FINAL REPORT

Histopathology of Embrace and a 2-part light cured material on the pulp – a subhuman primate study

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Description test material(s)

For use of test materials see worksheets.
Subhuman primates or other appropriate animals\(^1,2\)
In this study 3 baboons weighing approximately 14 - 20 Kg will be used.


Test Teeth
The teeth will be clinically intact, non-carious teeth with no more than superficial abrasion.
The histological sections cut from all test and control teeth will meet the following requirements: 3,4,5,6,7

They shall be free of histological artifacts due to shrinkage or tears interfering with the cavity/pulp relationship; the floor of the cavity shall be in the inner third of the dentin; the cavity shall be sufficiently wide so that dentinal tubules may be followed from the cavity to the pulp throughout at least sixty 5 gm sections.

Experiments, tests, or controls resulting in sections, which do not meet with these requirements, shall be replaced. The positive and negative controls from previous studies with known positive and negative tests in the same species will be used. This has been approved by the ADA council and has been done in the past by the investigators.

Clinical test procedure
The animal is administered short lasting anesthesia. (Ketamine 100 mg/ml/kg body weight) followed by general anesthesia by means of a mixture of 20% Halothane and 60 % O2 administered through intubation. All calculus and debris will be removed from the tooth surfaces during a prior treatment, at least 2-3 days before surgery.

A class V cavity is prepared with a carbide 331 bur at high speed with fiber optics, under a water spray, which simultaneously hits the contact area between the drilling instrument and the tooth surface.

The cavity is placed in the gingival area of the tooth and can be as wide as possible reaching into both proximal areas.

The cavity is prepared to a depth so that its floor is situated in the inner third of the dentin. An attempt is made to have an average remaining dentin thickness of 1.0 mm or slightly less in each category. This procedure is followed as well prior to making full crown preparations to assure similarity in RDT's in case crown preparations are indicated.

The choice as to which cavities receive the test filling materials can be seen on the attached work sheet and is presented as groups (systems). Each category of teeth should resemble each other with respect to tooth size and type of tooth. The materials shall, in all phases, be used accurately according to the manufacturer's directions. The manufacturer will provide written directions as to the use of the experimental materials or shall approve written instructions. The cavity shall be dried carefully with sterile cotton or short blasts of air at room temperature, preventing excessive drying of the dentin.

Period of evaluation (Modified for this experiment, see worksheets)

A minimum of 7 teeth shall be used for each system

**Short period:** 3-5 days.

To evaluate the immediate effect of the experimental material

**Intermediate period:** 25 - 30 days

To evaluate the intermediate effect after a period of 25 - 30 days.

**Long duration:** approximately 60-90 days, to study the long-term effect on the pulp. (Extended period: 120 days. May be added when pulp capping materials are being evaluated.)

When the information from the short, intermediate, and long period experiments is considered jointly, it can be determined whether any initial inflammation decreased over time.

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Preparation of the specimens for histological evaluation
The animals will be sacrificed by means of first inducing general anesthesia with (Ketamine 100 mg/ml/kg body weight and acepromazine (10 mg/ml/kg body weight) followed by (100 mg/2.5 kg) of Sodium pentobarbital (65 mg/ml). Under deep anesthesia perfusion fixation will be performed. After perfusion the jaws will be removed with a Stryker saw and with a carbide # 331 in a high speed hand piece the apices will be opened. Prior to removing the apex a radiogram may be taken to determine if radiographic changes have occurred.
The jaws will then immediately be immersed in 10% neutral buffered formalin which is replenished after 24 hours. The samples are further processed according to accepted histological techniques.
The end point of demineralization in 5% formic acid can be checked radiographically or determined by the ease of trimming with a razor blade. The specimens (teeth) shall be prepared for histological exam/nation by generally accepted methods, preferable providing sections through the entire pulp.\textsuperscript{12}
The specimens shall be coded so that the test and control teeth are not identified during the histological evaluation.
Serial sections shall be cut at 6 microns. The sections shall be cut so that dentinal tubules may be followed along the entire stretch from the cavity floor to the pulp.\textsuperscript{13}


Intermediate slides shall be stained according to Masson's trichrome stain for control of the effect of cavity preparation. Intermediate slides shall be stained according to Brown & Brenn for recognition of bacteria.
The distance from the floor of the cavity to the pulp shall be measured in a straight line and called the remaining dentin thickness. Average remaining dentin thickness values shall be obtained for test and control groups and they shall not be significantly different \textsuperscript{14,15,16}
The reaction of the test material shall be compared to a positive and negative control. The experimental material shall not be different from the negative control. The data from negative and positive controls are on file and have been conducted by the principal investigator. As a negative control IRM (Dentsply/Caulk Div., Milford DE USA) and as a positive control silicate cement (Kerr, Romulus, NJ USA) was tested.

The above described protocol conforms to the ISO 7405 and the Recommendations for guidelines of the ANSI/ADA, Specifications #41.
MATERIALS AND METHODS

Group 1. Class V preparations were prepared to a depth of 0.50-0.75 mm from the pulp using a high speed hand piece and a #331 carbide bur. Preparations were done using copious water cooling. An attempt was made to prepare the depth to a distance of 0.5-0.75 mm to the pulp. After preparation the cavities were washed thoroughly with an air/water spray. The experimental material (EMBRACE pit & fissure sealant) was applied as a liner with a small ball burnisher, spreading it to an even thickness on the floor of the preparation. Once applied, the material was light cured for 20s using a visible light-curing unit (Spectrum, Dentsply/Caulk Div. Milford DE). Output of the light was measured at the beginning of the experiment and set at 650 mW/cm². Light output was checked periodically during the experiment. The entire preparation was etched with 35% phosphoric acid gel (Etch-Rite, Pulpdent) for 15s. This was followed by thorough rinsing and gentle drying leaving the preparation moist. A dentin bonding agent was applied (PQ1, Ultradent Products Inc, South Jordan UT), which was air thinned and light cured for 20s. The cavity was restored with TPH composite resin (/Dentsply/Caulk Div.), which was light cured for 40s. The restoration was finished with fine fluted carbide burs under water cooling and the restoration and surrounding enamel was etched for 15s with 35% phosphoric acid gel, dried lightly, and Embrace Glaze (Pulpdent Corp) was applied. After gentle air thinning this was light cured for 20s.

Group 2.
This group was similar to Group 1 with exception of the experimental material being applied. Part A & B of a proprietary formula were mixed (Pulpdent) and applied as a liner. The remainder of the treatment was the same as described for Group 1.

At time intervals of 25 and 120 days, the animals were perfusion euthanised, the jaws dissected and further fixed in 10 % neutral buffered formalin for 48 hours. Using routine histological techniques the jaws were decalcified, imbedded in paraffin and sections of 6 microns were cut. This was followed by staining with hematoxylin and eosin (H&E) and Brown and Brenn for detection of bacteria.

RESULTS

Embrace Pit & Fissure Sealant- 25-day results
The results for this material were remarkable in that there was a lack of a superficial and deep inflammatory reaction. The protocol stated that an attempt would be made to have a remaining dentin thickness (RDT) of 0.50-0.75. The mean RDT measured for the 5 teeth that were tested measured 0.82mm. The mean score for the superficial inflammatory reaction was 0, the mean score for the deep inflammatory reaction was 0, measured on a scale of 0-4. No aspiration of nuclei in odontoblasts was observed (0). On a scale of 0-3, hyperemia recorded 0.4. After 25 days no reparative dentin was observed. There was an absence of edema and the odontoblasts appeared normal.
(The reason for having only 5 teeth instead of 7-10, is because the second animal of the 25-day observation period died within 24 hours of the experiment.
(Please note that the death of this animal was determined to be totally unrelated to the material that was tested.)
**Embrace Pit & Fissure Sealant- 120-day results**
The mean RDT after 120 days measured 0.61 mm. The mean superficial and deep response remained at 0. All other parameters (see raw data sheet) scored 0. If after 120 days a mean of only 0.02 mm reparative dentin is formed we are dealing with a bland material. The presence of reparative dentin formation may also have been due to trauma from the preparation rather than caused by the material itself.

**Conclusion**
Based on the histological reactions of the experimental material, Embrace Pit & Fissure sealant applied as a liner behaved as a very bland material. In comparison, a control material tested by the investigator, IRM (negative control) caused more irritation (Pameijer and Stanley), and scored in a range of 0.50-1.45 at a mean RDT of 0.81 mm.

**Embrace A & B Liner- 25-day results**
The results for this material Embrace A&B liner were not much different from the reaction, or the lack thereof, of Embrace Pit & Fissure sealant. The mean RDT measured for the 5 teeth that were tested was 0.76 mm. The mean score for the superficial inflammatory reaction was 0, the mean score for the deep inflammatory reaction was 0.4, measured on a scale of 0-4. No aspiration of nuclei in odontoblasts was observed (0). On a scale of 0-3, hyperemia recorded 0. After 25 days no reparative dentin was observed. There was an absence of edema except in one tooth and all odontoblasts appeared normal.

**Embrace Pit & A & B Liner 120-day results**
The mean RDT after 120 days measured 0.68 mm. The mean superficial response was 0, the deep response was 0.16. The odontoblasts were normal, there was no hemorrhage or edema and the mean secondary dentin (reparative dentin) was a mere 0.26 mm. Again, this may also have been due to trauma from the preparation rather than the material.

**Bacterial leakage**
No bacterial leakage was observed in any of the sections that were stained with Brown and Brenn for any time period.
The control sample, a human tooth with caries, showed the presence of bacteria.

**Conclusion**
Embrace Pit & Fissure sealant and Embrace A&B liner formula did not appear to cause a toxicological reaction to the pulp in subhuman primates. The reaction of both materials was very bland. Based on the histological data, it can be determined that the materials are biocompatible and are suitable to be evaluated in humans.

See raw data sheets: PulpSA#1 histo Embrace Pit&Fiss and PulpSA#1 histo Embrace A&B.

Databook/Pulpdent/PulpdSA#1 4 03 final rep histo


