Ph.D. THESIS

THE EFFECTS OF ADHESIVE MATERIALS ON THE PULPAL MICROCIRCULATION

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INTRODUCTION

Adhesive dentistry has become a routine method in the daily dental practice. The achieved adhesion between the tooth structures and filling material warrants the retention and the good marginal sealing.

To enhance bonding of a composite filling material the entire adherent surface of the cavity is bonded. Accordingly, no protective lining or base is applied on the pulpal cavity wall, thus the barrier for protection of the pulpal tissue against the effect of the bonding procedure is reduced. However, the pulpal toxicity of adhesive materials is still subject of controversy.

Several laboratories have investigated the biocompatibility of dental adhesive materials with conflicting findings. It is difficult to obtain accurate results, partly owing to technical difficulties when dealing with the pulp enclosed within rigid walls. It is further complicated by the fact that the adhesive procedures often entail several treatment phases, and are constantly subject to development and restructuring. The physical properties of the bonding material can also influence the results of the experiments done on the biocompatibility of the market available bonding materials. For instance, in case of weak adhesion, there will be a leakage between the filling material and tooth structures. This may lead to osmotic, bacterial, and thermal irritations, which can be taken mistakenly for the irritative effects of the bonding agents.

Several international tests have been established to evaluate the biocompatibility of restorative resins with contradictory experimental results. Though, some acknowledged shortcomings are attributed to these tests.

Circulatory changes are valuable predictors of pathophysiological changes. Thus the investigation of pulpal microcirculation provides valuable information about the adverse effect of dental materials.

Thus the investigation of pulpal circulatory reactions after bondmaterial application seems to be necessary and justified. The investigation of the effect of bondmaterial constituents on pulpal microcirculation and identifying the physiological process may contribute valuable information to the findings of the biocompatibility tests.
AIM OF THE STUDY

The aim of the study was to investigate the immediate circulatory effects of the adhesive materials applied directly onto a constant thin pulpal dentine. Experiments were carried out with the following goals:

1. to find out whether acid etching with different application time and with different composition alters the blood circulation of the pulp of the rat’s lower incisors.

2. to investigate the effect of a composite resin bonding system on the pulpal vascular diameters applied after acid etching or without etching.

3. to compare the pulpal circulatory effects caused by an acetone containing bondmaterial to the findings with acetone free ones.

4. to determine the acute circulatory effects of the chemical ingredients of bonding agents.

5. to clarify the role of substance P in regulation of pulpal microcirculation under basal conditions and during local administration of bonding ingredients.

6. to compare experimental data obtained by vitalmicroscopy to those provided by laser Doppler flowmetry.

METHODS AND MATERIALS

Experiments were carried out on the first lower incisor of male Sprague-Dawley rats (341 ± 4 g, SE; n=184), anaesthetized with pentobarbitone sodium. Breathing was facilitated by tracheal cannulation, and arterial blood pressure was monitored through a cannula inserted into the left femoral artery. Body temperature was maintained at 37°C using a heating lamp throughout the experiment.

Vitalmicroscopy:

The cartilaginous ligaments connecting the lower jaws were transected and the left part was cautiously cleared of soft tissues by gently pulling them toward the molar teeth. This jaw was fixed with a circular polyester matrix strip by a dental matrix holder to ensure a rigid grip for further preparation. The distal and mesial surfaces of the entire crown of the left lower incisor and some parts of the alveolar bone were ground away under a dissecting binocular microscope until the pulp vessels became clearly visible through the remaining dentin. When grinding was completed a thin plate of dentin still remained intact over the delicate pulpal tissue. To prevent dehydration or overheating the exposed dentin was kept wet at a constant temperature by a thermostat-driven continuous saline rinsing throughout the experiment. After one hour of equilibration the baseline vessel diameter of a suitable arteriole was measured on the monitor. Subsequently, bonding procedures or saline...
solution (control) was applied on the prepared dentine surface.

Changes in vascular diameter of the pulpal arteriole were measured at 5, 15, 30 and 60 minutes. Changes of arterial diameter were expressed as percentage of baseline. For statistical analysis two-way ANOVA was applied with treatment and time as factors (p<0.05).

With this method the pulpal reactions after acid etching were investigated. The pulpal effects of bonding agents (acetone-containing / acetone-free; after acid etching / without acid etching) were also evaluated. In addition, the pulpal circulation was observed after the application of chemical ingredients (HEMA, TEGDMA). This method was also used to clarify the role of substance P in regulation of pulpal microcirculation under basal conditions and during local administration of bonding ingredients.

_**Laser Doppler flowmetry:**_

A fixing rod attached to the central incisors and to the laser probe by dental acrylic immobilized the teeth and the jaw. For test material application on the buccal side of the lower left incisor a standardized deep Black class V cavity was prepared according to FDI standards. It was done under an operating microscope till the pulp vessels became visible through the floor of the cavity. The prepared dentin surface was kept wet at a constant temperature by thermostat-driven controlled continuous sterile saline rinsing. Upon completion of preparation the animals were allowed to rest for 1 hour as equilibration time before starting the experimental procedures. Subsequently, the laser Doppler signals were recorded prior to and at 1, 5, 15, 30 and 60 minutes after the bonding agents or saline (control) were administered on dentin. The changes of pulpal blood flow were expressed as percent of baseline at each time of examination. For statistical analysis two-way ANOVA was applied with treatment and time as factors.

With this method the effects of a forth generational bonding agent and the consequences of a glassionomer based bonding material application were investigated.
RESULTS AND CONCLUSIONS

Vitalmicroscopy:

- Under the present experimental conditions, the initial toxic effect of etching per se seems to be tolerable to the pulp, when applied as recommended by the manufacturers (15-20 seconds). Apparently the thin wall of dentin seems to enable the pulp to overcome the toxic effects of acid etching.

- Prolonged etching time (60 seconds) causes pulpal vasoconstriction and acute pulpal necrosis in 40% of rats, thus the prolongation of etching time is a serious hazard to the dental pulp.

- Bonding agents applied onto a thin layer of dentin have an acute vasodilatating effect, however they do not provoke irreversible stasis like changes. Acid etching prior to the bondmaterial application provokes more rapid pulpal reaction to the bonding agents. There was no statistical difference between the acetone containing and acetone free bonding material either in the degree of vasodilatation or the absence of irreversible changes.

- Dental adhesive ingredients applied for 20 seconds in commercial concentrations (HEMA [35%], TEGDMA [47%]) onto a very thin layer of dentin cause significant vasodilatation, however they do not seriously irritate the pulp. However, the observed stasis in one specimen after the application of TEGDMA [47%] indicates the possible hazard for the dental pulp.

- The application of TEGDMA [94%] caused vasoconstriction in the dental pulp, which ended in stasis in 33% of rats. It appears that these high concentration of TEGDMA is capable of provoking serious pulpal circulatory reactions.

- Under our experimental conditions SP antagonist administration prevented the development of the vasodilatation observed after HEMA application.

- During SP antagonist administration to the resting pulp, a slow vasoconstriction was observed, suggesting basal SP secretion.

Laser Doppler flowmetry:

- In the presence of a forth-generation bonding material significant blood flow elevation was observed in the pulpal circulation.

- A glassionomer based bonding agent did not cause significant circulatory changes.
NEW FINDINGS

• For the first time, it was shown; that bonding agents applied in the direct proximity to the pulp will bring an immediate vasodilatation and enhanced local blood flow within the dental circulation. However, it is not accompanied by an irreversible change (stasis). The observed vasodilatation and blood flow elevation can be considered as a protective response, and presumably does not lead to an irreversible pulpal damage.

• It was shown that conditioning of the dentin according to the manufacturer’s recommendations, does not lead to significant pulpal circulatory changes. Nonetheless, prolonged conditioning can bear serious effects on the pulp.

• It was documented that there are no significant differences between acetone-containing and acetone-free bond materials with respect to their effects on the pulpal circulation.

• For the first time, it was shown that the chemical contents (HEMA, TEGDMA) of the bonding material cause vasodilatation when used at the clinically applied concentrations, however, the application of greater concentrations will lead to irreversible adverse effects. In deep cavities the ingredients of bondmaterials can provoke pulpal circulatory reaction even if applied for only 20 seconds. The extremely high concentration of hydrophilic monomers in adhesive system can be a serious risk for the dental pulp. Careful selection of ingredients should precede the application of resin material in very deep cavities, eventually for pulp capping. On the other hand, the duration of pulpal exposure to the uncured adhesives should be minimized.

• The role of substance P in pulpal basal microcirculation was shown; and for the first time, it was documented that substance P is involved in the acute pulpal response to bondmaterial constituents.

• The experimental delivery of a substance P antagonist directly through the thin layer of dentin to the pulp seems to be a useful method to evaluate its local effect per se.

In summary it is concluded, that the proper application of clinically used bondmaterials do not cause acute serious irreversible changes in the dental circulation. However, the improper application of these materials can lead to serious pathology of the pulp.

The results presented in this thesis provide valuable information about immediate pulpal effects of dental materials. The long lasting consequence of this vascular alteration in the dental pulp remains to be elucidated by long-term histopathological tests.
LIST OF PUBLICATIONS

The current dissertation is based on work described in the following publications


I. Iványi, Á. E. Balogh, Á. Fazekas, L. Rosivall, I. Nyárasdy: Comparative analysis of pulpal circulatory reaction to an acetone containing and an acetone free bondmaterial as measured by vitalmicroscopy. *Operative Dentistry*, *accepted for publication* 2002 IF(2000): 1,411


Publications in progress


I. Iványi, B. Kispélyi, L. Rosivall, Á. Fazekas, I. Nyárasdy: Effect of Bondmaterial Constituents on Pulpal Microcirculation Measured by Vitalmicroscopy

I. Iványi, B. Kispélyi, L. Rosivall, Á. Fazekas, I. Nyárasdy: SP is involved in regulation of pulpal microcirculation under basal conditions and during local administration of HEMA

The current dissertation is related to the work described in the following publications

B. Kispélyi, L. Fejérda, I. Iványi, L. Rosivall, I. Nyárasdy: Dentin sealers effect on the diameter of pulpal microvessels – A comparative vitalmicroscopic study.
The current dissertation is based on work described in the following abstracts


Iványi, I. Nyárasdy, Á.E.Balogh, L. Rosivall(1996): In vivo examination of bondmaterials used in dentistry (vitalmicroscopic study). *Physiology* vol.6/ Nr. 2(10) 55 - ISSN 1223-2076

The current dissertation is related to the work described in the following abstracts


