and second milk molars were trepanned on the vestibular surface in the cervical region. The direction of the bur is perpendicular to the vestibular surface of the tooth with an apical inclination of 6–9 degrees. Preparation under water cooling until a sinking sensation, spraying the surface with saline. Probing the pulp before bleeding, after stopping the bleeding in each tooth, the trepanation hole was closed with various preparations (6 teeth each): materials based on MTA – Rootdent (Technodent, Russia), materials based on tricalcium silicate — Biodentin (Biodentine, Septodont, France) and glass ionomer cement (GIC, Vitremer, 3M, USA).

After withdrawal from anesthesia, during the first day, the animals were injected with non-steroidal anti-inflammatory drugs (Melocsicam 250 mg/kg). The animals were withdrawn from the experiment on the 28$^{th}$ day by an overdose of ether anesthesia, the teeth were extracted with the adjacent alveolar ridge and placed in a 2% formalin solution. The teeth were fixed during the day in 10% buffered formalin. The teeth were fixed within 24 hours in 10% buffered formalin. Then decalcification was carried out using an electrolytic decalcifier Medax Mod. 33.000 (Germany) in Electrolytic decalcifying solution (05–03004E, Bio Optica, Italy) within 3 weeks; then washed in 70% ethyl alcohol for 24 hours. After histological processing in the device Tissue-Tek VIP5Jr (Sakura, USA), teeth were enclosed in a histomix in the device Tissue-Tek TEC (Sakura, USA), sections with a thickness of 5–8 microns on a microtome Microm HM340E (Thermo Scientific, USA) were made. The obtained preparations were stained with hematoxylin and eosin. Using the method of light microscopy (Microscope Axioplan 2 imaging (Carl Zeiss, Germany)) we studied exudative-inflammatory changes in the dental pulp, such as the severity of infiltration, cellular composition, edema, changes in the histology of the pulp with the use of photofixation.

## RESULTS

The results of histological examination showed absence of inflammatory changes in the samples of teeth treated with MTA. Morphological examination of dentin and pulp of intact teeth showed a clear striation of dentin.

On the micrograph of the histological section of the tooth 8.5 after trepanation using Rootdent material to close the hole, the pulp has a normal structure, no inflammatory changes were detected. A strip of predentin or uncalcified dentin is determined between dentin and odontoblasts. Rows of odontoblasts with long narrow nuclei are oriented perpendicular to the inner surface of the dentin layer, forming a peripheral layer of pulp. The central layer of the pulp consists of loosely arranged mesenchymal cells, fibers and blood vessels (Fig. 1). In the preparation of the tooth 5.4 (tangentially cut tooth crown), when using Rutdent after trepanation we noticed inflammatory changes around the dentin fragment associated with the presence of foreign bodies in the pulp. (Fig. 2). In the pulp of the tooth, focal inflammatory infiltration around a foreign body (a fragment of hard tooth tissues) was revealed - pulpitis (stasis, plethora, edema, plasmolymphocytic infiltration, disruption of the pulp structure) caused by the intrusion of a foreign body into the pulp.

Outside this zone, the pulp has a normal structure: it is represented by loose fibrous connective tissue with many thin-walled, unevenly plethoric blood vessels; a layer of odontoblasts is located on the border with dentin. Thus, the use of the MTA-containing material after crown trepanation showed a pulp-saving effect, the revealed inflammatory changes in the pulp are associated with the penetration of a foreign body into the pulp tissue.

In the preparations of teeth, the trepanation holes of which were closed with Biodentin, no histological changes characteristic of pulpitis were found (Fig. 3).

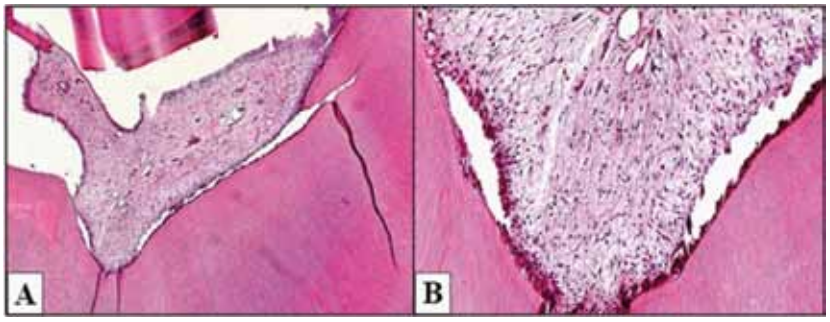


***Fig.1.*** *Longitudinal section of the premolar 8.5 in laboratory pigs after trepanation with MTA closure: A — general view of the crown of the tooth, magnification ×50. B — tooth pulp without damage, multicellular, normal odontoblast row, magnification. ×200. Staining with hematoxylin and eosin*

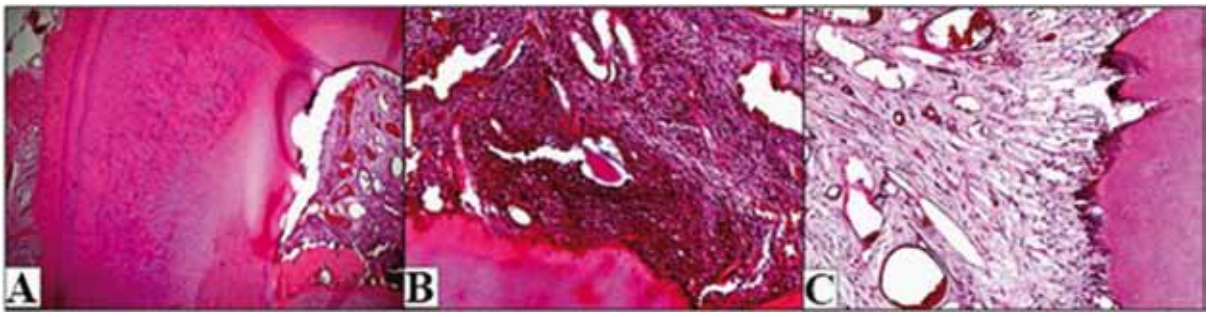


***Fig.2.*** *Tooth preparation 5.4 (Rootdent). A — general view of the tooth, magnification ×50. B — focal inflammation around a foreign body, magnification ×200. C — normal pulp of the tooth, magnification ×200. Staining with hematoxylin and eosin*

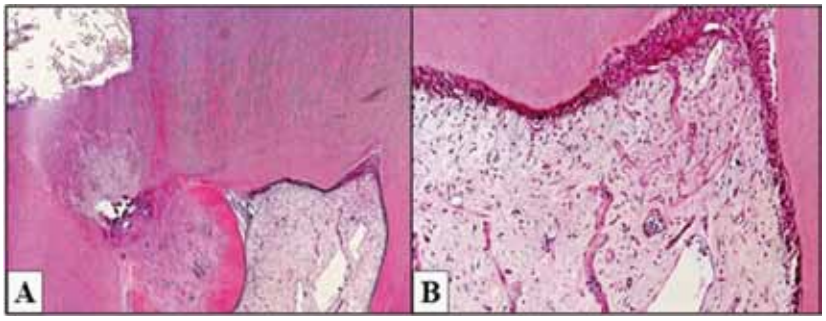


***Fig.3.*** *Pulp of the tooth 6.4 (Biodentin). A — general view of the crown of the tooth, fragments of the filling material are visible, magnification ×50 B — normal pulp of the tooth, magnification ×200. Staining with hematoxylin and eosin*

In the preparation of tooth 6.4 (Biodentin) a longitudinally cut tooth with fragments of the pulp, crown and two roots with fragments of filling material in the pulp tissues. In all areas, the pulp has a normal structure, no inflammatory changes were found.

In preparation of tooth 6.5, Vitremer glass ionomer cement was used to close the trepanation hole (Fig. 4). In the preparation of tooth 6.5 (Vitremer), the coronal part of the tooth and the orifice of the root canals are cut tangentially.

In one canal, the pulp has a normal structure, there are no inflammatory changes. In the second canal the pulp cells have a mesenchymal morphology, their number is increased, but there is no inflammatory infiltration, which indicates the development of physiological repair in the pulp.

## DISCUSSION

High reparative properties of the pulp were shown by Giraud T., Jeanneau C. et al. (2019). When using various models of pulpitis to study the effect of materials on the pulp, it was shown that the pulp has an innate anti-inflammatory potential and a high ability to regenerate in all teeth and at any age [3].

In research of Ricucci D., Siqueira J.F. Jr, Li Y. et al. (2019) on 264 carious teeth, histological and histobacteriological analysis carried out on the extirpated pulp showed a localized inflammatory reaction that usually occurs in the adjacent pulp tissue as soon as the enamel was affected by caries. If softened and infected dentin was completely excised and the cavity repaired, pulpal inflammation was often relieved. In teeth with a carious process, the degree of penetration of bacteria

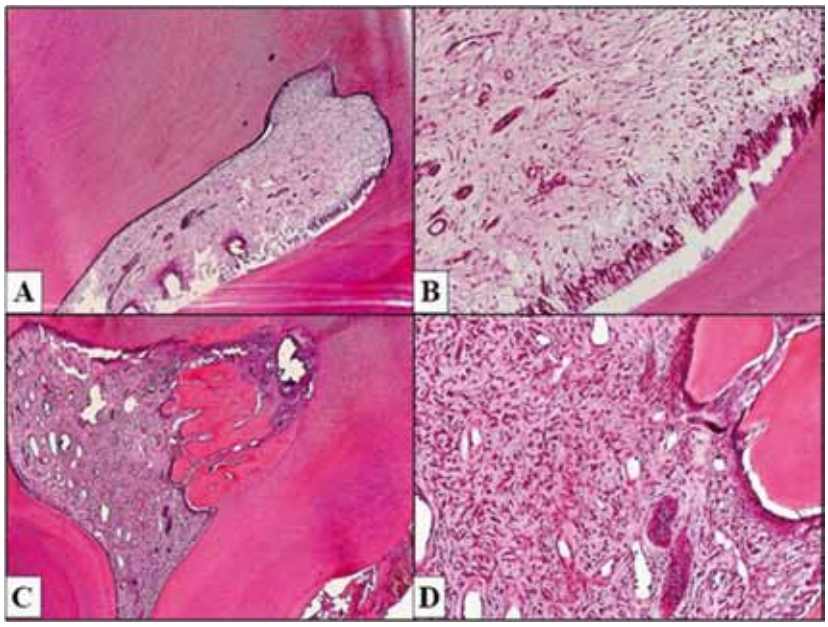


***Fig.4.*** *Tooth pulp 6.5 (Vitremer). A, B — canal of the tooth with normal pulp. C, D — cellular reaction in the pulp in the second canal. A, B — magnification ×50; C, D — magnification ×200.*

varied, and the areas of infection were pulp inflammation, including micro-abscesses. However, the pulp tissue in the root canals was usually non-inflamed and normal [4].

In experimental samples, simulated pulpitis, inflammation was detected in samples containing Rutdent material (tooth 5.4). The cause of inflammation in sample 5.4 is associated with the presence of a foreign body (dentin fragment) in the pulp chamber. In the sample (tooth 8.5) containing the MTA-based materials, no inflammatory changes were detected. In all other samples of teeth with experimental pulpitis, where MTA-containing material (Rootdent), tricalcium silicate materials (Biodentin)were used for direct pulp capping there were no inflammatory changes in the pulp tissues. The high efficiency of MTA material and tricalcium silicate cements has been confirmed by numerous clinical studies in the treatment of pulpitis in young patients with an unformed and uncovered root apex. Brizuela C., Ormeño A. et al. (2017) published the results of a study on 169 patients using MTA, calcium silicate cements and calcium hydroxide. After a year of follow-up, no significant differences were found between Biodentin and MTA. The authors noted some of the advantages of these cements over classical calcium hydroxide. [5].

Hegde S., Sowmya B., et al. (2017) treated reversible pulpitis in 24 permanent molars with carious lesions. There were two groups, group I - MTA and group II - Biodentin. Patients were seen at 3 weeks, 3 months, and 6 months for clinical and radiographic evaluation. The authors found that over 6 months MTA and Biodentin showed 91.7% and 83.3% success, respectively, based on subjective symptoms, pulp sensitivity tests, and radiographic control [6].

The results of our study showed that the reaction of the pulp to GIC (Vitremer), according to histological analysis, did not differ from the reaction to tricalcium silicate cement (Biodentin). Lipski M., Nowicka A., et al. (2018) published the results of predictive value of factors in relation to the results of direct pulp capping treatment with Biodentine (Septodont, Saint-Maur-de-Fosse, France). The overall success rate was 82,6%. The authors noted that age had a significant effect on pulp survival: the success rate was 90.9% of patients under 40 years and 73.8% of patients 40 years and older [7]. Ricucci D., Siqueira J.F. Jr, Li Y., et al. (2019) conducted observation of 757 clinical cases with a long follow-up period of 30 years. The authors found that direct pulp capping was successful in 73.2%, partial pulpotomy in 96.4% and complete pulpotomy in 77.8% of cases [4].

## CONCLUSION

Thus, on the basis of our data, we can conclude: 1. In traumatic pulpitis and the use of various materials for direct pulp capping, active reparative regeneration of pulp tissue was revealed. All researched covering materials contribute to the restoration of the pulp structure after damage, relief of the symptoms of pulp inflammation. 2. In case of damage to the pulp by fragments of a tooth during the modeling of pulpitis and the use of Rootdent material to cover the defect, incomplete regeneration of the pulp along the periphery of the inflammatory focus was revealed.

For the final decision on treatment methods – the methods that ensure the healing of the pulp and the formation of replacement dentin, further studies of the humoral and cellular factors of the inflammatory response in the pulp are required.

## REFERENCES


1. **Paula AB, Laranjo M, Marto CM, Paulo S, Abrantes AM, Casalta-Lopes J, Marques-Ferreira M, Botelho MF, Carrilho E.** Direct Pulp Capping: What is the Most Effective Therapy-Systematic Review and Meta-Analysis. J Evid Based Dent Pract. 2018 Dec;18(4):298–314. doi: 10.1016/j.jebdp.2018.02.002. Epub 2018 Feb 15. PMID: 30514444.
2. **Dhar V, Marghalani AA, Crystal YO, Kumar A, Ritwik P, Tulunoglu O, Graham L.** Use of Vital Pulp Therapies in Primary Teeth with Deep Caries Lesions. Pediatr Dent. 2017 Sep 15;39(5):146–159. Erratum in: Pediatr Dent. 2020 Jan 15;42(1):12–15. PMID: 29070150.
3. **Giraud T, Jeanneau C, Rombouts C, Bakhtiar H, Laurent P, About I.** Pulp capping materials modulate the balance between inflammation and regeneration. Dent Mater. 2019 Jan;35(1):24–35. doi: 10.1016/j.dental.2018.09.008. Epub 2018 Sep 27. PMID: 30269862.
4. **Ricucci D, Siqueira JF Jr, Li Y, Tay FR.** Vital pulp therapy: histopathology and histobacteriology-based guidelines to treat teeth with deep caries and pulp exposure. J Dent. 2019 Jul; 86:41–52. doi: 10.1016/j.jdent.2019.05.022. Epub 2019 May 21. PMID: 31121241.
5. **Brizuela C, Ormeño A, Cabrera C, Cabezas R, Silva CI, Ramírez V, Mercade M.** Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. J Endod. 2017 Nov;43(11):1776–1780. doi: 10.1016/j.joen.2017.06.031. Epub 2017 Sep 14. PMID: 28917577.
6. **Hegde S, Sowmya B, Mathew S, Bhandi SH, Nagaraja S, Dinesh K.** Clinical evaluation of mineral trioxide aggregate and biodentine as direct pulp capping agents in carious teeth. J Conserv Dent. 2017 Mar-Apr;20(2):91–95. doi: 10.4103/0972-0707.212243. PMID: 28855754; PMCID: PMC5564251
7. **Lipski M, Nowicka A, Kot K, Postek-Stefańska L, Wysoczańska-Jankowicz I, Borkowski L, Andersz P, Jarząbek A, Grocholewicz K, Sobolewska E, Woźniak K, Drożdzik A.** Factors affecting the outcomes of direct pulp capping using Biodentine. Clin Oral Investig. 2018 Jun;22(5):2021–2029. doi: 10.1007/s00784-017-2296-7
8. Epub 2017 Dec 12. PMID: 29234957; PMCID: PMC5945752