

Histological Study of Gingival Reattachment with Cementogenesis in Taiwan Rock Monkey (*Macaca Cyclopis*)

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The possibility of the reattachment of the periodontal membrane to cementum and bone after periodontal surgery was a controversial issue in 1942⁽¹⁾. One group believed that the periodontal membrane fibers cannot be reattached to the cementum. A number of reasons were given, one of which was that the tooth is under frequent movement through mastication and that what appears to be a reattachment is only a close physical adaptation of the gingival tissues to the tooth. Another group thought that reattachment occurs by the formation of new layers of cementum which engulf the free ends of the periodontal fibers.

The experiments in dogs showed that epithelium had the property of rapidly covering any interruption, it could not form an organic union with the root except at the extreme base of the attachment⁽³⁾. The epithelium proliferated so rapidly that all further reattachment of connective tissue was prevented⁽²⁾. In 1949 Dr. Morris found that surgical amputation of cementum might accelerate union sufficiently to block the ectodermal invasion⁽³⁾. In 1950, Dr. Linghorne and Dr. O'Connell found that following the surgical destruction of a section of the periodontal membrane and alveolar bone, connective tissue reattachment

of the gingival tissues to the tooth by a deposition of new cementum was repeatedly obtained. And this new cementum may be laid down on the old cementum or directly on dentin⁽⁴⁾.

Many investigators had demonstrated that new bone or cementum can be induced to form on the surface of transplanted allogeneic bone or dentin matrix that has been partially or totally demineralized in vitro by acid^(5,6,7,8).

In 1975, Dr. Register and Dr. Burdick designed an experiment to identify an optimum range of demineralization related to type of agent, pH, and time of application. Their selection for clinical evaluation was citric acid at pH 1 applied for 2 to 3 minutes. They demonstrated that new cementum can be induced to form on the surface of denuded dentin, thereby securing reattachment of flap tissues to the root⁽⁹⁾.

Many studies have been designed to determine the nature of a protein portion of the matrix that, when exposed by demineralization, induces mesenchymal cells in the approximating connective tissue to differentiate into cementoblast. Since collagen is the most important fibrous protein in connective tissue, and collagen has an unusual high content of glycine (25%),

and of proline and hydroxyproline (30%)⁽¹⁰⁾, it remains an interesting question that whether the accelerated reattachment with cementogenesis to dentin occurs, if we add proline into citric acid at pH 1 applied for 2 to 3 minutes. The present experiment was designed to find out a new technique so that predictable accelerated reattachment via cementogenesis can become an accepted clinical treatment regimen for the regeneration of the periodontium.

MATERIALS AND METHODS

To examine the hypothesis that if proline can induce rapid reattachment with cementogenesis, the following experiments were devised.

Adult Taiwan-Rock monkeys were anesthetized with ketamine and 5% sodium pentobarbital. Buccal full thickness flaps were reflected over $\frac{54}{65} | \frac{45}{56}$. Buccal bone about 5 mm horizontally and 7 mm vertically was removed, with a round bur and a sharp chisel, to expose the cementum of the distal roots of $\frac{4}{5} | \frac{4}{5}$ and of the mesial roots of $\frac{5}{6} | \frac{5}{6}$. The exposed cementum was removed with chisel so that the resulting wound might simulate a buccal periodontal pocket treated by splitflap exposure, root planning and replacement of the flap to its original position.

56 teeth of 7 monkeys were divided into three groups, each group was applied with different agent.

group I — applied with citric acid and 0.08% proline.

group II — applied with citric acid and 0.10% proline.

group III — applied with citric acid.

All agents were at pH 1.

Agents application to dentin surface was accomplished by rubbing the root surface with a cotton-tipped applicator soaked in the agents. Applicators were changed frequently during the demineralization period to approach a more constant concentration of acid throughout the application time.

Demineralized teeth were rinsed free of acid with normal saline at the end of the application time, and flaps were returned to their original height and were closed with interrupted sutures. In group I and group II, 1% proline were applied to the flaps and denuded dentin surface before flaps were closed. Taiwan-Rock monkeys were administered 300,000 units of benzylpenicillin intramuscularly for prophylaxis at the end of operations.

2-week and 3-week results were produced by operating one-half of the mouths, waiting one week, operating the other half and waiting two weeks to terminate the monkeys. Monkeys were terminated with perfusion of formalin. Fixation in vitro was continued for forty-eight hours following block sections were excised. The fixed specimens were demineralized for about twelve days in Plank's solution.

Specimens were processed for paraffin sectioning and blocks were cut bucco-lingually, parallel to the long axis of the root. Sections were stained with hematoxylin and eosin and were observed under light microscope.

RESULTS

In order to estimate the effect of proline, group I and group II are discussed

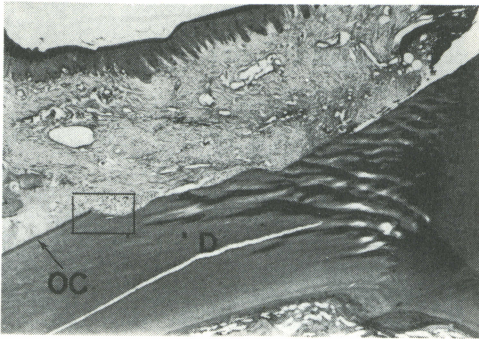


Fig. 1. specimen applied with citric acid and 0.08% proline at 2 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. oc: old cementum (H.E. 40x).

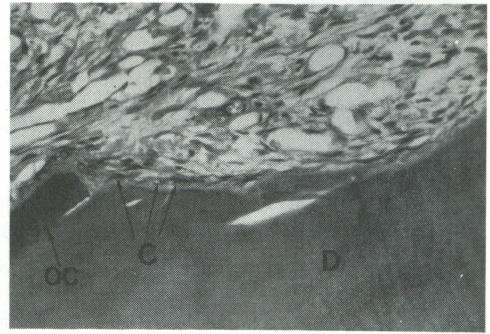


Fig. 2. high magnification of inset in Fig. 1, shows cementogenesis. d: dentin. oc: old cementum. c: cementoblast. (H.E. 400x).

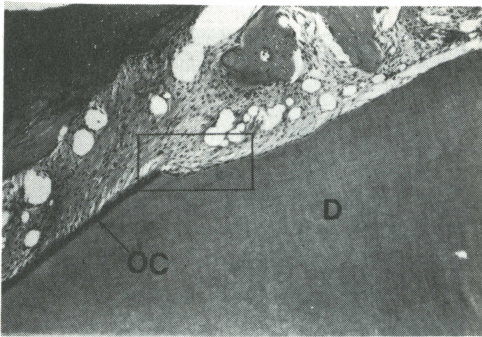


Fig. 3. specimen applied with citric acid and 0.10% proline at 2 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. oc: old cementum (H.E. 100x)

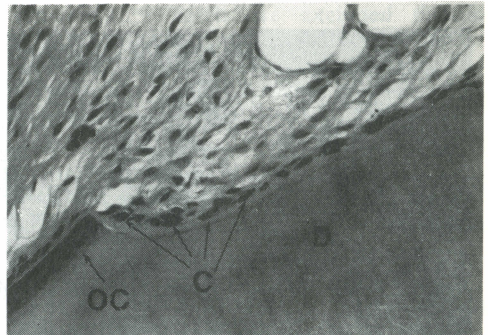


Fig. 4. high magnification of inset in Fig. 3, show cementogenesis. d: dentin. oc: old cementum. c: cementoblast. (H.E. 400x)

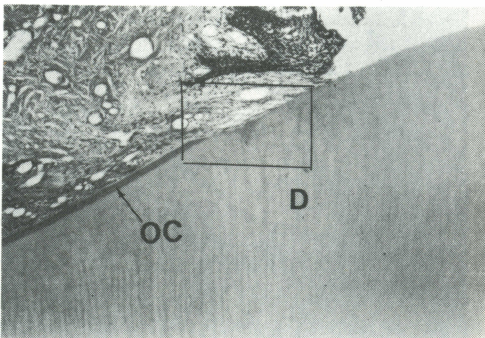


Fig. 5. specimen applied with citric acid at 2 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. oc: old cementum. (H.E. 100x)

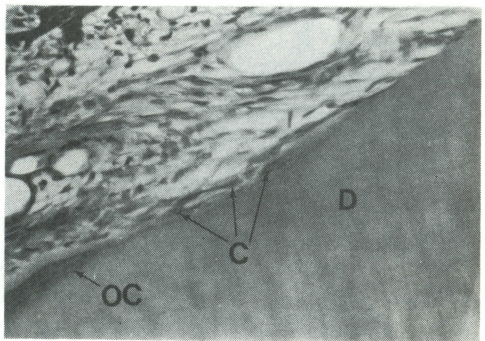


Fig. 6. High magnification of inset in Fig. 5, shows cementogenesis. d: dentin. oc: old cementum. c: cementoblast. (H.E. 400x)

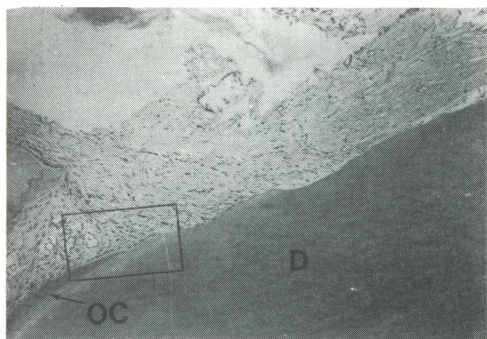


Fig. 7. specimen applied with citric acid and 0.08% proline at 3 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. oc: old cementum. (H.E. 100x)

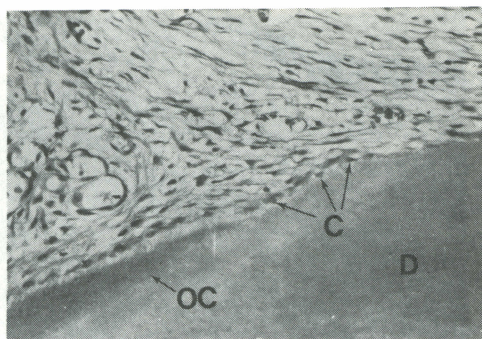


Fig. 8. high magnification of inset in Fig. 7, shows cementogenesis. d: dentin. oc: old cementum. c: cementoblast. (H.E. 400x)

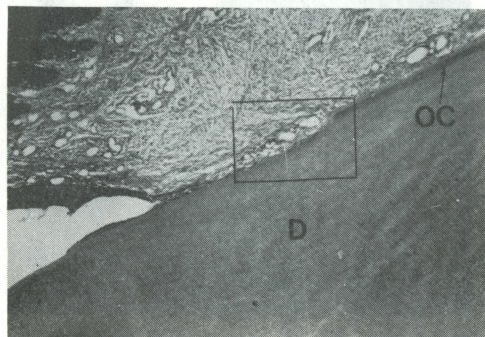


Fig. 9. specimen applied with citric acid and 0.10% proline at 3 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. oc: old cementum. (H.E. 100x)

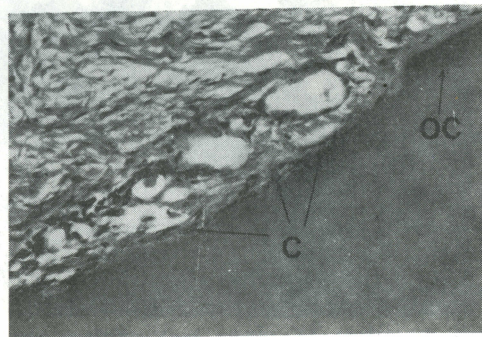


Fig. 10. high magnification of inset in Fig. 9, shows cementogenesis. d: dentin. oc: old cementum. c: cementoblast. (H.E. 400x)

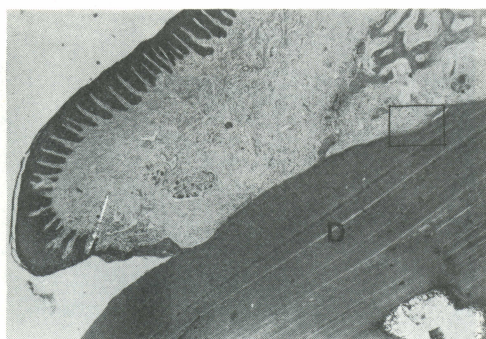


Fig. 11. specimen applied with citric acid at 3 weeks, shows the gingiva tissue had reattached to dentin. d: dentin. (H.E. 40x)

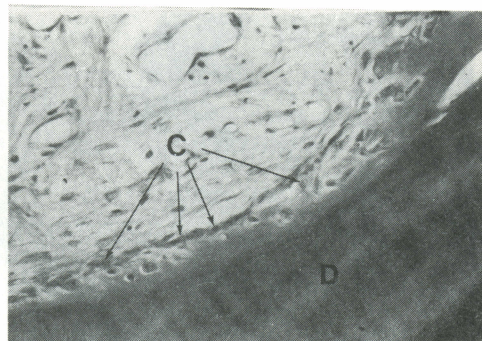


Fig. 12. high magnification of inset in Fig. 11, shows cementogenesis. d: dentin. c: cementoblast. (H.E. 400x)

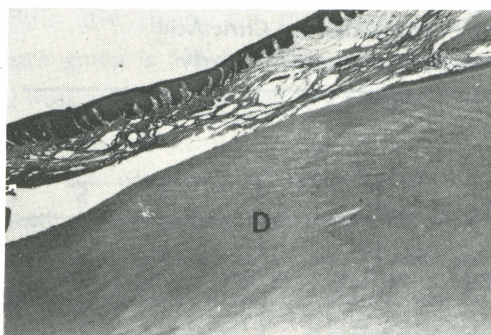


Fig. 13. Shows the gingiva was separated by slide processing at 2 weeks.
d: dentin. (H.E. 40x)

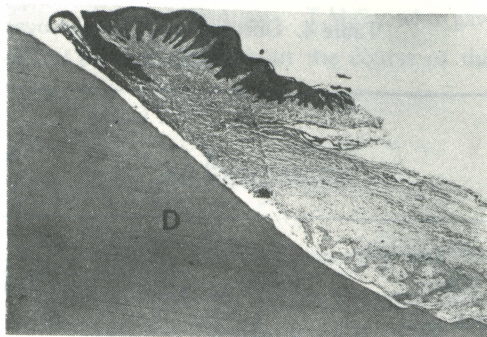


Fig. 14. shows the gingiva was separated by slide processing at 3 weeks.
d: dentin. (H.E. 40x)

Table 1. Occurrence of Gingival Reattachment Observed at 2 weeks and 3 weeks Post-operatively

	2 weeks		3 weeks	
	A	S	A	S
Group I	7	1	5	3
Group II	7	4	7	2
Group III	4	2	2	2
Total	18	7	15	7

* A: Attached
S: Separated

Table 2. Occurrence of Gingival Reattachment in Proline-added and Citric Acid Groups Observed at 2 Weeks and 3 Weeks Post-operatively

	2 weeks		3 weeks	
	A	S	A	S
Proline-added group	14 (73.68%)	5 (26.32%)	13 (72.22%)	5 (27.78%)
Citric acid group	4 (66.67%)	2 (33.33%)	2 (50%)	2 (50%)
Total	18 (72%)	7 (28%)	15 (68.18%)	7 (31.82%)

* A: Attached
S: Separated

together and called proline-added group. Table 1 shows the result of 47 teeth, the

remaining 9 teeth are excluded because of incomplete data.

1. In 2-week result, 18 out of 25 teeth (72%) the gingiva had reattached. In 3-week result, 15 out of 22 teeth (68.18%) the gingiva had reattached (see Table 1).
2. In 2-week result, 14 out of 19 teeth (73.68%) the gingiva had reattached in proline-added group, while citric acid group only 4 out of 6 teeth (66.67%) the gingiva had reattached (see Table 2).

Table 3. Occurrence of Cementogenesis in Teeth Whose Gingiva Had Reattached Observed at 2 weeks and 3 weeks Post-operatively

	2 weeks		3 weeks	
	\bar{C}	\bar{S}	\bar{C}	\bar{S}
Group I	2	5	5	1
Group II	5	2	5	2
Group III	3	1	2	0
Total	10	8	12	3

* \bar{C} : with cementogenesis
 \bar{S} : without cementogenesis

Table 4. Occurrence of Cementogenesis in Proline-added and Citric Acid Groups Observed at 2 Weeks and 3 Weeks Post-Operatively

	2 weeks				3 weeks			
	A		S		A		S	
	\bar{C}	\bar{S}	\bar{C}	\bar{S}	\bar{C}	\bar{S}	\bar{C}	\bar{S}
Proline-added group	7 (50%)	7 (50%)	0 (0%)	5 (100%)	10 (76.92%)	3 (23.08%)	0 (0%)	5 (100%)
Citric acid group	3 (75%)	1 (25%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)
Total	10 (55.56%)	8 (44.44%)	0 (0%)	7 (100%)	12 (80%)	3 (20%)	0 (0%)	7 (100%)

* A: Attached S: Separated

\bar{C} : with cementogenesis \bar{S} : without cementogenesis

3. In 3-week result, 13 out of 18 teeth (72.22%) the gingiva had reattached in proline-added group, while citric acid group only 2 out of 4 teeth (50%) the gingiva had reattached (see Table 2).
4. In 2-week result, 10 out of 18 teeth (55.56%) had cementogenesis whose gingiva had reattached (see Table 3).
5. In 3-week result, 12 out of 15 teeth (80%) had cementogenesis whose gingiva had reattached (see Table 3).
6. In 2-week result, 7 out of 14 teeth (50%) had cementogenesis whose gingiva had reattached in proline-added group, while citric acid group 3 out of teeth (75%) whose gingiva had reattached (see Table 4).
7. In 3-week result, 10 out of 13 teeth (76.92%) had cementogenesis whose gingiva had reattached in proline-added group, while citric acid group had 2 out of 2 teeth (100%) whose gingiva had reattached (see Table 4).
8. Connective tissue adhesion with no

cementogenesis in the middle third and coronal third of the wound with occasional cementogenesis in the apical third of the wound.

DISCUSSION

As far as gingival reattachment is concerned, we could not find any significant difference between proline-added and citric acid groups, for the occurrence of each group is 73.68% and 66.67% respectively in 2-week result, 72.22% and 50% respectively in 3-week result. Our conjecture is that the topical application of 1% proline interfered with the metabolism of aminoacids on applied areas and did not get the accelerated effect to gingival reattachment as we expected. Oral administration may be considered in further experiments.

From histological observation, cementogenesis did occur as early as 2 weeks post-operatively both in proline-added and citric acid groups. We could not find any significant difference between proline-added and

citric acid groups, for the occurrence of each group is 50% and 75% respectively in 2-week result, 76.92% and 100% respectively in 3-week result.

The occurrence of cementogenesis in teeth whose gingiva had reattached in 2-week and 3-week results are 55.56% and 80% respectively, higher occurrence may be resulted if observation was continued for 4 weeks or longer to allow mesenchymal cells to differentiate into cementoblasts.

It has been known for a long time that alveolar process may be regenerated if attachment of the soft tissues has taken place⁽⁵⁾. Fig. 13 and Fig. 14 show that the gingiva flaps were separated although there were bone regeneration.

External resorption occurred more frequently near the base of the wound. Resorption was possibly related to bur removal of buccal bone and cementum that generated much heat and could be minimized by smooth chisel preparation of roots.

CONCLUSIONS

1. From data collected no significant difference in rate of gingival reattachment occurs between proline-added and citric acid groups in either 2-week or 3-week result.
2. If cementogenesis is present, gingival reattachment will occur.

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牙骨質生成誘致牙齦再附著之組織學研究

林胡讓

摘 要

為探討如何使已剝離的牙齦再附着於牙齒原來位置的過程能加速進行，曾經過一段長時間的研究，認為若能在牙齦分離之後，在適當的 pH 值及適當的時間下，於牙齒表面塗以某種酸，在對周圍組織刺激性最小的情況下，而能達到最大的牙齦再附着效果。目前為止被認為 pH 值 1 之檸檬酸 (citric acid) 塗予 2 ~ 3 分鐘的效果最好。今見於脯氨酸 (proline) 對結締組織的形成為一不可或缺的要素，若將脯氨酸加入於 pH 值 1 的檸檬酸內塗在裸露的牙本質上，是否更能加速牙齦的再附着，原此而作本實驗。本實驗係將 7 隻台灣野猴的 56 顆牙齒 (每隻猴子的 $\frac{54}{65} | \frac{45}{56}$)，在麻醉下，翻開頰側牙齦之後，除去頰側齒槽骨及牙骨質，使牙本質暴露，實驗組分兩群各分別塗以含有 0.08 % 及 0.10 % 脯氨酸的檸檬酸，對照組係僅塗予檸檬酸，均係在 pH 值 1 的條件下，塗予 2 ~ 3 分鐘，然後以生理食鹽水沖乾淨，實驗組的牙齦於縫回之前，再塗予 1 % 之脯氨酸，最後再將牙齦縫回原來的高度，手術完了再肌肉注射 300,000 單位的 Benzylpenicillin 預防細菌的感染。術後二星期及三星期在顯微鏡下觀察結果。我們觀察得之，牙骨質生成的確在二星期時就產生，在實驗組及對照組比較上並沒有不同，或許是因局部的塗予大量的胺基酸會增加組織代謝的負擔所致，我們考慮在以後的實驗中用口服的方法，是否會有不同的結果。