

# Pulpal Response to Direct Pulp Capping with Collagen Bioresorbable Membrane

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## Summary

*The study evaluates the effect of collagen bioresorbable membrane as a pulp capping material. Experiments were carried out on mongrel dogs. The dogs were anesthetized and teeth were isolated by rubber dam. In all animals, along the buccal side, class V cavities were prepared on two maxillary and mandibular incisors. The pulp chambers were exposed and bioresorbable collagen membrane Bio-Gide was placed on the pulp wound in the first group of the teeth. In the second group the pulp wound was covered with a sterile Teflon disk and in the third group the cavities were closed by Ketac-Silver cementum only. The cavities in the first two groups of teeth were permanently closed by Ketac-Silver cementum. After six weeks the specimen teeth were extracted and prepared for histological examination. The histological findings of the specimens treated only with Ketac-Silver cementum showed complete necrosis of the pulp tissue. Two samples treated with Teflon disks showed complete necrosis of the pulp, while on six samples partial loss of morphologic structure could be seen. On the specimens treated with collagen bioresorbable membranes the pulp tissue preserved its vitality to a much greater extent. Particularly noticeable was a better blood supply in the pulp, with an increased number of blood vessels. The creation of a reparatory bridge was not noticed in any of the tested samples.*

**Key words:** collagen bioresorbable membrane, dental pulp, odontoblasts, dentinal reparatory bridge.

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## Introduction

The maintenance of tooth vitality has been a constant concern in the practice of dentistry. When pulp exposure occurs, the clinician should select the appropriate treatment to preserve pulp vitality when-

ever possible (1). The result desired in this clinical procedure is the formation of a reparative dentine bridge over the exposed pulp surface (2). The most commonly used pulp-capping material is calcium hydroxide. Although capping with calcium hydroxide frequently results in the formation of necrotic

and acute or chronic inflammatory zones and dystrophic calcification (3). It also destroys the underlying healthy pulp tissue, leaving a necrotic layer (4). Today it is known that the formation of a dentinal bridge is not caused uniquely by calcium hydroxide but can also occur under many other materials (5). Collagen possesses hemostatic properties and the ability to aggregate platelets, so it can facilitate wound maturation by enhancing the initial blood clot and fibrin linkage formation (6). It is chemotactic for fibroblasts and improves their migration and attachment through its fibrillar structure (7). This property may enhance cell migration into the space between collagen membrane and the pulp wound. Collagen fibres are able to induce mineral formation and to orient hydroxyapatite crystals which form directly on or within collagen fibres of the mineralizing tissue, although the precise molecular mechanism involved has not been determined. The advantages of the collagen bioresorbable membrane include its handling properties, ease of placement and postoperative maintenance. Collagen is a natural protein, and human body enzymes break it down into natural amino acids, eliminating "second generation" materials. The purpose of this study was to evaluate the effect of collagen bioresorbable membrane in the direct pulp-capping procedure compared to teflon and Ketac-Silver glass ionomer cement.

## Material and methods

### *Clinical treatment of samples*

Experiments were carried out on 6 mongrel 2-year-old dogs of approximately 20 kg in weight. Before the experiment all dogs were examined by a veterinarian. The dogs were anesthetized by medetomidin-hydrochloridum (38 µg per kilo of body weight) and cetamine-hydrochloridum (3 mg per kilo of body weight). Teeth were isolated by rubber dam and cleaned with 2.5% sodium-hypochloride solution. In all animals class V cavities were prepared along the buccal side, on two maxillary and two mandibular incisors, 2 mm above marginal gingiva, using a high speed water cooling diamond drill. Dimension of all cavities was 2x2 mm. The pulp chamber was exposed by steel bores in all cavities. The bleeding from the pulp tissue was stopped by

rinsing with 10 ccm sterile saline solution. The dogs were then randomly divided into three groups. A bioresorbable collagen membrane Bio-Gide (Ed. Geistlich Sohne Ag, Wolhusen, Switzerland) was placed on the pulp wound in the first group of teeth. Prefabricated membrane was 25x25mm in size, and the smallest pieces (1x1 mm) were obtained by cutting with sterile scissors. In the second group of teeth the pulp wound was covered with a sterile Teflon disk 1x1mm. In the third group of teeth the cavities were closed by Ketac-Silver cement (ESPE, Seefeld, Germany) without pulp capping. After having been covered, the cavities in the first two group of teeth were permanently closed by Ketac-Silver cement. For awakening from anesthesia, dogs were treated with atipamezol-hydrochloridum. Each group of animals was marked with special medals. During the experiment all animals were kept under the same conditions and veterinary control. There was no sign of body weight or health change in any of the tested animals. After six weeks they were anesthetized by the procedure mentioned above, and all specimen teeth were extracted. Following extraction they were prepared for further histological examination. Because of particularity of denture in dogs, the nature of the defects caused by teeth extraction was not clinically significant, and therefore none of the animals were sacrificed after the experiment.

### *Histological preparation of samples*

Following extraction the teeth were fixed in 10% buffered formalin solution with the ratio of the fixative and tooth mass being 1:50 in favor of the fixative. Previously the apex of the root of each tooth has been cut off by a small osteotom in order to facilitate penetration of the fixative, whereby a better quality of adhesion was achieved. During a two-week period, the fixative was changed twice, and in the first week the teeth were stored in a refrigerator in order to minimize possible postmortem autolytic alterations. After fixation the teeth were demineralized by a technique using formic and nitric acid reported elsewhere (8). Demineralization solutions were changed every day during the procedure and after two weeks, the level of demineralization was tested by X-rays in all samples. The samples were then cut vertically into smaller pieces. Each included the prepared cavity and surrounding tissue,

3-4 mm coronally and apically. The obtained samples were additionally demineralized during the following three days, after which they were rinsed in distilled water and then refixed in a 10% neutral buffered formalin. After the additional fixation, the samples were dehydrated in ascending concentrations of alcohol and chloroform, perfused with paraffin in the thermostat, and finally embedded into paraffin blocks. Horizontal cuts of 6 $\mu$  in depth were made from these blocks on a grinding microtome Leitz 2000 (Ernst Leitz, Wezlar GMBH, Wetzlar, Germany). They were then mounted on the microscope slide, deparaffined and stained with the hematoxylin-eosin coloring method according to Harris. Once the samples had been stained, they were covered with Canada balm (Kemika, Zagreb, Croatia) and evaluated under a microscope by standard magnification. The fields of significant alterations were photographed.

## Results

### *Histological findings of the samples treated with Ketac-Silver cement only*

The histological findings of the specimens treated only with Ketac-Silver cementum showed complete necrosis of the tissue. The natural morphology of the samples was lost, and the basic structure of the pulp could not be histologically discerned. There was complete absence of the odontoblastic layer which, with the dental pulp, formed an amorphous mass of dead tissue. An uneven layer of predentin surrounded the pulp chamber, in which only the dead residue of the pulp was visible. The dark pigment in the preparations was probably a remnant of the pulp capping agent (Ketac-Silver) (Figs. 1 and 2).

### *Histological findings of the samples treated with Teflon disks*

Of the total of 8 preparations treated with Teflon disks, 2 showed complete necrosis, while on the 6 remaining preparations partial loss of the morphologic structure could be seen. Although the morphological structure of the vital cells had disappeared, the pulp tissue still showed its basic structure. Particularly noticeable were particles on the

periphery of the preparations. According to their position, size and shape, they may have been the residues of odontoblast cells. Although in central parts of the samples amorphous necrotic tissue mass prevailed, such results were still not so absolute as they were in the samples treated with Ketac-Silver cement (Figs. 3 and 4).

### *Histological findings of the samples treated with bioresorbable collagen membrane*

The pulp tissue preserved its vitality to a much greater extent on the preparations treated with collagen bioresorbable membranes with preservation of the morphology of all histological structures. Almost all pulp structures were clearly visible and could still be very well discerned histologically. A marked layer of the odontoblast could be noticed along the whole periphery of the pulp chamber, very close to the predentine itself, uniformly and evenly arranged, surrounding the pulp chamber. This leads to the conclusion that the vitality of the odontoblasts was preserved for a long time. Very typical findings for this group of samples were the unusually large quantity of extravasated erythrocytes and the large number of newly formed blood vessels. Both clearly indicate the very good blood supply in these preparations. With such strong vascularization, there appeared to be an increased number of fibroblasts and a tendency to the forming of granulation tissue. The basic features of this group of samples were the very large number of cells (remarkable even for an intact pulp), unusual amount of blood supply, and especially the clear distinction of the pulp. (Figs. 5, 6, 7).

## Discussion

Collagen is very important in the development and healing process of tissue. Different branches of medicine have been using collagen for therapeutic purposes for a long time. Bimstein and Shoshan (9) applied collagen as a means of direct pulp capping. In a one-month period, the pulp of tested teeth remained vital and histologically similar to the pulps of intact teeth. In all tested samples, a thin, newly created dentinal bridge was found. In her study, Fuks et al. (10) obtained similar results. She carried out

tests on 25 monkey teeth that were subjected to vital amputation and later treated with collagen solution. She proved that dentine bridges occurred in 73% of the tested teeth. Rutheford et al (11) tested the direct pulp capping reaction to human osteogenetic protein. To do this he used collagen matrix as the carrier for its application. While human osteogenetic protein showed good characteristics as a direct pulp capping material, the collagen matrix itself did not at all initiate mineralization or the creation of dentine bridge. Such differences can be explained by a statement of Cox et al (12) published in 1987. According to the author, some kind of a stimulative chemical factor is necessary for the development of the protective reaction of traumatized dental pulp, and mere contact with biocompatible non-stimulative matter is not sufficient. The differences between data obtained by Bimstein and Shoshan (9) and Fuks et al. (10) on the one hand, and Rutheford et al (11) on the other, can be explained by the fact that Bimstein and Shoshan, and Fuks in both cases used an enriched collagen solution (Medium 199) whereas Rutheford did not. That solution, apart from collagen, also contained some vitamins, amino acids and other nutritive factors. We can speculate that, in their tests, precisely those secondary matters were a more significant initiator of the positive pulp reaction than the collagen itself.

Nevertheless, the results of this research differ from all of the above mentioned results. None of the samples in this study treated with collagen bioresorbable membrane developed tissue necrosis. Particularly noticeable was the highly increased vascularization of tissue. Such findings are compatible with collagen characteristics already known and with its effect on tissues in general. However, in these conditions, collagen obviously leads to the forming of new blood vessels and increased extravasation of erythrocytes. In this study the creation of a dentine bridge was not observed. Collagen, although possessing the capacity to initiate *in vitro* mineralization, does not show that feature in *in vivo* conditions. A possible reason for the non-occurrence of reparatory dentin is the collagen preparation which he used. The bioresorbable membranes contained type I collagen and type III collagen. Type

III collagen was not noticed in dentine, and, according to some research, it may even inhibit mineralization (13). Type III collagen is present in dental pulp in 50% of the cases together with type I collagen, and pulp is a soft non-mineralizing tissue. There were doubts whether collagen could have the effect of an antigen. Today a specially treated collagen is used, and its immunological activity is clinically without significance. In clinical testing (14), 13% of the patients with enlarged alveolar ridge caused by the preparation of collagen and calcium-hydroxyapatite, developed antibodies to bovine collagen, although they were not reactive to human collagen, and did not cause any kind of allergic reaction. Considering the results of these studies, the possible antigen quality of collagen used for therapeutic purposes can be excluded as a cause of possible failures. In the samples treated with Teflon disks, complete pulp necrosis was observed. This is in accordance with the results of previous research on similar materials for direct pulp capping (15-20). Non-occurrence of a chemical stimulus decreases the positive pulp reaction to trauma, and creation of a dentinal reparatory bridge. In the best case closing of the pulp wound occurs by a thin soft tissue membrane (17). In his study of calcium hydroxyapatite in 1993 Subay (15) confirmed this material as highly biocompatible, but without dentinogenetic activity of clinical importance. Similar results were obtained by Jaber (17) in 1991 and Stanley (19) in 1989. In numerous studies materials that in some way chemically stimulate pulp tissue have given much better results as a means for direct pulp capping, although the mechanism of the stimulation of odontoblasts to produce reparatory dentin is still not sufficiently known. Nevertheless the fact remains that initiating chemical irritation has a favorable effect for that process.

### Conclusion

From the results obtained in this study, it can be concluded that collagen, although a highly biocompatible material, is not a suitable means for direct pulp capping.