Project Title:
Histopathological Evaluation of a RMGI cement, auto and light cured, used as a luting agent – A subhuman primate study

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Date: March 28, 2011
Ref: Pulpdent/PlpdMX#7RMGI/PulpmX#7_RMGI FINAl report

Signature: CeesHPameijer
Objectives:

Aims of the study
This experiment was conducted to investigate the biocompatibility of a new Resin Modified Glass Ionomer luting agent (RMGI). The RMGI was tested in vivo in subhuman primates using two curing methods, an auto-polymerizing and a light cure mode.

Luting agents have to exhibit biocompatibility properties and have to be kind to the pulp without causing irritation leading to post-cementation hypersensitivity.

The principal investigator has conducted animal experiments on a large variety of materials and methods for more than 40 years, which has resulted in numerous publications in peer reviewed journals (A Curriculum Vitae can be provided upon request).

For this RMGI cement study 2 rhesus macaques were used.
The primates were sedated i.m. with Ketamine, and further anaesthetized IV with Thiopentone, incubated and maintained on 2% Halothane in Oxygen. Alternatively anesthesia was maintained with ketamine.

The methods for this test followed the recommendations of DOCUMENT NO. 41 FOR RECOMMENDED STANDARD PRACTICES FOR BIOLOGICAL EVALUATION OF DENTAL MATERIALS. The contents of this document can be found at the end of this report.

Materials and methods
Prior to any preparation radiographs were made of the teeth to establish a baseline. Each primate received Cl V composite resin inlays, which were cemented in Class V inlay preparations in the posterior teeth or canines of the four quadrants. The preparations were made with tapered carbide burs and a high-speed hand piece under copious water-cooling. Below is an example of an inlay preparation in an upper canine.
Using saliva as a separating medium, direct composite resin inlays were fabricated in Filtek resin composite (3M ESPE, St. Paul MN USA).
The preparations were rinsed and dried and then disinfected with Consepsis, a 2% chlorhexidine (Ultradent Products, Inc. South Jordan, UT USA). The Consepsis was rinsed and the preparations were dried before cementation. The composite inlays were cemented with:

1. RMGI self-cured (n=6-8 per animal),
2. RMGI light cured (n=6-8 per animal). An LED light was used for curing of the light cure group (Valo, Ultradent Products, Inc).

Below is a clinical picture of the resin composite inlay after cementation. Note excess cement at the margins.
Excess cement was removed after setting and the inlay finished flush with the tooth surface. See below, the completed cemented inlay.

**Experimental material and restorative materials**

Experimental RMGI 05, Pulpdent Corporation, Lot 110329
Filtek: 3M/ESPE, St. Paul MN USA, Lot N182171, Exp. 2013-05

**Clinical Observations**

There were no clinical complications with the use of the experimental materials.
The RMGI mixed easily and had good flow properties. Light curing was effective and auto curing required 4 minutes intraorally and 8 minutes on the bench. All restorations were satisfactorily placed. Recovery of the animals post surgery was uneventful.

In order to alleviate as much post-operative discomfort as possible the following administration of Scheduled Medicinal Substances (Medicines Control Act) was adhered to:

Ketamine 10mg/kg i.m. Five times.
Thiopentone to effect IV (± 25mg/kg) once.
Halothane 2% in Oxygen to effect twice.
Buprenorphine 0.01mg/kg i.m. 12 hourly 8 times.
Procaine / Benzyl Penicillin i.m. 1ml/10kg once. (Duplocillin)
Pentobarbitone I.V. 200mg/kg once.

**Euthanasia**

At postoperative intervals of 30 and 100 days, medium and long-term observation period respectively, the animals were administered i.m. Ketamine to induce general anesthesia as described above. They were then perfusion euthanized with 10% neutral buffered formalin. Subsequently the jaws were dissected and with a high speed bur and copious water cooling a groove was cut through the bone and apical one third of the root. This method promoted rapid and better fixation. Radiographs were made of all teeth that had been restored to determine if periapical changes had occurred. The jaws were then processed for routine histology. Decalcification, imbedding in paraffin and 6 micron sections and H&E staining were performed. Additionally a Brown & Brenn stain was used for detection of bacteria.

**RESULTS**

All radiographs showed normal and healthy bone without periapical involvement. An example of a representative radiograph can be found at the end of this document.
**Histological interpretation**

The raw data scores are listed in the attached Excel spread-sheet (2x). They represent the evaluation parameters as outlined in the Document No. 41 (see below).

**Evaluation parameters according to:**

**AMERICAN NATIONAL STANDARDS INSTITUTE/AMERICAN DENTAL ASSOCIATION**

**DOCUMENT NO. 41 FOR RECOMMENDED STANDARD PRACTICES FOR BIOLOGICAL EVALUATION OF DENTAL MATERIALS**

(See appendix)

**Histological interpretation for the 30-day observation period**

The RDT for subsequently Groups 1 and 2 were 0.46 and 0.62mm. Under any clinical situation that means that the cement was placed under hydraulic pressure in close proximity to the pulp. The mean superficial response was 1.8° for the light cured group and 1.6° for the auto cure group. Both are slightly above the 1.5° that is recommended, however at RDT’s of less than 0.5 and even 0.2mm it can be anticipated that reactions occur, especially as the cement was placed under hydraulic pressure. Hyperemia for both groups indicated that the healing process was active and that the defense mechanisms of the pulp had taking its normal course. Since no hemorrhage or edema was recorded we are observing a normal defense reaction that needs time to resolve the irritation.

**Histological interpretation for the 100-day observation period**

The Remaining Dentin Thickness (RDT) for Groups 1 and 2 is low and highly standardized. Under normal clinical conditions in humans a 1 mm RDT is the norm, except in cases of deep caries lesions. However, at a mean distance of 0.67 and 0.48 mm of subsequently Group 1 and 2, the RMGI was tested under challenging circumstances, i.e. in close proximity to the pulp. Nevertheless the mean of the superficial and deep responses for Group 1 were subsequently 0.6 and 0.2 degrees. Document No. 41 allows a response of 1.5°. Therefore these low scores are favorable and of little concern. The same can be stated for Group 2. Superficial and deep response of subsequently 0.86 and 0
degrees are well below of what is determined acceptable. When we look at this data of the 100-day period and correlate it with the 30-day observation we can conclude that over time the initial irritation that was observed after 30 days resolved to acceptable levels. The other parameters, hyperemia, hemorrhage, edema were all acceptable, while secondary dentin formation was within acceptable limits and can be considered normal. They are either the result of the trauma from preparation or a result of chemical composition of the cement, or a combination of both.

The results are remarkably consistent with the medium-term results and demonstrate that a decrease in irritation occurred over time, which can be considered normal if a material is biocompatible and demonstrates that whatever irritation occurred, the pulp was able to cope with it and reacted using its normal defense mechanisms (inflammatory response) without being overly stressed.

**CONCLUSION**

It can be concluded from clinical observations and the histological data that the RMGI cement that was tested is biocompatible. Whatever initial irritation was present resolved over time to acceptable very low levels.

References

3. Experimental Procedures

3.1. Treatment of teeth

Place the cavity in the gingival area of the tooth and make it as wide as possible reaching toward the proximal surfaces. If a product or material is to be used as a luting agent, one can cut standardized Class V cavity preparations to receive inlays. On stone models used for making the castings, Class V resin templates are fabricated reproducing the cervical contour of the teeth. Another technique is the direct fabrication of Class V composite resin inlays in vivo. Saliva or other suitable lubricants may be utilized as separating agents. It is recommended to use light cured composite resins, which after direct placement are cured for the recommended period of time using a visible light source. After curing the inlays are removed. Overfilling facilitates removal of the inlay, while the excess can be cut back following hardening of the luting agent. The use of a disinfecting agent in case saliva is used as a separating material is strongly recommended. These inlays are then luted under pressure for the length of time necessary for the initial set of the cement thus simulating the hydraulic forces of full crown or only cementation (Eames et al 1979; Pameijer et al 1991). In small animals ensure that the cavities reach into the inner 1/3 of the dentin without exposure of the pulp.

All that has been said concerning the reaction of the pulp to cutting procedures applies to both cavity and full-crown preparations. Provided the crown preparation remains 2.0 mm or more from the pulp chamber, little or no pulp response results. However, when shoulder preparations are made or pulp horns are approached, the same conditions apply as in cavity preparations. Extensive shallow cavity preparations involving the entire surface of a tooth or a full-crown preparation produce pulp responses of a mild nature as compared with the more remarkable responses resulting from deeper but more confined cavity preparations. To ensure proximity to the pulp it is recommended to first prepare a Class V cavity, essentially serving as depth cuts, followed by full crown preparation.

3.2. For each time period restore at least seven cavities with the test material and four cavities with a negative-control material on the basis of a random allocation. If necessary, for each time period, restore up to four cavities with a positive material. For laboratories, which possess a data bank for previous positive-control materials, further studies are unnecessary other than on an occasional basis to confirm a positive reaction.

3.4. Assessment of dentine and dental pulp

Examine the sections without prior knowledge of whether the specimen is experimental or control. For each series of sections, record a full description of all the histological features in the dentine, pulp and periapical tissues, including any that may have arisen from the cavity preparation technique. From the serial sections, select at least five evenly spaced through the cavity and grade separately the inflammatory infiltrate in the superficial tissues (odontoblast layer, cell-free zone and cell-rich zone) and the remainder (deeper) pulp tissue on the following scale.

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<th>Scale</th>
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<th>Description</th>
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<tr>
<td>0</td>
<td>No inflammation</td>
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<tr>
<td>1</td>
<td>Mild inflammation</td>
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<td>2</td>
<td>Moderate inflammation</td>
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<tr>
<td>3</td>
<td>Severe inflammation</td>
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<tr>
<td>4</td>
<td>Abscess formation or extended lesions not localized to the tissue beneath the cavity floor</td>
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For each section graded, record the minimum remaining dentine thickness, either in a straight line at right angles from the floor of the cavity to the pulp or by measuring along the course of the dentinal tubules.

Calculate an index of response for both sites of inflammation at each time interval by summing the individual grades and dividing by the total number of observations. Present the data separately for cavities filled with the test material, including the lining material or cavity treatment agent if recommended by the manufacturer, cavities filled with the negative control alone, and cavities used as positive controls (the last data may be obtained from previous studies). In addition, record the mean remaining dentine thickness and the number of cavities containing bacteria at each time interval.

3.5. Remaining Dentin Thickness (RDT)

The distance from the floor of the cavity to the pulp shall be measured in a straight line called the remaining dentin thickness (RDT). The microscopic sections with the smallest RDT value will be utilized for grading pulp responses. The RDT in any specimen that exceeds 2.00 mm is to be rejected. Any specimen with an RDT over 1.00 mm begins to disguise pulp responses from restorative procedures because of the reduced diffusion gradient of the dentin and the consequent dilution of the extraction products of the materials being tested. When evaluating cutting techniques, RDT values greater than 1.5 mm provide sufficient insulating factors to reduce the pulp responses. Dentin tubular length from the floor of the cavity preparation to the pulp can also be measured. However, more time is required to do so and the mean RDT will have a higher value. Experience has shown that the results (trends) will be similar.

An investigator can unintentionally bias the results of an operative technique by having in a category a preponderance of specimens with large or small average RDTs or excess numbers of anterior and posterior teeth.

3.6. Confinement of the pulp reaction to cut dentin tubules

Lesions resulting from cutting procedures are usually confined to that portion of the pulp underlying the inner ends of the cut dentin tubules. At the same time that one is determining the appropriate section for measurement of the RDT, one can be observing whether or not a lesion, if present, extends beyond the cut dentin tubules. This type of lesion can result from cutting techniques that utilize inadequate coolants and from certain exceptionally toxic restorative materials.

Frictional heat, if not excessive, can increase the intensity of the pulp response without expanding the lesion beyond this region. If it is excessive, however, a burn lesion can develop which extends beyond the cut tubules, even reaching across to the opposite side of the pulp chamber. Such lesions present coagulation necrosis and often develop intrapulpal abscesses. Provided the lesion is confined to the cut tubules, one can be fairly certain that frictional heat was controlled (Stanley, 1962). No burn lesion will result at any cavity depth, provided an adequate water coolant accompanies the cutting procedures.

3.7. Reparative dentin formation (RDF)
Nature attempts to protect the pulp with (a) sclerosis of dentin, either as a natural process of aging or induced by the irritation from caries, attrition, abrasion and erosion; and (b) reparative dentin formation, induced by the above factors and also by tooth cutting and restorative procedures.

If the production of sclerotic (peritubular) dentin has not been adequate to protect the pulp, the inherent reaction of pulp tissue is to seal cut or diseased dentin tubules at the pulp surface with reparative dentin and protect the pulp tissue from most subsequent dental procedures; the seepage of irritating substances into the pulp tissue are blocked. In clinical practice when there is doubt as to the uniform presence of reparative dentin beneath an area of the restoration, exogenous substances must be employed to seal or plug the opened dentin tubules.

Because the prevalence of reparative dentin formation is so low with the high-speed cutting techniques, even after extended periods of time, it is unrealistic to wait for reparative dentin to form. A more practical approach is to apply an adequate and effective cavity liner or create a hybrid layer.

Reparative dentin formation has become increasingly significant because mildly traumatic, high-speed, water-cooled cutting techniques have greatly decreased the prevalence of reparative dentin formation (Stanley, 1971). The average prevalence of reparative dentin formation for any high-speed, water-cooled technique is 18.7%; this value decreases as the RDT beneath the cavity preparation increases. Furthermore, if the injured pulp is not stimulated to form reparative dentin within the first 50 days in humans following a cutting and restorative procedure, reparative dentin will not form from that procedure (Stanley, 1971; Stanley 1997).

The greater the degree of initial response due to the irritation caused by cutting and placing of a restorative material, the greater the subsequent prevalence of reparative dentin. With many high-speed techniques, however, a very low prevalence of reparative dentin results, leaving many primary dentin tubules patent for the subsequent seepage of toxic products into the pulp. In order to compare the prevalence of reparative dentin, one first establishes the number of specimens with reparative dentin at each time interval. In most mammals and sub-human primates it is seldom seen before 5-7 days. In humans, reparative dentin is seldom seen before 20 days beneath a non-exposed cavity preparation. The specimens are arbitrarily graded 0-3; 0.5 represents an isolated focus of reparative dentin; 1.0 a thin layer of reparative dentin; 2.0 a thicker layer of reparative dentin; and 3.0 a very thick, bulky layer of reparative dentin.

Once formed, the regenerated odontoblasts begin to deposit dentin in humans at the rate of 3.6 μm/day, slowing down after about 20 days of production to less than 1.0 μm/day and averaging overall about 1.5 μm/day of dentin. In about 50 days following cavity preparation and restoration, a ribbon like layer of reparative dentin about 70 μm thick will have formed (Stanley, 1962; Stanley et al 1966).

The greater the frequency of reparative dentin and the greater its bulk formation in long-term postoperative intervals, the greater the initial injury and the inflammatory response. This is a hallmark of early injury especially when the subjacent pulp tissue is completely normal at longer PTIs. Cellular inclusions within the newly formed reparative dentin adjacent to the normal pulp are another indication of an early severe response (Stanley, 1994).

It must be remembered that with some procedures the lesions may so persist in intensity of inflammatory response that the differentiation of new odontoblasts is difficult and the incidence of reparative dentin will be low, giving the false
impression of a mild response. This can also occur in the presence of an abscess. A preferable result provides a significant prevalence and quantity of reparative dentin and, at the same time, a decreasing order of inflammatory cells. The interface between secondary and reparative dentin usually is clear-cut.

3.8. Regeneration of odontoblasts
One should keep in mind that, with resolution of a lesion, most or all of the inflammatory cells may disappear and leave behind an atrophic or degenerated odontoblast layer, even exhibiting foci completely lacking in primary odontoblasts. In other instances, only regenerated odontoblasts will be found. A distinction needs to be made between (1) a degenerating, atrophying odontoblast layer with short irregular odontoblasts and (2) a regenerating odontoblastic layer in which the individual cells are quite large.

After the initial trauma from whatever source has triggered an inflammatory response sufficient to destroy completely the odontoblast layer, the fibroblasts in the cell-rich zone, underlying the former layer of primary odontoblasts, begin to multiply (Fig 6-7) (Kuwait p. 144). These cells move toward the injured predentin area, develop into odontoblasts, and begin to deposit reparative dentin (Fig. 6-8) (Kuwait, p. 144). If the cell-rich zone is itself destroyed because of greater destruction, granulation tissue will fill the entire defect and eventually, through gradual maturation of cells, new odontoblasts are formed (Stanley, 1962 - The cells of the dental pulp.).

Cavity preparation with dry-cutting, low-speed methods usually triggered sufficient pulp inflammation to induce a high prevalence (about 60%) of reparative dentin formation. Cutting dry at any speed or with air alone can lead to burn lesions and abscess formations that make these clinical procedures unacceptable. Only one abscess was found in 600 teeth prepared with air-water spray techniques, but about 25% formed abscesses in teeth prepared with dry or air-cooled cutting techniques (Stanley 1960, Stanley 1960).

4. Histologic features to be considered in the full description:
The features that are graded are selected on the basis that a characteristic usually increases in intensity as the RDT decreases in value. Such a relationship has never been satisfactorily established with cytoplasmic vacuolization. Therefore, this feature has not been emphasized as a significant criterion.

No doubt a quantitative evaluation of a lesion is necessary, but a statistical evaluation at this moment seems to be meaningless, since the identification of the inflammatory type cells is dependent upon the examiner’s ability and experience.

The following illustrations emphasize these characteristics in varying degrees (0 to 3). Estimation of the degrees of response is purely a subjective exercise, and disagreement can occur between examiners but these differences usually nullify each other and the means will be quite similar. All grades or degrees of response do not necessarily parallel each other.

4.1 Histologic requirements
The histologic sections shall meet the following requirements:
They shall present minimal histologic artifacts that would interfere with an interpretation of the cavity/pulp relationship.

The cavity shall be sufficiently wide so that dentin tubules may be followed from the cavity to the pulp throughout at least five sections of thickness 5-10 μm. The critical point is to have serial sections of the area where the RDT is the narrowest because one usually finds the severest pulpal response in that area.
Replace experimental test teeth or control teeth when such sections do not meet these requirements.

4.2. Grading of Pulp Responses: (See Addendum II for illustrations of gradations).

The pulp tissue is divided into the superficial tissues representing the combined odontoblastic layer, the cell-free zone of Weil, and the cell-rich zone and the deeper tissues beneath the cell-rich zone.

The severity of the pulp response shall decide the acceptability of the restorative procedure. The severity of the response shall be arrived by recording findings according to the following criteria:

a. The number and intensity (density) of acute neutrophilic and eosinophilic leukocytes, mononuclear inflammatory cells (lymphocytes, monocytes, plasma cells, macrophages, and foreign body giant cells) in the superficial and deeper tissues is graded 0-4. Rarely, does the intensity of the response reach 4 except in the presence of an abscess.

b. Predominating inflammatory cell. The predominating type of infiltrating (trafficking) inflammatory cell (neutrophil leukocyte, lymphocyte, eosinophil, monocyte, or plasma cell) is usually recorded at the same time as the intensity of the cellular inflammatory response is noted. Eosinophils are grouped with the chronic cells because they usually appear at the same time in pulp lesions. As the lesion resolves, the acute inflammatory cells diminish in number and are replaced by smaller numbers of mononucleated or chronic cells in both the superficial and deeper tissues. With extended postoperative intervals, the mononuclear cells and Eosinophils gradually disappear in a resolving lesion with the formation of reparative dentin. In some situations, the maximal initial response is reached in 1 to 2 days, in others it takes 3 to 6 days. With some restorative materials, postoperative periods of 20 days or longer are required before the total expression of the initial lesion is manifested.

c. The number of cells displaced into the dentinal tubules exposed by the cavity preparation is graded 0-3. When the response reveals only one or two displaced cells or infiltrating inflammatory cells, a grade of 0.5 is given.

d. Dilatation and congestion of capillaries in the superficial and deeper tissues (localized to the affected area) is graded 0-3.

e. Hemorrhage is graded 0-3 (minimal, moderate, severe) with recognition of focal or diffuse features (Langeland & Langeland, 1965).

f. An abscess formation in or adjacent to the affected area beneath the cavity preparation is graded 4.0

g. The quantity of reparative dentin subjacent to the pulp ends of cut dentinal tubules is graded 0-3. Some attention should be paid to the quality of the reparative dentin. (See section under Reparative Dentin Formation).

4.3. Other Pathologic characteristics to be considered:

a. Dentin discoloration

The occurrence of discoloration of the cavity floor and walls is evidence of a dentin burn caused by frictional heat of the cutting instrument in combination with a lack of sufficient and efficient water spray. This discoloration with hematoxylin and eosin stained sections can be confirmed as a bright red margin with a Masson trichrome stain.

b. Blood stasis and pigment

Capillaries filled with blood simultaneously exhibiting brown pigment which is birefringent in polarized light, in and around vessels in the reaction area is evidence of stasis and a circulatory disturbance.

c. Foci of necrosis
Initially, in burned pulp tissue due to inadequate cooling techniques and in lesions induced by highly toxic restorative materials or chemicals, a blanching out of all histologic features with loss of cellular detail and a paucity of inflammatory cells and erythrocytes is characteristic. When this occurs, a novice may interpret the lesion as insignificant because of the absence of inflammatory cells. Subsequently, these lesions will become heavily infiltrated by inflammatory cells and may either resolve with granulation tissue replacement and organization or undergo abscess formation.

d. **Eosinophilic staining**

With an H and E stain an area which is mainly stained pinkish is due to the release of transudate from a dilated capillary or arteriole. The pink represents albumen in the transudate and is not related to the presence or eosinophilic leukocytes.

5. **Assessment of results**

All information gathered in the test shall be taken into account in assessing the test results, particularly any differences in results between the experimental and control groups. The results of the assessment shall be recorded in the test report. After selecting the microscopic sections with the narrowest RDT values, a full description of all the histopathologic features are noted and recorded. Score the sections of each specimen blindly.

When completed, calculate the mean RDT for each experimental and control group. Then calculate the index of response for each parameter (histopathologic feature) for each material or cutting procedure for each time interval by summatting (prevalence) the individual scores and dividing by the total number of specimens examined (percentage) within each group.

Present the data separately for cavities filled with the test material alone, those filled with the test material and recommended cavity treatment or lining materials, and those filled with the control material alone. In addition, record the number of cavities exhibiting the penetration of microorganisms (microleakage) onto the cavity walls, floor of the cavity and into the dentinal tubules (utilizing the appropriate bacterial stains).

One should strive to have a mean RDT value of 1.00 mm. or less in order to compare categories and time intervals. Mean RDT values shall also be obtained for control groups and they shall not be significantly different. If the mean RDT values in each category is not comparable, a reasonable interpretation must be made. In small animals, instead of a numerical value for the RDT, one should divide the thickness of the dentin of small teeth into thirds (outer 1/3, middle 1/3, and inner 1/3). Instead of striving for a mean value of 1.0 mm of RDT, in small animals one should try to develop a mean RDT equal to the thickness of the middle third.

A favorable situation requires that the mean grades of responses be higher in the initial short-term group and decrease in value in the long term groups. If the values in the long term postoperative group are higher than or equal to the initial group, it is indicative of prolonged responses, a situation that is not acceptable.

Other features to observe are the number of specimens with no response and the number of specimens with the responses of 2 degrees or more. This type of appraisal permits a quick evaluation. As lesions heal, the incidence of specimens with responses of 2 degrees or more decreases. If the reverse should happen, the technique is unsatisfactory.
With these values, one can compare the experimental categories of teeth with the control category and determine which restorative procedures are acceptable and which ones require modification or elimination. Usually an average (intensity) score of 1.5 (0-4) or less is acceptable. However, if an occasional abscess occurs under any conditions or with any material, the data must be scrutinized for an explanation. Usually a category with more than one abscess will register a mean response rate higher than 1.5 and cause rejection of the material.

The severity of the pulp response determines the acceptability of the restorative procedure. At one time, all experimental materials had to equal the results of the negative control category. However, with experience it became apparent that an initial response could rapidly fade and become acceptable by the intermediate period. Consequently, the guidelines have changed.

Obtaining mean scores for prevalence, and percentages of characteristics is recommended. When differences between testing categories are not clear cut, with the previously presented grading system, it is recommended that the evaluator measure roughly the length or extent of the entire lesion beneath the cut dentin tubules and what percentage of the lesion underlies the floor of the cavity preparation: 10 50 100%. Then count the number of serial sections through which the pulp lesion persists: 3 30 50 microscopic sections. Then multiply the number of sections (allowing for unstained sections) times the thickness of the microscopic section (Pameijer & Stanley, 1988).

6. **Statistical Approach**

Although many statisticians claim there are too few specimens in any category to develop statistical significance, some feel an attempt to apply statistical analysis is warranted.

**Remaining Dentin Thickness (RDT).** This data is recorded in mm and can be analyzed by means of an Analysis of Variance (ANOVA) repeated measures corrected for ties. This method will determine if there is a statistically significant difference between the RDT’s of various groups and is reported as P< 0.05 for instance or “there is a statistically significant difference at a 95% confidence level”.

<table>
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<tr>
<th>Histologic Characteristics</th>
<th>Observations</th>
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<tr>
<td>Cellular Displacement (0-3 degrees)</td>
<td>0 None (no inflammation)</td>
</tr>
<tr>
<td>Superficial Response (0-4 degrees)</td>
<td>1 Minimal (mild inflammation)</td>
</tr>
<tr>
<td>Deep Response (0-4 degrees)</td>
<td>2 Moderate (moderate inflammation)</td>
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<tr>
<td>Reparative Dentin Formation (0-3 degrees)</td>
<td>3 Severe (severe inflammation)</td>
</tr>
<tr>
<td>Dilatation and Congestion (0-3 degrees)</td>
<td>4 Abscess and/or extended lesions</td>
</tr>
<tr>
<td>Hemorrhage (0-3 degrees)</td>
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When the data is reported in degrees (nonparametric data) in categories such as those that are listed above under Histologic Characteristics, a nonparametric test (Friedman test) corrected for Chi-Square ties, is indicated to establish statistical significance. Once statistical significance has been established the data can be further subjected to an ANOVA repeated measures to determine statistically significant differences between the groups. The Friedman test only determines if there is a statistically significant difference but does not tell how significant the
difference is. The ANOVA may be used only after the nonparametric test has determined the existence of a significant difference.

Data also can be analyzed in the form of contingency tables, where the frequencies of each out come (0, 0.5, 1, 1.5, etc) are tabulated for each group and each time. Fisher’s exact test (1984)\(^1\) is used to test the null hypothesis of independence of rows and columns.

Also, the nonparametric Friedman test, corrected for Chi-square ties, can be utilized to determine the Total Inflammatory Response (TIR) a combination of the displacement of cells, superficial and deep inflammatory response. The combination of these groups basically allows for an easier comparison of groups that determine the degree of inflammatory response.

7. References

Ref: Prestigio/Pulpdent/PulpdMX#7_RMGI FINAL report
Histopathological Evaluation of RMGI, auto and light cured, when used as a luting agent – A subhuman primate study

Company: Pulpdent Corp
Ref: PulpdMX#7 RMGI 6 11

Date: 6/29/11 Animal ID: #50 (11-01) Operator: Dr. Pameijer

MATERIALS AND METHODS

*Group 1. Experimental Resin - VLC.* Class V inlay preparation, rinsed with water. Fabrication of Class V inlay in Filtek (3M ESPE). Cementation with RMGI, light cured for 20s.

*Group 2. Experimental Resin - Auto cure.* Class V inlay preparation, rinsed with water. Fabrication of Class V inlay in Filtek (3M ESPE). Cementation with RMGI, self curing

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</tr>
<tr>
<td>4.3 xxxx</td>
<td>3.3 xxxx</td>
<td></td>
</tr>
<tr>
<td>4.4 xxxx</td>
<td>3.4 xxxx</td>
<td></td>
</tr>
<tr>
<td>4.5 xxxx</td>
<td>3.5 xxxx</td>
<td></td>
</tr>
<tr>
<td>4.6 xxxx</td>
<td>3.6 xxxx</td>
<td></td>
</tr>
<tr>
<td>4.7 xxxx</td>
<td>3.7 xxxx</td>
<td></td>
</tr>
</tbody>
</table>

Date Scaling: N.A. Date Operative: 06/2911 Date Sac 100 day: 10/06/11

Required materials:
Experimental RMGI 05; Lot 110329
Filtek: Lot N182171, Exp. 2013-05

Prestigio/Pulpdent/PulpdMX#7+RMGI ws 6 11
Histopathological Evaluation of RMGI, auto and light cured, when used as a luting agent – A subhuman primate study

Company: Pulpdent Corp
Ref: PulpdMX#7 RMGI 6 11
Date: 6/30/11
Animal ID: (11-3)(Pedro) Operator: Dr. Pameijer

MATERIALS AND METHODS

**Group 1. Experimental Resin- VLC.** Class V inlay preparation, rinsed with water. Fabrication of Cl V inlay in Filtek (3M ESPE). Cementation with RMGI, light cured for 20s.

**Group 2. Experimental Resin- Auto cure.** Class V inlay preparation, rinsed with water. Fabrication of Cl V inlay in Filtek (3M ESPE). Cementation with RMGI, self curing

| 1.1 Group 1 | 2.1 Group 1 |
| 1.2 Group 1 | 2.2 Group 1 |
| 1.3 Group 1 | 2.3 Group 1 |
| 1.4 Group 1 | 2.4 Group 1 |
| 1.5 xxxx | 2.5 xxxx |
| 1.6 xxxx | 2.6 xxxx |
| 1.7 xxxx | 2.7 xxxx |

| 4.1 Group 2 | 3.1 Group 2 |
| 4.2 Group 2 | 3.2 Group 2 |
| 4.3 Group 2 | 3.3 Group 2 |
| 4.4 Group 2 | 3.4 Group 2 |
| 4.5 xxxx | 3.5 xxxx |
| 4.6 xxxx | 3.6 xxxx |
| 4.7 xxxx | 3.7 xxxx |

Date Scaling: NA
Date Operative: 06/30/11
Date Sac 30 day: 7/30/11

Required materials:
Experimental RMGI 05; Lot 110329
Filtek: Lot N182171, Exp. 2013-05
Liquid filled resin AR 704 Lot 110510, Exp.
Histopathological Evaluation of RMGI, auto and light cured, when used as a luting agent – A subhuman primate study

Company: Pulpdent Corp  Yes _X_ No_  Ref: PulpdMX#7 RMGI 6 11

Date: 6/30/11  Animal ID: (11-4)(Capone)  Operator: Dr. Pameijer

MATERIALS AND METHODS

**Group 1. Experimental Resin- VLC.** Class V inlay preparation, rinsed with water. Fabrication of Cl VI inlay in Filtek (3M ESPE). Cementation with RMGI, light cured for 20s.

**Group 2. Experimental Resin- Auto cure.** Class V inlay preparation, rinsed with water. Fabrication of Cl V inlay in Filtek (3M ESPE). Cementation with RMGI, self curing

| 1.1 Group 2 | 2.1 Missing |
| 1.2 Group 2 | 2.2 Group 2 |
| 1.3 Group 2 | 2.3 Group 2 |
| 1.4 Group 2 | 2.4 Group 2 |
| 1.5 xxxx | 2.5 xxxx |
| 1.6 xxxx | 2.6 xxxx |
| 1.7 xxxx | 2.7 xxxx |

| 4.1 xxxx | 3.1 xxxx |
| 4.2 xxxx | 3.2 xxxx |
| 4.3 xxxx | 3.3 xxxx |
| 4.4 xxxx | 3.4 xxxx |
| 4.5 xxxx | 3.5 xxxx |
| 4.6 xxxx | 3.6 xxxx |
| 4.7 xxxx | 3.7 xxxx |

Date Scaling: NA  Date Operative: 06/30/11  Date Sac 100 day: 10/06/11

Required materials:
Experimental RMGI 05; Lot 110329
Filtek: Lot N182171, Exp. 2013-05

Prestigio/Pulpdent/PulpdMX#7+RMGI ws 6 11
Representative X-ray made immediately post-euthanasia. The radiopaque features at the cervical of the teeth represent the cemented inlays, while the black horizontal line at the one third apical depicts the groove that was cut through the bone and the root after perfusion euthanasia. This method improved fixation of the pulp tissue.
Representative histological appearance of a 30-day specimen. A grade 2/C inflammatory reaction score was assigned. The “C” is for the presence of chronic inflammatory cells. (Tooth #3.1-30-day period)

Ref: Prestigio/Pulpdent/PulpdMX#7_RMGI FINAL report