

## Histological Study on Dentinopulpal Complex Response to Glass Ionomer Cement Filling in Taiwan Rock Monkey (*Macaca Cyclopis*)

GUANG-SHIUN LIN

### INTRODUCTION

The filling material and cavity preparation in cervical abrasion and erosion cavities to give something trouble to the dentist. Bis-GMA series composite resin was advocated recently, it irritated the pulp tissue was also reported<sup>(1)</sup>. The glass ionomer cement, ASPA cement; is fluorine containing aluminosilicate glass powder and aqueous solution of poly alkenylcarboxylic acid, in particular poly acrylic acid to give the hardening reaction. The clinical use of this material in abrasion and erosion cavities by McLean and Wilson<sup>(2)</sup>, and in caries in primary teeth by Plant<sup>(3)</sup> has been advocated. Dahl and Tronstad 1976 observed mild pulp reactions after 8 days of observation in monkeys<sup>(4)</sup>. They used the ASPA III formula and in vitro cell culture, they found that the glass ionomer cement was toxic, when freshly prepared. The toxicity decreased with increased setting time. Tobias et al found ASPA cement showed greater reaction than the control materials, Zinc oxide-eugenol cement and Kalzinol (reinforced ZOE cement<sup>(5)</sup>). Nordenwall et al research pulp reaction and microorganism under ASPA and concise composite filling<sup>(6)</sup>, they concluded that ASPA cement material provides considerably better cavity adaptation than composite materials.

### MATERIALS AND METHODS

The materials used in this study were all based on the glass ionomer cements reported by Wilson and Kent (1972, 1973). ASPA cement is a newly product glass ionomer cement. It contained powder (aluminosilicate glass powder) and liquid (polyacrylic acid). The adhesion to tooth structure was of a high order, the actual adhesive forces are polar, hydrogen bridging and ionic<sup>(2)</sup>. Powder was stored in the refrigerator and liquid was stored at room temperature<sup>(7)</sup>. Mixing the ASPA cement with plastic spatula, chilled slab, and power/liquid ratio is 3:1 g/ml<sup>(5,7)</sup> mixing time 30 seconds. The experiment animals are six Taiwan Rock Monkeys (*Macaca Cyclopis*) of body weight 3000 g to 5500 g, in every molars (except third molar) buccal cervical area prepared a 1.5-2 mm depth cavity, with air turbine No. 806/018 inverted cone diamond bur in full length, dry the cavity with air blast and cotton-wool, isolate the teeth with rubber dam. The monkeys were divided to two groups.

#### Group I

Four monkeys, anesthesia with Ketamine 10 mg/kg, I.M. induce and 5% sodium pentobarbital 25 mg/kg, I.P. 32

healthy molars, experiment group 14 molars, control group 18 molars, prepared a cavity as described as before. The experiment group filled with ASPA cement, without any other lining or basing material, hold the material with cervical matrix for 5 minutes, then applied the copal ether vanish over the filling material. The control group were normal saline solution topical application, dried with cotton, covered with calcium hydroxide and filled with amalgam. After 24 hours, 1 week, 2 weeks, 4 weeks, the monkeys were sacrificed (tooth were not extracted), and formaline perfusion through the body, fixation with 10% neutral buffer formalin 48 hours, decalcification with Plank's solution, dehydration, paraffin embedding, section and observe under microscope. One monkey died within 24 hours before sacrificed, so we could not make formaline perfusion, only formalin fixation.

## Group II

Two monkeys, 16 healthy molars, experiment group 16 molars (all experiment group). The procedures and materials were the same as group I, except after cavity preparation, etching solution, ASPA conditioner (50% citric acid) was applied to the cavity for 30 seconds, the teeth then washed with water, dry the cavity, isolated the teeth, filled the ASPA cement as group I. The monkeys sacrificed and specimen prepared as group I.

## RESULTS

The major observation was the dentinopulpal response to chemical and mechanical stimulation. All section showing the minimum

residual dentin in the floor of the cavity as measured at right angles to the pulpo-dentinal junction. The distance of residual dentin was measured under micrometer. Thickness of remaining dentin were all under 2.0 mm (Table 1).

Table 1. Thickness of Residual Dentin

|               |    |
|---------------|----|
| 0 - 0.5 mm    | 12 |
| 0.51 - 1.0 mm | 21 |
| 1.01 - 1.5 mm | 13 |
| 1.51 - 2.0 mm | 2  |

## Group I 32 teeth, 14 teeth experiment group, 18 control group

There were 5 teeth under 0.5 mm, 18 teeth between 0.51-1.0mm, 7 teeth between 1.01-1.5 mm, 2 teeth over 1.5 mm. After 24 hours and 1 week restoration, there was not any histopathologic change being found (Fig. 1-3), only 2 case of experiment group 24 hours had secondary dentin formation under the occlusal surface, it could be considered as normal physiological stimulation (Fig. 4). After 2 weeks restoration, both control group and experiment group were no significant change in the pulp (Fig. 5, 6), 4 weeks control group 5 teeth, there were 3 teeth had secondary dentin formation beneath the restoration (Fig. 7). Experiment group 4 teeth were all showed secondary dentin formation under cavity, and loss of odontoblast (Fig. 8, 9), but no change on the same level of lingual pulpal wall, one tooth showed severe venous stasis, because it could not make formalin perfusion (Fig. 9).

## Group II 16 healthy teeth were divided to 4 groups, each group had 4 teeth

There were 7 teeth under 0.5 mm

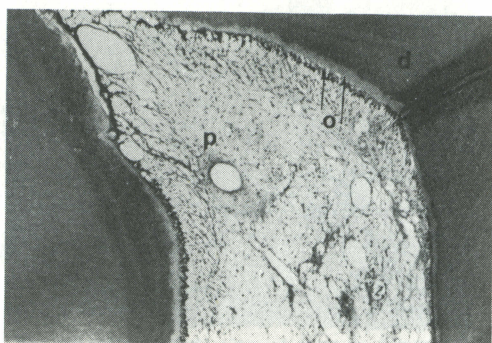


Fig. 1. control group 24 hours, normal pulp constituents.  
p: pulp chamber. o: odontoblast. d: dentin (H.E. 100x)

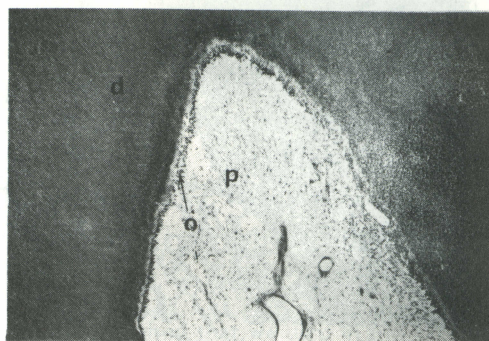


Fig. 2. control group 1 week, normal pulp constituent.  
p: pulp chamber. o: odontoblast. d: dentin (H.E. 100x)

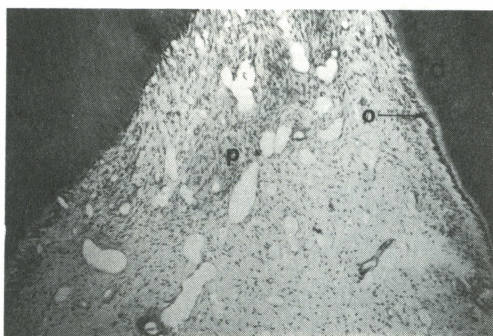


Fig. 3. experiment group I 1 week, normal pulp constituent.  
p: pulp chamber. o: odontoblast. d: dentin (H.E. 100x)

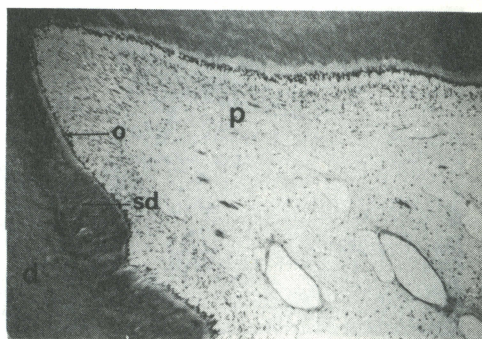


Fig. 4. experiment group I 24 hours, secondary detin with physiological stimulation.  
p: pulp chamber. o: odontoblast. d: dentin sd: secondary dentin (H.E. 100x)

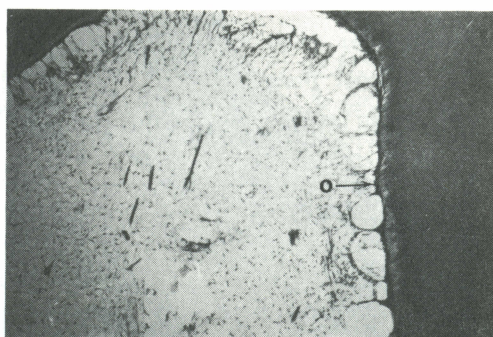


Fig. 5. control group 2 weeks, decrease of number of odontoblast.  
o: odontoblast. (H.E. 100x)

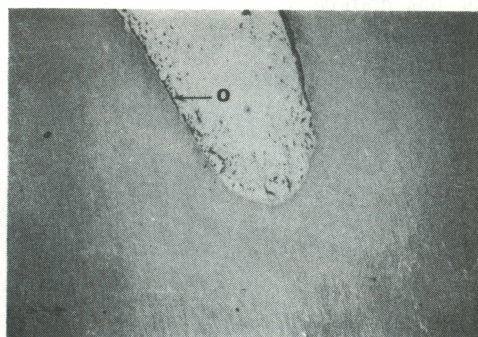


Fig. 6. experiment group I 2 weeks, decrease of number of odontoblast.  
o: odontoblast. (H.E. 100x)

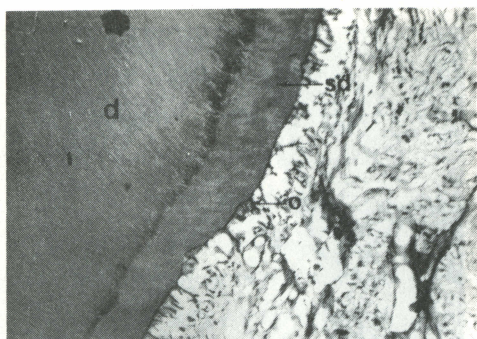


Fig. 7. control group 4 weeks, secondary dentin formation under cavity and loss of odontoblast.  
o: odontoblast. d: dentin.  
sd: secondary dentin. (H.E. 200x)

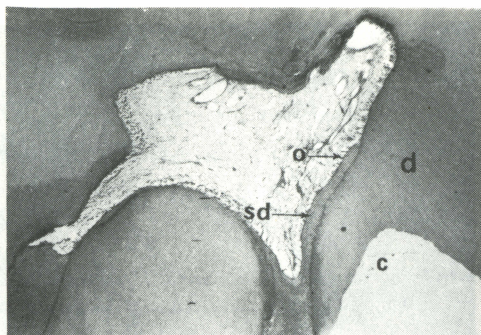


Fig. 8. experiment group I 4 weeks, loss of odontoblast, secondary dentin formation  
o: odontoblast. d: dentin.  
sd: secondary dentin. c: cavity (H.E. 40x)

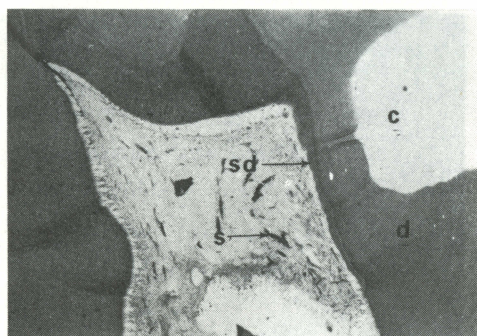


Fig. 9. experiment group I 4 weeks, loss of odontoblast, secondary dentin formation and blood stasis.  
s: blood stasis, sd: secondary dentin.  
d: dentin. c: cavity (H.E. 40x)

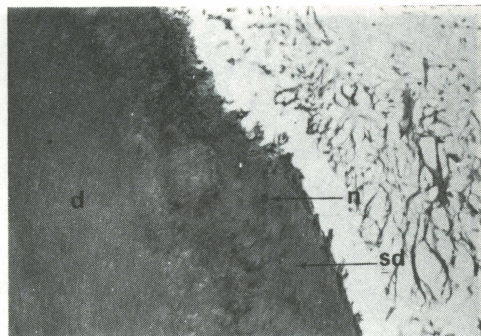


Fig. 10. experiment group II 4 weeks, with 50% citric acid, secondary dentin formation, odontoblast aspirated into dentinal tubule.  
sd: secondary dentin. d: dentin.  
n: odontoblast nucleus. (H.E. 100x)

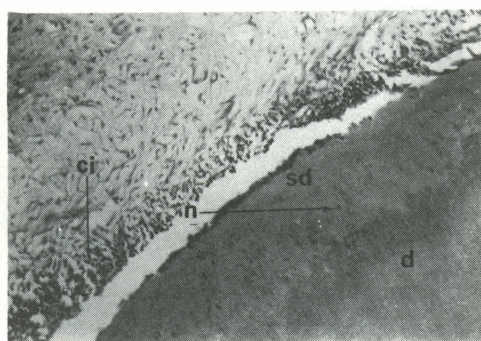


Fig. 11. experiment group II 4 weeks, with 50% citric acid, secondary dentin formation, odontoblast aspirated into dentinal tubule, localized inflammatory cell infiltration.  
d: dentin sd: secondary dentin.  
n: odontoblast nucleus.  
ci: chronic inflammatory cell. (H.E. 200x)

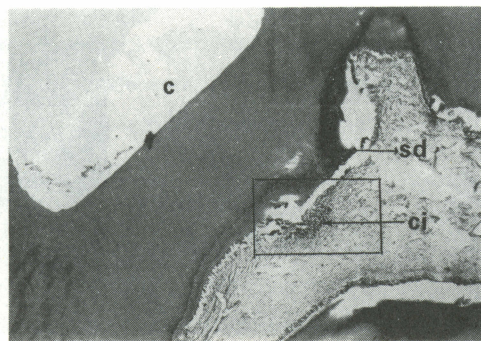


Fig. 12. experiment group II 4 weeks, with 50% citric acid, secondary dentin formation, localized inflammatory cell infiltration.  
c: cavity sd: secondary dentin.  
ci: chronic inflammatory cell infiltration (H.E. 40x)

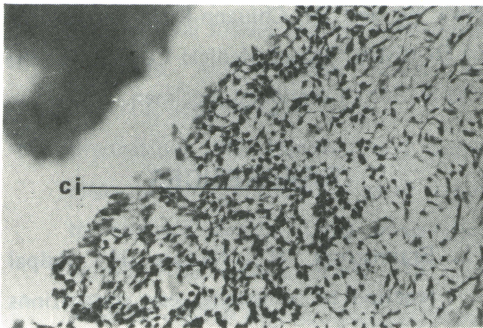


Fig. 13. high magnification of inset in Figure 12 shows chronic inflammatory cell.  
ci: chronic inflammatory cell. (H.E. 400x)

3 teeth between 0.51-1.0 mm, 6 teeth between 1.0-1.50 mm. 24 hours and 1 week experiment group were no response in the pulp. 2 weeks later there was 1 teeth showed a narrow band of secondary dentin just beneath the cavity. 4 weeks specimen that all of the 4 teeth showed evidence of marked secondary dentin formation, loss of the odontoblast, or aspirated it nucleus into dentinal tubule (Fig. 10, 11). Two teeth in 4 weeks experiment group showed localized chronic inflammatory cell infiltration (Fig. 12, 13). The inflammatory cell appeared in the pulp chamber in one tooth was just under the cavity, the other tooth the inflammatory cell was present far from prepared cavity. In 48 specimen, there were

12 teeth (11 teeth in 4 weeks, 1 tooth in 2 weeks) showed secondary dentin formation under the cavity, 2 teeth were localized chronic inflammatory cell infiltration (Table 2).

## DISCUSSION

The method that we used in this experiment was followed a pattern established in earlier experiments<sup>(4,5,6)</sup>. Some modification has been done. A good dentinopulpal response to protect the pulp tissue was reparative dentin formation as a barrier to interrupt the continued irritant to the pulp. Whether we should thus induce the formation of reparative dentin is open to question. However, there is evidence that reparative dentin can protect the pulp against exogenous injury<sup>(12)</sup>. The formation of reparative dentin after 1 to 3 months indicates a loss of odontoblasts; this is consistent with earlier observations and proposals made by Gottlieb<sup>(13)</sup>. The loss of odontoblast, a consistent finding in these thin dentin covering the pulp cases in 4 weeks, was probably caused by dehydration, a result of cavity preparation and air spray; or caused by cutting of the odontoblastic processes themselves, which are present mainly in the inner 0.5 mm of the dentin<sup>(9)</sup>. Furthermore, in very deep cavities, a chemical irritation of the odontoblasts may have occurred from the acid-etching or from the filling material itself. The loss of odontoblasts as a result of dehydration or some other injurious agent seems to be followed by the deposition of irregular secondary dentin withing two to three months<sup>(8,10)</sup>, mild localized inflammation was found in

Table 2. Result of Microscopic Finding

|    | Control Group |     |     |     | Experiment Group |     |     |     |          |     |     |     |
|----|---------------|-----|-----|-----|------------------|-----|-----|-----|----------|-----|-----|-----|
|    |               |     |     |     | Group I          |     |     |     | Group II |     |     |     |
|    | 24h           | 1w  | 2w  | 4w  | 24h              | 1w  | 2w  | 4w  | 24h      | 1w  | 2w  | 4w  |
| I  | 0/4           | 0/4 | 0/5 | 3/5 | 0/3              | 0/3 | 0/4 | 4/4 | 0/4      | 0/4 | 1/4 | 4/4 |
| II | 0/4           | 0/4 | 0/5 | 0/5 | 0/3              | 0/3 | 0/4 | 0/4 | 0/4      | 0/4 | 0/4 | 2/4 |

I: Secondary dentin formation

II: Inflammatory cell infiltration

two cases, that was probably caused by degeneration of aspirated odontoblast in the tubules produce inflammation. The other reason of inflammation may be explained by passage of toxins of filling material toward the pulp cause a chemotactic migration of leukocytes. The greater inflammatory response to ASPA with citric acid than ASPA without citric acid confirms the observations of previous workers (Eriksen, 1974; Vojinovic et al, 1973; Stanley et al, 1975) that use of 50 per cent citric acid as a cavity conditioner enhanced the pulpal inflammatory response to filling materials. It is not clear whether the more marked changes observed followed the use of citric acid are a consequence of additional irritation from the acid itself or from greater penetration of the toxic constituents of the filling materials. In all of the specimen, it is not clear whether the glass ionomer cement being toxic itself or the citric acid is the main factor that damaged the pulp tissue, but it seems that ASPA gives more irritant to the dentinopulpal complex in the deep cavities than routine amalgam restoration.

### CONCLUSIONS

1. ASPA cement is suitable for shallow cavity filling, if there is a deep cavity, it is better to have a lining material than direct filling of ASPA cement.
2. A reduction in the number of odontoblasts lead to the formation of reparative dentin after 4 weeks.

### ACKNOWLEDGEMENT

The author was grateful to Miss Ching-

Hwa Yu and Dr. Chiuann-Kuen Leu School of Dentistry, Taipei Medical College, for the preparation of sectioned specimen.

### REFERENCES

1. Brannstrom M., Nyborg H.: Pulpal reaction to composite resin restorations. *J. Prosthet. Dent.* February, 27, 2; 181-189, 1972.
2. J W. McLean and A.D. Wilson: Fissure Sealing and filling with an adhesive glass ionomer cement. *J. Brit. Dent.*, 136; 269-276, 1974.
3. C.G. Plant, David, S. Shovelton, J.R. Vlietstra, J.M. Wartnaby: The use of a glass ionomer cement in deciduous teeth. *J. Brit. Dent.* 143; 271-274, 1977.
4. Dahl, B.L. and Tronstad, L.: Biological tests of an experimental glass ionomer (silicoly acrylate) cement. *J. Oral Rehab.* 3; 19-24, January, 1976.
5. R.S. Tobias, R.M. Browne, C.G. Plant, D.V. Ingram: Pulp response to a glass ionomer cement. *J. Brit. Dent.* 144; 345-350, 1978.
6. Karl-John Nordenvall, Martin Brannstrom, Bodil Torstensson: Pulp reactions and Microorganisms under ASPA and concise composite fillings. *J. Dent. for children* Nov.-Dec. 17-21, 1979.
7. G.J. Mount and O.F. Makinson: Clinical characteristics of a glass ionomer cement. *J. Brit. Dent.* 145; 67-71, 1978.
8. M. Brannstrom: The effect of dentin desiccation and aspirated odontoblasts on the pulp. *J. Prosthet. Dent.* August. 20, 2; 165-170, 1968.
9. Brannstrom, M. and Garberoglio, R:

- The dentinal tubule and the odontoblast processes. *Acta, Odont. Scand.* 30; 291-311, Sept. 1972.
10. Brannstrom, M.: Dentinal and pulpal response III. application of an air stream to exposed dentin. Long observation period. *Acta Odont. Scand.*, 18; 235-252, Nov. 1960.
11. Williamson J.J.: Chemotactic migration.
12. El-Kafrawy, A.H., Mitchell, D.F.: Pulp reaction to open cavities later restored with silicate cement. *J. Dent. Res.*, 42; 874-884, 1963.
13. Gottlieb, B.: The formation of secondary dentin and related problems. *J. Dent. Res.*, 25; 29, 1946.

## 牙髓牙本質對Glass Ionomer Cement 充填反應之組織學研究

林光勳

對齒頸部楔形機械性磨耗及齶蝕的填補，在窩洞的形成及材料的選擇上是一大困擾。近年來臨床報告顯示出 Glass Ionomer Cement 可解決大部份的問題。本研究現就以 Glass Ionomer Cement 對活體牙髓牙本質的刺激所產生的反應，提出報告。實驗是以六隻臺灣野猴的健康大白齒為研究材料。30 顆實驗組，18 顆對照組，經由苯基巴比特魯納麻醉，在每一臼齒頰側近齒頸部三分之一處以氣動式鑽頭 806/018 鑽石鑽針磨製一深 1.5—2.0 毫米之窩洞，以消毒棉球及壓縮空氣吹乾，掛上防濕橡皮布。對照組 18 顆以生理食鹽水沖洗過，乾燥，塗以氫氧化鈣齒髓覆蓋劑，再填以銀汞齊。實驗一組 14 顆牙齒，窩洞不塗佈任何襯底材料而直接填以 ASPA 水門汀；實驗二組 16 顆牙齒，窩洞以 50 % 檸檬酸擦拭 30 秒後洗淨，再填以 ASPA 水門汀。實驗動物在牙齒填補後 24 小時，1 週、2 週、4 週後將其殺死，全身福馬林灌流，固定，脫灰，包埋，染色，鏡下觀察。實驗的目的是在於觀察牙髓牙本質對化學性及機械性刺激的反應，殘留牙本質的厚度是以窩洞底部至牙髓間垂直的最短距離為準。所有檢體牙本質之厚度皆小於 2 毫米。實驗一組及對照組 24 小時及 1 週，窩洞底之牙髓牙本質並無變化，2 週之結果亦同。4 週對照組 5 顆牙齒中，3 顆有再生牙本質的生成，實驗一組全部 4 顆牙齒都有再生牙本質的生成，且有造牙本質細胞減少的現象。實驗二組 16 顆牙齒，24 小時及 1 週與實驗一組同。2 週 4 顆牙齒中，1 顆在窩洞下有一狹長帶狀再生牙本質生成。4 週 4 顆牙齒皆有再生牙本質的生成，部份造牙本質細胞喪失，細胞被吸入牙本質管中，2 顆牙髓中有慢性發炎細胞浸潤。再生牙本質的生成，牙本質的脫水，或造牙本質細胞突的斷裂都可能使造牙本質細胞喪失。造牙本質細胞在牙本質管中之退化，及填補材料之毒性都可能是產生發炎現象之原因。