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Katsumi Nishijima*

Kenichiro Kiguchi†

*Okayama University,

†Okayama University,

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Katsumi Nishijima and Kenichiro Kiguchi

Abstract

Exfoliative cytologic studies on normal oral cavity and on the postexodontic wound healing of infants were carried out and the following results were obtained: 1) The keratinization of cells was found marked in such regions as buccal mucosa, mucobuccal fold, gingiva and palate in that order. 2) As for changes in the distribution of cells and leucocytes, the cell distribution in the period of 15-21 postexodontic days proved to be identical with that of normal exfoliated cells. Namely, the wound healing from exfoliative cytologic aspects takes place during the period of 15-21 postexodontic days.

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EXFOLIATIVE CYTOLOGIC STUDY ON NORMAL CHILDREN'S ORAL CAVITY AND THEIR POSTEX- ODONTIC WOUND HEALING

Katsumi NISHIJIMA* and Kenichiro KIGUCHI**

Department of Oral Surgery, Okayama University Medical School, Okayama, Japan
(Director: Prof. Y. Watanabe)

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Cytologic study of the oral cavity dates back to MILLER's paper (1890) on the epithelial cells and leucocytes in the human saliva (10), and there are studies on the relationship between keratinization of the epithelial cells and cellular components by WEINMANN (1935, 1936, 1940), ORBAN and WEINMANN (1939), MÜLLER (1940), KUME (1941, 1944), and BRADLEY (1948) (12, 13, 15). Keratinization of the gingiva was investigated by AURER-KOZELJ (1967) (1) recently. WATANABE (1951) (11) made a study on the roentgen effect upon the cellular elements in the human saliva by means of sialogram.

MILLER (1951) (5), MONTGOMERY (1951) (6) and HIDAKA (1960) (4) made comparative studies on the difference of the percentage of keratinized cells at different regions of the normal oral mucosa statistically by Papanicolaou's staining technic. In addition, there are studies on histologic changes in normal oral mucosa according to the age by SUZUKI *et al.* (8), and those on the changes due to mouth washing and gurgling in newborn and infants by SHIRAISHI (1951) (9) of our Department, and those by MATSUI (1958) (7) on oral exfoliated and keratinized cells by scraping.

Though there have been many descriptions on the keratinization of the oral mucosa, only a few studies on the exfoliative cytologic study in postexodontic healing were made by HAHN (1958) (3), FUKUI and MAKI (1958) (2), WATANABE and TOMOZAWA (1963) (14). And no study can be found on oral cytology after extraction of deciduous teeth. In order to make this point clear, exfoliative cytologic observations were made in normal oral cavity and in wound healing after extraction of deciduous teeth of infants.

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* Assistant Professor and **Clinical Assistant of Oral Surgery, Okayama University Medical School

MATERIALS AND METHODS

A. Study on Exfoliated Cells in Normal Oral Cavity of Infants

1. *Collecting and Staining*: Subjects of the study were 20 infants of the age ranging 2—5 years with apparently normal oral mucosa who saw us at the Department of Oral Surgery in Okayama University Hospital. Materials were collected from the following 5 sites: a) Buccal mucosa corresponding to the position of the crown of the upper first deciduous molar. b) Palatal mucosa corresponding to the apical area of the root of the upper first deciduous molar. c) Mucobuccal fold of the lower first deciduous molar. d) Buccal gingival mucosa of the lower first deciduous molar (about 1—2 mm below the neck of the tooth). e) Lingual gingival mucosa of the lower first deciduous molar (about 1—2 mm below the neck of the tooth).

The materials collected from these sites by scraping the mucosa softly with a cotton swab about 2 mm in diameter were thinly smeared over a clean slide glass as evenly as possible about 2 cm² in the area and immersed quickly in a solution of 95 % ethyl alcohol and equal part of ether to avoid drying. After fixing over 2 hours the smears were stained by Papanicolaou method.

2. *Cell Classification*: Microscopic observations of smears were made at 200—400× magnification and all the cells were classified into Type I to V according to the morphology and stainability of cytoplasm and nucleus.

Type I: Cells with round small cytoplasm and somewhat large nucleus stained deep (Fig. 1). Type II: Cells with ovoid cytoplasm and a nucleus slightly larger than Type I. Spindle-shaped cytoplasm is included in this group (Fig. 2). Type III: Cells with polygonal cytoplasm of medium to large size, and the nucleus showing no degeneration (Figs. 3, 4-a). Type IV: Cells with polygonal large cytoplasm and the nucleus showing degeneration (Figs. 3, 4-b). Cells with very poorly stained nucleus. Type V: Cells with nucleus mostly destroyed (Fig. 4-c).

B. Study on Exfoliated Cells in Postexodontic Wound Healing

1. *Collecting and Staining*: Subjects of this study were 74 postexodontic wounds in case of infants of the age ranging 2—5 years who saw us at the Department of Oral Surgery, Okayama University Hospital, and extractions of their deciduous teeth were indicated clinically and radiologically.

Daily follow-up observations were carried out on these cases and smears were taken from the center of the postexodontic wounds by the same method as in the normal control, avoiding hemorrhage as much as possible, and they were stained by Papanicolaou technic.

2. *Cell Classification*: Cells were classified in the same way as mentioned above.

3. *Classification of Leucocytes*: Smears were examined at 200—400× magnification and cell counts were made in 5 different fields at random. They were divided into the following 5 groups according the number of cells: 1) one under 4 cells, 2) 5—9 cells, 3) 10—19 cells, 4) 20—49 cells and 5) one with over 50 cells. No red blood cells were counted.



1. Fig. 1	Type I	2. Fig. 2	Type II
3. Fig. 3	Types III & IV	4. Fig. 4	Type V
	a: Type III		a: Type III
	b: Type IV		b: Type IV
			c: Type V

RESULTS

In the central portion of the specimen where cells were smeared evenly cell counts were taken under the microscope (200—400 $\times$) and classified according to the classification mentioned above. In the normal oral cavity of infants cells of Type I and Type II were observed in each of the five sites of the oral cavity, though they were a few in number. Cells

of Type III were found lowest in the number in the palatal region (25.6 per cent) and were highest in the buccal mucosa (40.4 per cent). These cells of Type III were observed about 36—37 per cent both in the mucobuccal fold and gingival region. They hardly showed any difference in the number. Cells of Type IV were highest in the number in the palatal region showing about 60.5 per cent, followed by the mucobuccal fold (56.8 per cent), and no differences were observed in other sites. Cells of Type V were most predominant in the palate showing 13.9 per cent, followed by 11.1 per cent in the buccal gingiva. A minimal number of leucocytes and no erythrocytes were observed.

After tooth extraction the distribution pattern of cells appearing in oral changed with lapse of time.

No cells other than blood cells could be observed immediately after tooth extraction. Cells of Types I and II appeared prominently 2—3 days after extraction and showed a decrease with lapse of time (as the cell count of Type I was so small, it was included with that of Type II). Cells of Type III were numerous. But they reached their peak in about 4—5 days later, and gradually decreased in the number thereafter. The cells of Type IV showed a tendency of increase in number till 2 postexodontic weeks, and during about 10—21 days their number became higher than that of Type III cells. Up to the period of 10—21 days, while the cells of Types I and II were decreased in number and those of Type IV were increased more than the number of Type III cells (Fig. 5).

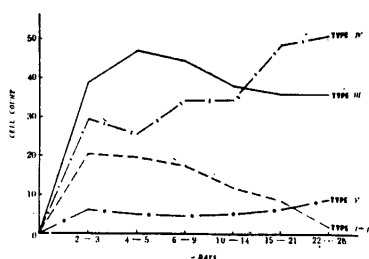


Fig. 5

During 2—3 postexodontic days the groups of over 50 cells occupied more than half of the total cell count. During 4—5 days the groups of over 50 cells grew less in number, and groups of 5—9, 10—19, and 20—49 cells were seen scatteringly. In more than 10 days the groups of over 50 cells could no longer be observed and the groups of less than 4 cells grew more numerous. The groups of less than 4 cells became predominantly marked 15 days later. Leucocytes were hardly visible 22 days later.

Table 1 NUMBER PER CENT OF EACH SPECIES, EXFOLIATED CELL FROM NORMAL CAVITY OF 20 INFANTS

Case No.	Type																								
	I					II					III					IV					V				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
1						2					36	23	35	32	40	54	62	59	58	48	8	15	6	10	12
2								1			40	30	35	32	34	51	60	60	59	55	9	10	5	8	11
3								2	1		41	27	35	32	37	56	67	60	47	54	3	6	5	19	8
4								1	2		45	17	35	52	42	49	72	60	38	53	6	11	6	8	5
5											42	27	35	19	35	52	61	59	59	54	6	12	6	21	11
6										1	47	35	39	46	39	48	59	56	51	55	5	6	5	3	5
7											44	14	37	40	35	49	67	60	56	54	7	19	3	4	11
8											43	25	37	37	38	53	57	54	53	53	4	18	9	10	9
9			1			2					45	32	31	31	38	49	59	62	52	53	4	9	7	16	9
10								1			44	31	37	39	27	52	61	59	50	61	4	8	4	10	12
11											36	21	36	45	31	58	59	60	52	58	6	20	4	3	11
12											43	17	36	26	39	52	66	61	52	56	5	17	3	22	5
13											47	29	41	33	36	49	59	55	47	55	4	12	4	20	9
14	1										47	34	42	45	38	49	58	53	50	56	4	8	5	5	6
15			1								33	22	38	33	39	56	56	57	54	59	11	22	5	12	2
16											31	25	38	33	37	62	53	55	62	56	7	22	7	5	5
17											37	30	43	35	36	48	58	51	52	57	15	12	6	13	7
18											37	29	40	37	42	56	50	51	54	50	7	21	9	9	8
19											34	32	43	29	35	60	55	53	59	61	6	13	4	12	4
20											35	12	42	40	37	57	71	50	50	58	8	17	8	10	5
average	0.1	0.1	0.2	0.1	0.3	0.1					40.4	25.6	37.7	35.8	36.9	53.0	60.5	56.8	52.8	55.3	6.5	13.9	5.6	11.1	7.8

Note: a: Cheek b: Palate c: Mucobuccal Fold d: Buccal Gingiva e: Lingual Gingiva

During 2—3 postexodontic days numerous erythrocytes could be observed but they gradually decreased in number thereafter (Table 2).

Table 2 FLUCTUATION OF THE NUMBER OF LEUCOCYTE GROUPS DURING THE POSTEXODONTIC PERIOD

groups of leucocytes	under 4 cells	5—9 cells	10—19 cells	20—49 cells	over 50 cells
days					
2 — 3		2	2	2	8
4 — 5		3	5	4	3
6 — 9	2	3	6	4	3
10 — 14	6		3	2	
15 — 21	5	1	1		
22 — 28	9				

Note: The leucocyte count in each group showing the average of five fields selected at random

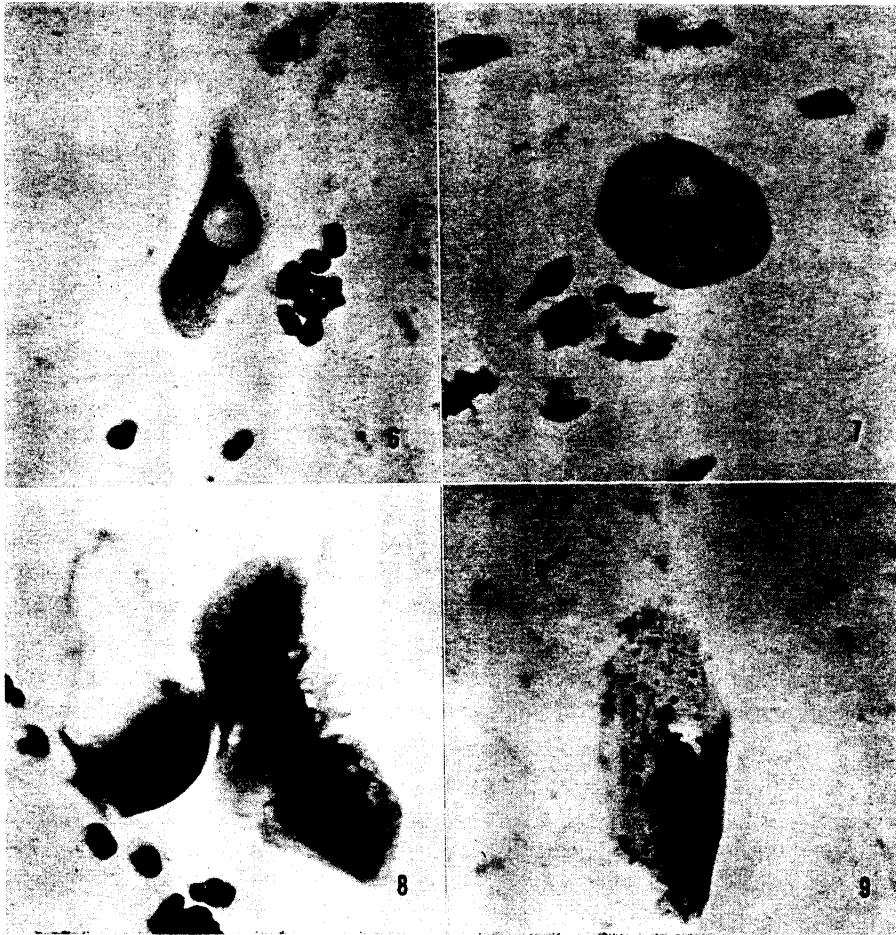
Signs of degeneration were seen during inflammatory changes, and vacuolization in cytoplasm or nucleus was prominent feature: Cells showing vacuolization in cytoplasm and in nucleus (Fig. 6), cells showing vacuolization in cytoplasm (Figs. 7, 8), cells showing large granules in cytoplasm (Fig. 9).

DISCUSSION

There are many studies dealing with problems of postexodontic wound healing, but the majority of them are histopathologic or roentgenologic study, and exfoliative cytologic studies in this field are few in number, such as the papers by HAHN (3), FUKUI and MAKI (2) and WATANABE and TOMOZAWA (13). And exfoliative cytologic study on the wound healing after extraction of human deciduous teeth in normal oral cavity is not available so far.

According to MATSUI (7) the epithelial cells in various layers of the epithelium were stained blue, red and yellow in the order as the keratinization advances. WATANABE and TOMOZAWA (13) reported on stainability of the cytoplasm of postexodontic wound healing in dogs and classified the epithelial cells into deep-layer cells, intermediate cells, precornified cells and keratinized cells.

From the viewpoint of the appearance of parabasal cells which can hardly be observed in normal exfoliated cells, it was considered that the stainability of the cells alone would not serve as a sufficient clue to pursue postexodontic wound healing. Therefore, further observations were made on exfoliated epithelial cells and they were classified into Types I, II, III,



6. Fig. 6

8. Fig. 8

7. Fig. 7

9. Fig. 9

IV and V, according to the shape and stainability of the cytoplasm and morphology of the nucleus by the method of WATANABE and TOMOZAWA.

WEINMANN (10) stated that the percentage of keratinized cells increased in the order of buccal mucosa, tongue root, gingiva and hard palate, and MILLER (5) obtained practically similar results. MONTGOMERY (6) observed that the order of keratinization was soft palate, buccal mucosa, antrum of oral cavity, the anterior part of tongue and gingiva, while MATSUI (7) in the order of buccal mucosa, tongue root, gingiva and hard palate.

It is difficult to make comparison of our results directly with theirs because of difference in the age of the subjects, but the fact that Type

IV and V cells are more predominant than in other regions, showing the total of 74.4 per cent, indicates marked keratinization in this region agreeing with those findings of MATSUI, WEINMANN and others, both gingiva and mucobuccal fold show Type IV and V cells in total of 62—64 per cent with little difference between the two regions and the buccal mucosa least with 59.5 per cent. The fact that in a few cases Type I and II cells which do not generally appear among exfoliated cells of normal oral cavity were observed, may imply a marked metabolism of mucosa in infant or wandering of the cells out of the periodontal pockets.

WATANABE and TOMOZAWA (13) described that the wound became covered with a new regenerated epithelium 15—20 days after extraction of canine teeth.

In the authors' study, up to 10—14 postexodontic days Type I and II cells appeared at the wound site relatively many which decreased in number with lapse of time. In the meantime Type III cells usually appeared more numerous than Type IV cells, and the latter tended to increase gradually, while Type III cells tended to decrease during 4—5 days. In other words, the result in the present observation was reverse of that observable in the distribution of normal exfoliated cells. During 10—14 days when Type I and II cells started to decrease in number, Type IV cells grew more than Type III cells, showing normal cell distribution.

On the other hand, as for leucocytes during 2—3 postexodontic days more than half of the cases belonged to the group of over 50 cells and during 10—14 days more than half of the cases belonged to the groups of less than 4 cells. And during 15—21 days most of the groups became under 4-cell group and all leucocytes during 22—29 days belonged to the under 4-cell group. As for erythrocytes, although the specimens were obtained avoiding bleeding as much as possible, red blood cells appeared up to one postexodontic week in every case.

From these changes in the exfoliated cells and leucocytes, the wound healing after deciduous tooth extraction must be completed during the period of 15—21 days.

SUMMARY

Exfoliative cytologic studies on normal oral cavity and on the post-exodontic wound healing of infants were carried out and the following results were obtained :

- 1) The keratinization of cells was found marked in such regions as buccal mucosa, mucobuccal fold, gingiva and palate in that order.

2) As for changes in the distribution of cells and leucocytes, the cell distribution in the period of 15—21 postexodontic days proved to be identical with that of normal exfoliated cells. Namely, the wound healing from exfoliative cytologic aspects takes place during the period of 15—21 postexodontic days.

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