Folia biologica (Kraków), vol. **54** (2006), No 1-2

Early Odontoblastic Layer Response to Cavity Preparation and Acid Etching in Rats

Lidija NEMETH, Andreja ERMAN and Draga ŠTIBLAR-MARTINČIČ

Accepted January 25, 2006

NEMETH L., ERMAN A., ŠTIBLAR-MARTINČIČ D. 2006. Early odontoblastic layer response to cavity preparation and acid etching in rats. Folia biol. (Kraków) **54**: 23-30.

The aim of this study was to establish the early odontoblastic layer response and quantitatively to estimate the number of odontoblasts after cavity preparation with and without acid etching. Half of 56 cavities prepared on rats' first upper molars were acid etched. Qualitative and morphometric analyses were made on histological and ultrathin sections 5 min, 6 h, 24 h and 72 h post-operatively. Under the etched cavity, a greater disarrangement of odontoblasts was found, modifications in nuclear shape and condensed chromatin 5 min. post-operatively. An additional reduction of odontoblast number was detected and an increase of aspirated cell number 5 min, 6 h and 24 h post-operatively, pronounced hyperaemia 6, 24 and 72 hours post-operatively and increased odontoblast number 72 hours post-operatively, compared to unetched cavities. In conclusion, injury to the odontoblastic layer was greater, but numerical renewal of the odontoblastic layer began earlier in etched cavities compared to unetched cavities.

Key words: Dental pulp, odontoblast, cavity preparation, acid etching.

Lidija NEMETH, Department of Restorative Dentistry and Endodontics, Faculty of Medicine, Section of Stomatology, Hrvatski trg 6, 1000 Ljubljana, Slovenia.
E-mail: lidija.nemeth@mf.uni-lj.si
Andreja ERMAN, Institute of Cell Biology, Faculty of Medicine, Lipičeva 2, 1000 Ljubljana, Slovenia.
ŠTIBLAR-MARTINČIČ, Institute of Histology and Embryology, Faculty of Medicine, Korytkova 2, 1000 Ljubljana, Slovenia.

Odontoblasts are the first cells to respond to cavity preparation with cell membrane injury or amputation of the odontoblastic process at different levels of its course, depending on the cavity depth. Amputation of the peripheral part of the process causes reversible damage to the cell, while amputation of the process near the cell body causes death of the odontoblast (SEARLS 1967). In the case of insufficient water-cooling, excessive dentine desiccation can occur. If the irritant is very strong, odontoblasts, erythrocytes or other cells from the superficial layers of the dental pulp can be displaced deep into the dentinal tubules, where they die (MJÖR 2001). Heavy pressure of the cutting drill, hypertonic liquid or hygroscopic filling material can cause a similar effect (MJÖR 1983). The extent of cell aspiration into the dentinal tubules after cavity preparation depends on the degree of additional stimuli, such as dentine desiccation with air blast or acid etching (HIRVONEN & NÄRHI 1986). The phenomenon called the outward movement of the dentinal tubules contents can even result from so-called non-traumatic cavity preparation with the use of a turbine with abundant water spray (MJÖR 2001).

The early odontoblastic layer response is a direct result of noxious stimuli and includes degenerative processes with a surrounding tissue reaction, while the late odontoblastic layer response represents regenerative and reparative processes. The early odontoblastic layer response to cavity preparation involves ultrastructural changes of odontoblasts, especially the disruption of the nuclear membrane and intercellular junctions, which leads to disorganization of the layer. In addition, there is an inflammatory exudate between the odontoblastic layer and predentine, and cellular fragments in the exudate and odontoblastic layer. Movement of capillaries from the subodontoblastic zone just beneath the predentine, increased fenestration of capillaries and the presence of macrophages and polymorphonuclear leukocytes near the capillaries, indicate the tissue response to cell destruction (OHSHIMA 1990). One day after cavity preparation, dendritic cells and macrophages gather on the dentin-pulp border, extend their processes into the

dentinal tubules and act as antigen-presenting cells at the site of possible noxious factor invasion towards the pulp (OHSHIMA *et al.* 1995). The permeability of the capillaries beneath the cavity is temporarily increased. The presence of fibrinogen in the dentinal tubules indicates the activity of fibrin in the mechanism of dentine permeability reduction (IZUMI *et al.* 1998). The predentine is thinned directly beneath the cavity, demonstrating the reduction of odontoblastic activity (MJÖR 1983). Hyperaemia (MJÖR 1983), temporarily increased blood flow in the dental pulp (MJÖR 2001), inflammatory cell infiltrates (ZACH 1972), necrosis and apoptosis of cells in the odontoblastic layer beneath the cavity (BRONCKERS *et al.* 1996), disruption and rarefaction of Korff's fibrils (ZACH 1972), sprouting of nerve endings (BYERS *et al.* 1988) and diminished protein synthesis of odontoblasts (SEARLS 1975) are also observed among early dental pulp responses to cavity preparation.

The majority of reports from the literature are qualitative or semi-quantitative studies of the odontoblastic layer response to cavity preparation, followed by acid etching, with the use of different adhesive techniques and restorative materials (ZACH 1972; FUJITANI *et al*. 1992; LAURELL *et al.* 1995; BALDISSARE *et al.* 1997; HEBLING *et al.* 1999; CAMPS *et al.* 2000; SIX *et al.* 2000). Opinions on the influence of acid etching on dental pulp are divided. One group of researchers presumes that acid, with its low pH value, hypertonic and osmotic effect, acts as a strong chemical irritant to dental pulp. This may cause irreversible damage to underlying odontoblasts and severe inflammatory reaction (VOJINOVIĆ *et al.* 1973; RETIF *et al.* 1974; MACKO *et al.* 1978; GWINNET & TAY 1998). The other group claims that bacteria with their toxins, penetrating along marginal gaps of restoration towards the etched dentine, are the main factor provoking a histologically detectable odontoblastic layer response, as well as postoperative sensitivity or even pulpitis and necrosis (FUJITANI *et al.* 1992; NAGAOKA *et al.* 1995; BERGENHOLTZ 2000; IMAZATO *et al.* 2000; GOTO & JORDAN 1973).

The purpose of this study was to establish the early odontoblastic layer response and to estimate the number of odontoblasts after cavity preparation with and without acid etching.

Material and Methods

The early odontoblastic layer response was studied on 28 female Wistar rats, 45 days old and weighing from 150 to 200 g. All procedures followed Veterinary standards for the care and use of laboratory animals in the Republic of Slovenia. The animals were anaesthetized with xylasyne (Rompun®, Bayer, Leverkusen, Germany; 8 mg/kg, i.p.) and with ketamine-hydrocloride (Ketanest®, Parke – Davis, Berlin, Germany; 60 mg/kg, i.p.). Two different restorative procedures – cavity preparation with and without acid etching – were prepared freehand on the mesial surface of the intact first molar in both quadrants of the upper jaw. For each animal, a new sterile, round 0.7 mm diamond burr (DEPHA-DIAM, ISO 806 204 001), attached to the handpiece of a dental electromotor with 14000 RPM, was used. The cavity and the burr were constantly cooled with sterile saline solution. The second molar in the same quadrant, intact and left without any restorative procedure, served as a control (BYERS *et al.* 1988).

The procedure in the first experimental group was performed on the upper right molar and included cavity preparation approximately to the depth of the radius of the burr and a width not more than its diameter (allowing the cavities to extend into the middle third of the dentine), acid etching of the cavity walls with 37% phosphoric acid Total Etch Gel® (Vivadent, Schaan, Liechtenstein) for 15 sec., saline solution rinsing for 15 sec., gentle air and cotton-pellet drying and temporary restoration with Cavit®. The procedure in the second experimental group was performed on the upper left molar and included identical cavity preparation with only saline solution rinsing for 15 sec., drying and the same temporary filling.

The animals were killed in a CO_2 chamber at different post-operative time intervals and were divided into four time groups: 5 min, 6, 24 and 72 hours (BYERS *et al* 1988), each including 7 animals. The blocks of three molars with surrounding soft and hard tissues were dissected and fixed in 4% neutral buffered formalin for 24 hours. Decalcification took place in 10% ethylenediaminotetraacetic acid (EDTA) for 45 days at room temperature. After embedding in paraffin and orienting the tissue to the sagital plane of the first molar's long axis, serial 5 m thin sections were cut. Four serial sections from the deepest region of the cavity were stained with haematoxylin/eosin (HE), with periodic-acid-Schiff's (PAS) reaction, using the Giemsa and Goldner methods.

Tissue samples from each group were also processed for transmission electron microscopy. They were fixed in a mixture of 4.5% paraformaldehyde and 2% glutaraldehyde for 3 h at 4°C, counterstained in uranyl acetate for 1 hour at room temperature, rinsed in 0.2 M cacodylate buffer overnight and decalcified in EDTA together with the specimens for light microscopy. They were then postfixed in buffered 1% OsO_4 for 1 h at 4°C, dehydrated in series of ethanols, oriented and embedded in Epon. Ultrathin sections, cut from the

deepest region of the cavity, were stained with lead citrate.

Qualitative analysis

The odontoblastic layer beneath the cavity, in both experimental groups through the four time intervals and the control group (Fig. 1a), was observed under a light microscope (Reichert, Austria) at magnifications of 63-, 160-, 400- and 1000-times. Four serial sections, stained with HE, PAS, Giemsa and Goldner, were used for this purpose. The shape and the arrangement of the odontoblasts, the presence of cellular fragments and hyperaemia in the odontoblastic layer were assessed. The ultrastructural changes of odontoblasts beneath the cavity in the experimental groups were examined with a transmission electron microscope (JEOL 100 CX, Japan).

Quantitative analysis

Four serial sections from the deepest region of the cavity were quantitatively analyzed with the semiautomatic picture analyzing system IBAS-1000 (Kontron, Germany), at magnifications of 63- and 400-times. The cavity length and the remaining dentine thickness along the course of the dentinal tubules from the deepest part of the cavity were measured, thus indicating the dimensions of the cavities (Fig. 1b). The early odontoblastic layer response was morphometrically assessed by determining the following:

(a) the odontoblastic layer length beneath the cavity, being the distance between the uncut dentinal tubules from the gingival and the occlusal margins of the cavity (Fig. 1);

(b) the number of morphologically normal odontoblasts beneath the cavity;

(c) the number of aspirated cells in the dentinal tubules;

(d) the predentine width from five measuring points beneath the cavity and from five measuring points on the same level in the control (Goldner stained sections);

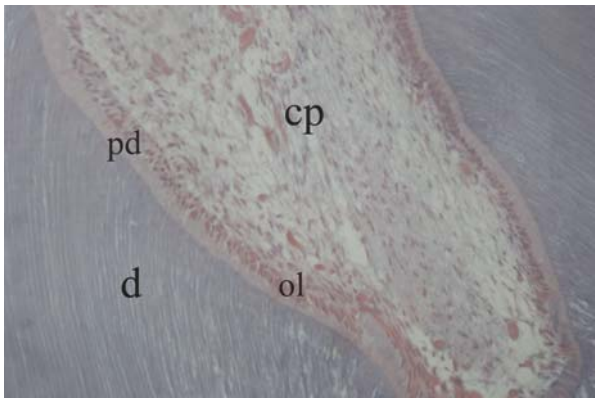
Statistical analysis

The data from quantitative and semi quantitative analyses were statistically processed using the program SPSS®. The results were represented as mean values with standard deviations. The normality of the distribution for each attribute was confirmed with the Kolmogorov-Smirnov test. The statistical significance between the two experimental groups and the control was examined with General Linear Model analysis of variance for repeated measurements, with between and within subjects' factors and with the *post hoc* comparison test (Fisher's LSD test), considering a P-value of less than 0.05 to be significant.

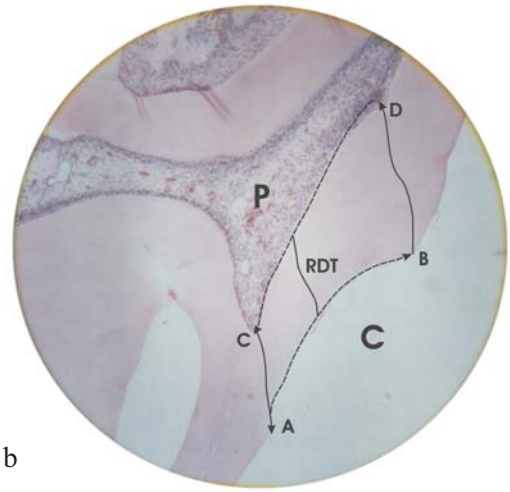
Results

Qualitative analysis

Electron microscopic (EM) analysis 5 min postoperatively in the specimens of etched and unetched cavity showed odontoblasts of normal shape and structure, with some missing intercellular junctions and lying in disorder (Fig. 2a). Beneath the etched cavity, a few separate odontoblasts



a



b

Fig. 1. (a) Histological section of control (not operated) molar. d – dentine, pd – predentine, ol – odontoblastic layer, cp – central pulp (HE), × 160; (b) Histological section of first molar with cavity. The curved line between A and B represents the cavity length, the curves between A and C and between B and D the course of the uncut dentinal tubules, and the curved line between C and D the odontoblastic layer length beneath the cavity. C – Cavity, RDT – remaining dentine thickness, P – dental pulp (HE), × 26.

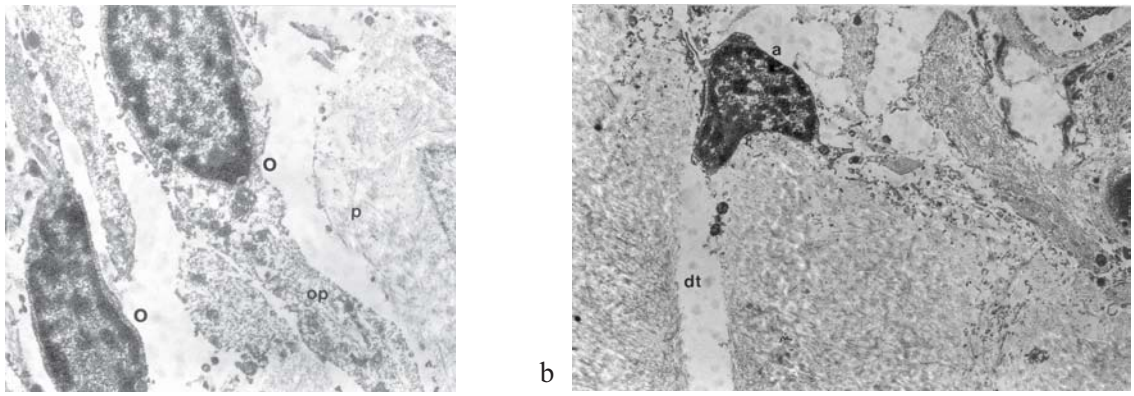


Fig. 2. TEM micrography of odontoblasts beneath etched cavity 5 min post-operatively. (a) Enlarged intercellular spaces between odontoblasts (o) without intercellular junctions, × 8400; (b) Aspirated cell (a) with condensed chromatin of untypically shaped nucleus, aspirated in the dentinal tubule (dt), × 5000. op –odontoblastic process, p – predentine.

had altered nuclear shape and condensed chromatin. The chromatin condensation was also visible in cells, aspirated in the dentinal tubules (Fig. 2b). Light microscopic (LM) analysis 5 min post-operatively showed a more obvious disarrangement of odontoblasts in etched cavity specimens. A large number of blood-filled capillaries was found between the odontoblasts, directly beneath the predentine, and also in the subodontoblastic zone in both experimental groups (Fig. 3).

Six and 24 hours post-operatively, cellular fragments were observed between odontoblasts, in the dentinal tubules and in the subodontoblastic zone

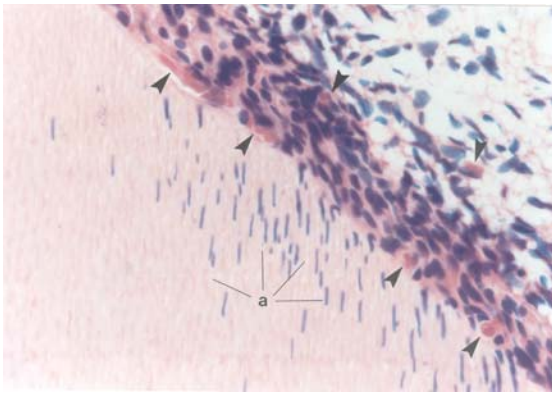


Fig. 3. Odontoblastic layer beneath etched cavity 5 min post-operatively. Arrows indicate blood-filled capillaries. a – aspirated cells in dentinal tubules (HE), × 400.

in both experimental groups (Figs 4a, Fig. 4b). On the EM level, the cytoplasm of odontoblasts contained several vacuoles. The chromatin showed compaction at the nuclear periphery (Fig. 5). In the odontoblastic layer and subodontoblastic zone, hyperaemia was present in both experimental groups. In etched cavities, it was also present in the central part of the mesial root and crown pulp.

Ultrastructural analysis 72 h post-operatively showed a distinctive endoplasmic reticulum in some of the odontoblasts of both experimental

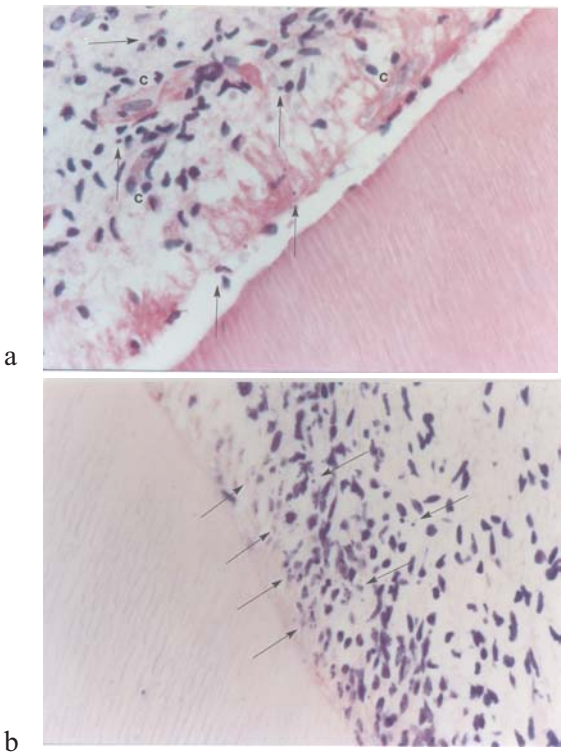


Fig. 4. Odontoblastic layer twenty-four hours post-operatively. (a) Beneath etched cavity (PAS), × 400. (b) Beneath unetched cavity (Giemsa), × 400. Arrows – cellular fragments, c – blood-filled capillaries.

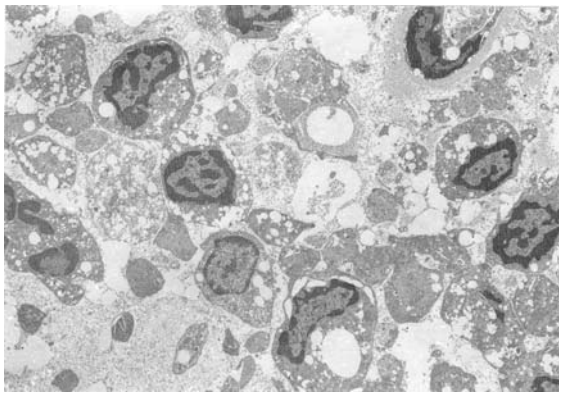


Fig. 5. TEM micrography of odontoblasts beneath etched cavity 24 h post-operatively. Note numerous vacuoles in the cytoplasm and chromatin compaction at the nuclear periphery, × 3900.

groups (Fig. 6). LM analysis of both experimental groups showed some cell fragments between the odontoblasts, and the latter began to rearrange. Several cells with large, less basophilic nuclei and a few mitoses were noticed between odontoblasts and in the subodontoblastic zone. Hyperaemia was still present in the odontoblastic layer in both experimental groups, as well as in the subodontoblastic zone in the etched cavity group (Fig. 7a). In some places near the capillaries beneath unetched cavities, a widening of the predentine and the beginning of tertiary dentine synthesis with erythrocyte inclusions was found (Fig. 7b).

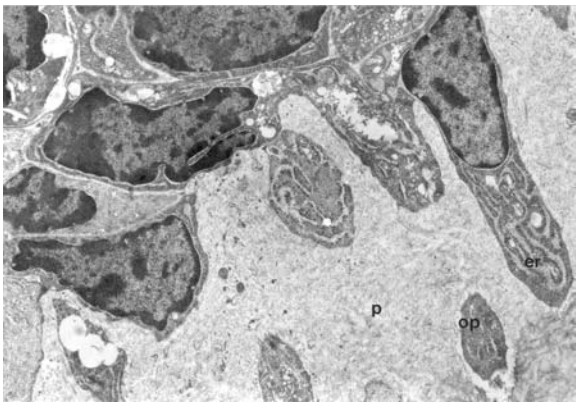


Fig. 6. TEM micrography of odontoblasts beneath unetched cavity 72 h post-operatively. Note odontoblast with marked endoplasmic reticulum (er). p – predentine, op – odontoblastic process, × 6300.

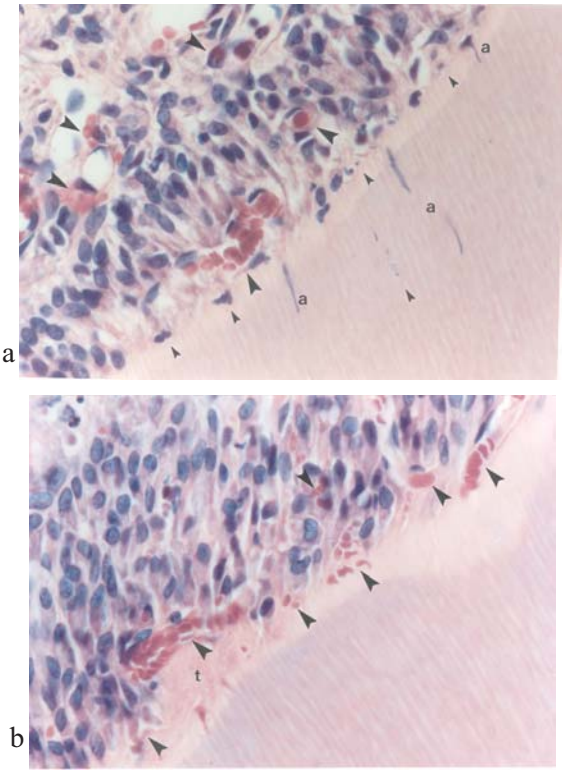


Fig. 7. Odontoblastic layer seventy-two hours post-operatively. (a) beneath etched cavity. (b) beneath unetched cavity. Small arrows – cellular fragments, large arrows – blood-filled capillaries, a – aspirated cells in dentinal tubules, t – tertiary dentin (HE), × 400.

Quantitative analysis

Cavity dimensions

The cavity length was 859 ± 32 μm, the remaining dentine width beneath the cavity was 119 ± 20 μm in both experimental groups and the dentine width in the control was 360 ± 27 μm, following the course of the dentinal tubules.

Number of odontoblasts (Fig. 8)

The number of odontoblasts per ěm of odontoblastic layer length (Nod_L) beneath the cavity and in the control group was calculated. Comparison between etched and unetched cavities showed Nod_L significantly lower 6 h ($P<0.001$) and 24 h ($P<0.005$) post-operatively and Nod_L significantly higher ($P<0.027$) 72 hours post-operatively in etched cavity. In etched cavity, Nod_L was significantly lower (P from 0.001 to 0.023) at all post-operative time intervals in comparison with the control. In unetched cavity, Nod_L was also significantly lower 6 h ($P<0.002$), 24 h ($P<0.003$) and 72 h ($P<0.001$) post-operatively in comparison with the control.

Number of aspirated cells (Fig. 9)

There were no aspirated cells in the control. At all time intervals post-operatively, the number of aspirated cells per odontoblastic layer length unit (Nas_L) in etched cavity was significantly higher than in unetched cavity (5 min: $P<0.004$, 6 h: $P<0.031$, 24 h: $P<0.007$, 72 h: $P<0.030$). In etched cavity, Nas_L was significantly higher than in the control (5 min: $P<0.004$, 6 h: $P<0.013$, 24 h: $P<0.005$, 72 h: $P<0.015$). In unetched cavity, Nas_L was significantly higher than in the control only 6 hours post-operatively ($P<0.009$).

Predentine width

The predentine width beneath the cavity was significantly lower in etched cavity than in the control (5 min: $P<0.003$, 6 h: $P<0.015$, 24 h: $P<0.035$, 72 h: $P<0.004$), and in unetched cavity than in the control (5 min: $P<0.005$, 6 h: $P<0.003$, 24 h: $P<0.031$). Seventy-two hours post-operatively, there were no significant differences between unetched cavity and the control. Differences between the two experimental groups were not significant.

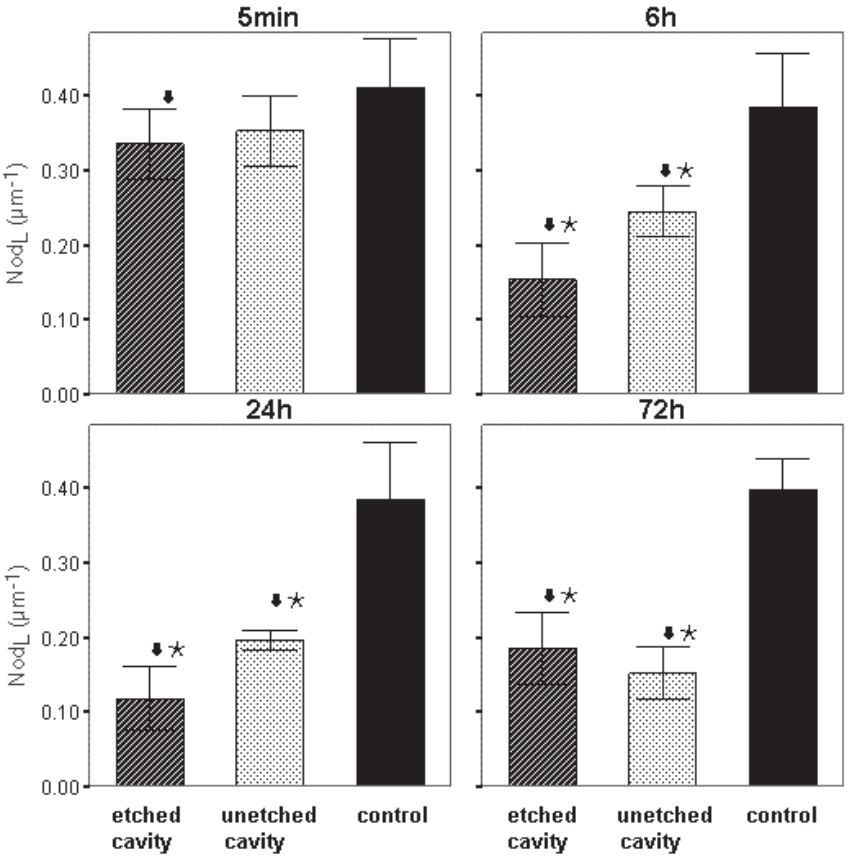


Fig. 8. Number of odontoblasts: ⬇ – significant difference between experimental group and the control, ⋆,– significant difference between etched and unetched cavity.

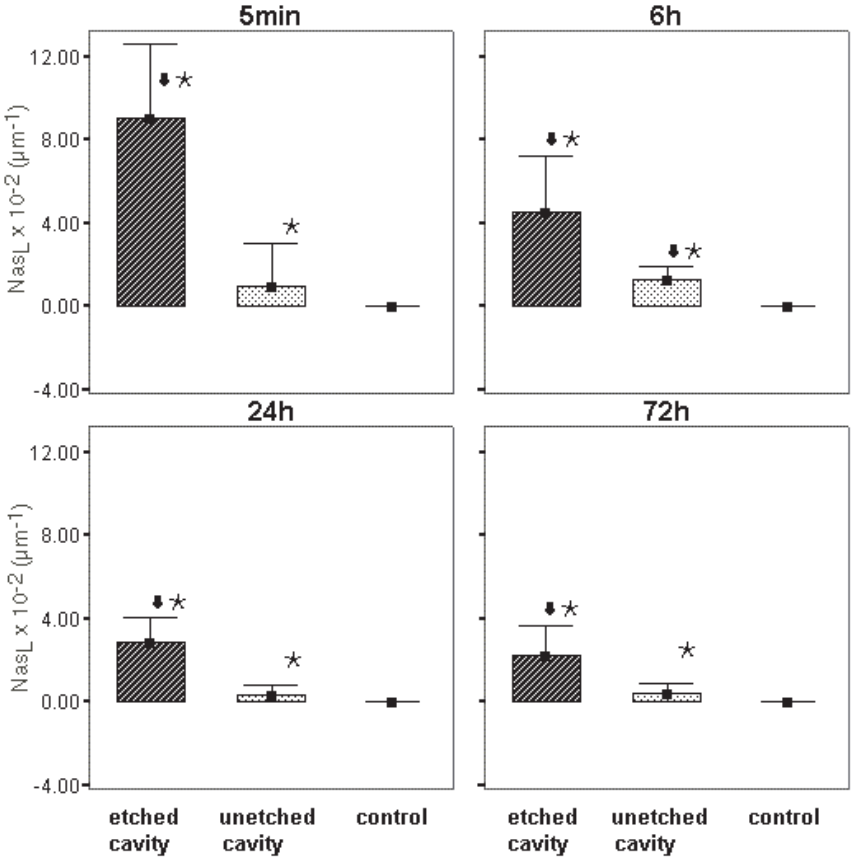


Fig. 9. Number of aspirated cells: ⬇ – significant difference between experimental group and the control, ⋆,– significant difference between etched and unetched cavity.

Discussion

The long-term success of a dental procedure depends on preserving the odontoblasts vitality and functioning, therefore on preserving the natural dental pulp's repair mechanisms (ABOUT *et al.* 2001). In this study, two factors influencing the vitality of odontoblasts were examined, i.e. cavity preparation and acid etching, , and their effects analyzed and compared. Cavit® (ESPE Dental AG, Seefeld, Germany) was used, since it has been reported that it is not noxious to dental pulp if placed gently into the moist cavity, but having appropriate mechanical characteristics and giving a tight marginal seal for the time of the experiment (PROVANT & ADRIAN 1978).

Qualitative analysis revealed a disarrangement of odontoblasts after procedures, cavity preparation and acid etching, 5 min post-operatively and the most extensive damage in the odontoblastic layer, 6 and 24 hours post-operatively. The degenerative changes of individual odontoblasts and the presence of cellular fragments at the site of injury indicate that one of the cell death pathways is present. The definition of the form of cell death is not possible on the basis of ultrastructural changes solely. Further studies have to be done to ascertain whether the degeneration of the odontoblasts is a part of apoptosis, necrosis or any other type of cell death. Cytoplasmic vacuolation, which was detected in the odontoblastic layer 24 hours post-operatively, has been reported as one of the morphological features of nonapoptotic cell death, called paraptosis (SPERANDIO *et al.* 2000). Apoptosis has already been demonstrated in the odontoblastic layer and in the subodontoblastic zone in studies of different types of cell death (BRONCKERS *et al.* 1996; KITAMURA *et al.* 2001). The reparative processes started 72 hours post-operatively in etched and unetched cavities. At this time, a rearrangement of the odontoblasts, newly differentiated cells, hyperaemia between the odontoblasts and in their vicinity, and tertiary dentin formation was observed, as in other similar studies (OHSHIMA *et al.* 1995; IZUMI *et al.* 1998; SATO 1989).

The main element of the quantitative analysis was the assessment of odontoblast numbers. The method of counting morphologically normal odontoblasts under the cavity gave relative, but relevant results to compare the two experimental groups. Analyzing the odontoblastic layer response quantitatively, it was found that acid etching of the cavity causes an additional reduction in odontoblast numbers, which was especially marked 6 and 24 hours post-operatively. In this experiment, the smear layer was removed by acid etching, thus causing increased permeability of the dentine in comparison with unetched cavity. In etched cavity, the increased number of aspirated cells was coincident with the decreased number of odontoblasts, demonstrating this phenomenon. The number of aspirated cells decreased in time post-operatively. In unetched cavity, an increased number of aspirated cells was only observed 6 hours post-operatively. According to data from the literature, hygroscopic temporary filling material Cavit can also cause a stronger outward movement of the dentinal liquid (PROVANT & ADRIAN 1978), thus contributing to the phenomenon of cell aspiration.

Reparative processes in rat molar dental pulp begin 72 hours post-operatively (IZUMI *et al.* 1998; GWINNET & TAY 1998). It has been suggested that acid etching of the cavity releases the growth factor TGF- β1 (SMITH & SMITH 1998) or other biologically active molecules from the dentinal matrix, promoting dental pulp repair. Owing to the demonstrated higher number of odontoblasts 72 hours post-operatively under etched cavity in comparison with unetched, an earlier initiation of odontoblastic numerical renewal was presumed. It is interesting that cavity etching initially caused the destruction of more odontoblasts until 24 hours post-operatively. Cavity preparation with acid etching represents a stronger irritant to the dental pulp than only cavity preparation. A stronger irritant seems to be needed for faster initiation of cell differentiation (BJŘRNDAL *et al.* 1998) from pulpal progenitor cells to new odontoblasts.

Another finding of this survey was the thinning of the predentine in both experimental groups at all post-operative time intervals, except in unetched cavity 72 hours post-operatively. The cause of its thinning is probably associated with the liquid moving outward from the predentine after the operative procedure. The increase in predentine width in unetched cavity 72 hours post-operatively could indicate a faster recovery of primary odontoblasts, with the beginning of tertiary dentine synthesis after the weaker injury, caused by cavity preparation only. Seventy-two hours post-operatively, the number of odontoblasts under etched cavity was higher than under unetched cavity. Therefore, an increase in predentine width may also be expected. The possible explanation is that there was a higher number of newly differentiated odontoblasts under etched cavity, which 72 hours post-operatively have not yet begun tertiary dentine synthesis and the predentine was still thin.

In conclusion: quantitative analysis of the early odontoblastic layer response demonstrated greater injury to the odontoblastic layer, but numerical renewal of this layer began earlier under etched cavity compared to unetched cavity. Further investigation will be needed to elucidate the reparative processes after mechanically and chemically induced injury.

References

ABOUT I., MURRAY P. E., FRANQUIN J-C. 2001. The effect of cavity restoration variables on odontoblast cell numbers and dental repair. J. Dent. **29**: 109-17.

BALDISSARE P., CATAPANO S., SCOTTI R. 1997. Clinical and histological evaluation of thermal injury tresholds in human teeth: a preliminary study. J. Oral Rehabil. **24**: 791-801.

BERGENHOLTZ G. 2000. Evidence for bacterial causation of adverse pulpal responses in resin-based dental restorations. Crit. Rev. Oral Biol. Med. **11**: 467-80.

BJŘRNDAL L., DARVANN T., THYLSTRUP A. 1998. A quantitative light microscopic study of the odontoblast and subodontoblastic reactions to active and arrested enamel caries without cavitation. Caries Res. **32**: 59-69.

BRONCKERS A. L. J. J., LYARUU D. M., GOEI W. 1996. Nuclear DNA fragmentation during postnatal tooth development of mouse and hamster and during dentin repair. Eur. J. Oral Sci. **140**: 102-11.

BYERS M. R., NÄRHI M. V. O., MECIFI K. B. 1988. Acute and chronic reactions of dental sensory nerve fibers to cavities and desiccation in rat molar. Anat. Rec. **221**: 872-83.

CAMPS J., DÉJOU J., RÉMUSAT M. 2000. Factors influencing pulpal response to cavity restorations. Dent. Mater. **16**: 432-40.

FUJITANI M., INOKOSHI S., HASODA H. 1992. Effect of acid etching on the dental pulp in adhesive composite restorations. Int. Dent. J. **42**: 3-11.

GOTO G., JORDAN R. E. 1973. Pulpal effects of concentrated phosphoric acid. Bull. Tokyo Dent. Coll. **14**: 105-12.

GWINNETT J. A., TAY F. R. 1998. Early and intermediate time responses of the dental pulp to an acid etch technique in vivo. Amer. J. Dent. **11**: 35-44.

HEBLING J., CIRO E. M. A., COSTA C. A. S. 1999. Human pulp response after an adhesive system application in deep cavities. J. Dent. **27**: 557-64.

HIRVONEN T. J., NÄRHI M. V. O. 1986. The effect of dentinal stimulation on pulp nerve functions and pulp morphology in the dog. J. Dent. Res. **65**: 1290-3.

IMAZATO S., TARUMI H., EBI N. 2000. Cytotoxic effects of composite restorations employing self-etching primers or experimental antibacterial primers. J. Dent. **28**: 61-7.

IZUMI T., YAMADA K., INOUE H. 1998. Fibrinogen/fibrin and fibronectin in the dentin-pulp complex after cavity preparation in rat molars. Oral Surg. Oral Med. Oral Pathol. **86**: 587-91.

KITAMURA C., KIMURA K., NAKAYAMA T. 2001. Primary and secondary induction of apoptosis after cavity preparation of rat molars. J. Dent. Res. **80**: 1530-4.

LAURELL K. A., CARPENTER W., DAUGHERTY D. 1995. Histopathologic effects of kinetic cavity preparation for the removal of enamel and dentin. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **80**: 214-25.

MACKO D. J., RUTBERG M., LANGELAND K. 1978. Pulpal response to the application of phosphoric acid to dentin. Oral Surg. Oral Med. Oral Pathol. **45**: 930-45.

MJÖR I. A. 1983. Reaction Patterns of Human Teeth. 1st ed. Boca Raton, Florida: CRC Press, Inc. 74-82.

MJÖR I. A. 2001. Pulp-dentin biology in restorative dentistry. Part 2: Initial reactions to preparation of teeth for restorative procedures. Quintessence Int. **32**: 537-51.

NAGAOKA S., MIYAZAKI Y., LIU H. J. 1995. Bacterial invasion into dentinal tubules of human vital and nonvital teeth. J. Endod. **21**: 70-4.

OHSHIMA S. 1990. Ultrastructural changes in odontoblast and pulp capillaries following cavity preparation in rat molars. Arch. Histol. Cytol. **53**: 423-38.

OHSHIMA S., SATO O., KAWAHARA I. Responses of immunocompetent cells to cavity preparation on rat molars: an immunohistochemical study using OX6-monoclonal antibody. Connect. Tissue Res. **32**: 303-11.

PROVANT D. R., ADRIAN J. C. 1978. Dental pulp reaction to Cavit temporary filling material. Oral Surg. **45**: 305-10.

RETIEF D. H., AUSTIN J. C., FATTI L. P. 1974. Pulpal response to phosphoric acid. J. Oral Pathol. **3**: 114-22.

SATO O. 1989. Responses of pulpal nerves to cavity preparation in rat molars: an immunohistochemical study using neurofilament protein (NFP) antiserum. Arch. Histol. Cytol. **4**: 433-46.

SEARLS J. C. 1967. Light and electron microscope evaluation of changes induced in odontoblast of the rat incisor by high-speed drill. J. Dent. Res. **46**: 1344–51.

SEARLS J. C. 1975. Radioautographic evaluation of changes induced in the rat incisor by high-speed cavity preparation. J. Dent. Res. **54**: 174-82.

SIX N., LASFARGUES J. J., GOLDBERG M. 2000. In vivo study of the pulp reaction to Fuji IX, glass ionomer cement. J. Dent. **28**: 413-22.

SMITH A. J., SMITH G. 1998. Solubilization of TGF-β1 by dentine conditioning agents. J. Dent. Res. **77**: 1034.

SPERANDIO S., DE BELLE I., BREDESEN D. E. 2000. An alternative, nonapoptotic form of programmed cell death. Proc. Natl. Acad. Sci. USA **97** (26): 14376-14381.

VOJINOVIĆ O., NYBORG H., BRÄNNSTRÖM M. 1973. Acid treatment of cavities under resin fillings: bacterial growth in dentinal tubules and pulpal reactions. J. Dent. Res. **52**: 1189-93.

ZACH L. 1972. Pulp lability and repair; effect of restorative procedures. Oral Surg. **33**: 111-21.