Evaluation of the Mineralized Tissue in the Pulp of Retained Human Deciduous Teeth (Histological and Immunohistochemical Study)

Zoba H. Ali

[1]Oral Biology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
rawya_h2a@yahoo.com

Abstract: Retention of primary teeth beyond their expected exfoliation date in human is encountered relatively frequently. Little information is known about the apposition of mineralized tissue in the resorped dentine surface of the retained deciduous teeth. **Objective:** The purpose of the present study is to evaluate and investigate the histological structure of the mineralized tissue deposited on previous resorped coronal and radicular dentine surface in human retained deciduous teeth. **Study design:** Sixteen sound upper maxillary deciduous canines and second molars extracted for orthodontic reason used in this work, eight teeth were demineralized, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Some demineralized sections from each tooth were photographed and morphometric analysis was measured. Moreover other sections were stained with the primary antibody osteonectin for immunohistochemical examination. Ground sections of eight teeth were prepared and all sections were viewed in a light microscope. **Results:**. Dentin resorption of the retained deciduous teeth was often followed by deposition of various amounts of cementum-like repair tissue. The cells responsible for the formation of cementum-like tissue are believed to be undifferentiated ectomesenchymal cells of the vital pulp. Mineralized tissue with a varied morphology and thickness was observed. Mineralization seemed to start in the incisal region or at the pulp horns of canines and molars and the central part of the pulp appeared the last part to be obliterated. The whole pulp chamber was sometimes completely obliterated by the calcified tissue especially in case of retained canine. Cementum-like tissue was composed of multiple alternating and irregular light and dark bands. Immunohistochemical examination revealed positive osteonectin immunoreactivity in the deposited hard tissue that indicate this hard tissue is cementum-like tissue. **Conclusions:** In the pulp chambers and in the root canals of retained deciduous teeth resorption had often occurred, indicating that signals giving rise to odontoclasts were present in the pulp tissue. Cementum-like tissue can be deposited within these resorped areas. Also a longer retention time generated more cementum-like tissue deposition in the pulp of the retained deciduous teeth.
[Zoba H. Ali. **Evaluation of the Mineralized Tissue in the Pulp of Retained Human Deciduous Teeth (Histological and Immunohistochemical Study)**. Journal of American Science 2012; 8(1):398-407].(ISSN: 1545-1003). http://www.americanscience.org.57

Keywords: Retained deciduous teeth**;** Cementum-like tissue**;** Osteonectin**;** Light microscope.

1. Introduction:

Shapira and Kuftinec (1998) stated that retained deciduous teeth are teeth that remain in the dentition along with the permanent teeth. This condition can cause malocclusion. Primary canines that are retained beyond the age of 13 years and have no significant mobility were strongly indicate displacement and impaction of permanent canines. **Kurol and Thailander (2000)** reported that in physiologic root resorption the roots of a deciduous tooth undergo resorption before the tooth exfoliates. This is a normal physiologic phenomenon. Resorption can occur with or without the presence of a permanent successor tooth. **Sletten et al. (2003)** retained primary teeth with no permanent successors present a unique challenge to the dentist. These retained primary teeth are mostly lost due to caries or periodontal breakdown. Root resorption is not the primary cause for loss of these teeth. Authors added that the retained mandibular primary second molars more durable than their maxillary counterparts. **Suprabha and Pai (2006)**

suggested that any portion of a tooth may be resorbed as long as such surfaces are associated with other living tissues for example, bone or pulp. Thus tooth resorption can occur from the internal surface of a tooth or from the external surface of a tooth.

Barberia et al. (2005) found that the condition in which a permanent tooth erupts in wrong and undesirable position is termed ectopic eruption. This is one of the most complicated phenomena in a dental system. **Sabri (2008)** describes treatment alternatives for patients who exhibit retained primary molars. When the underlying premolar is present and there is severe disruption to the occlusion and the underlying premolar, extraction and space management may be appropriate **Marimo (2009)** the delayed exfoliation of primary teeth among children is a common and frequent dental problem whose most sited cause is misalignment of the crown of the successional permanent tooth with the root apex of the primary tooth. Treatment is often extraction of the over retained

primary teeth and reassurance of the guardian that the normal tongue movement will push the misaligned permanent successional teeth into line in case of mild misalignment or orthodontic in sever cases.

In the later stages of exfoliation, resorption occurs at the pulpal surface of coronal dentin **(Sahara et al., 1992)** . However **Randi (2001)** found that resorption at the pulpal surface of coronal dentine occurred even if large parts of the roots were unresorped. **Yasutaka et al. (2005)** suggested that physiological root resorption is a characteristic feature of human deciduous teeth. The resorption is not continuous, and has resting periods. In the resting period, cementum deposits in resorbed root surface. The deposited cementum in permanent teeth has been reported in detail. However, the deposited cementum in deciduous teeth is unclear.

The aim of the present study was to show and investigate the histological structure of deposited cementum –like tissue on the resorped coronal dentine surface in human retained upper deciduous canines and upper second molars.

2. Material and Methods:

Sixteen sound human upper retained deciduous teeth eight canines and eight second molars that had been extracted under infiltration local anesthesia were used in this study. The mean of patients' age at the time of extraction was 15. 9 years (range, 14.3-17.6 years). All of these teeth were extracted for orthodontic treatment. After extraction, the teeth were immediately fixed in a mixture of 4% formaldehyde and 0.5 glutaraldehyde in 0.1 M sodium cacodylate buffer.

1- Four canines and four second molars were decalcified with 10% ethylene diamine tetra-acetic acid (EDTA), pH. 7.3, for 4 weeks at 4°C. After decalcification, the teeth rinsed with buffer solution, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections longitudinal sections or transverse sections of 6-7um were obtained and mounted on clean glass slides and subjected to the following investigations to be examined by light microscope:

1-A-Haematoxyline and Eosin stain: For examining the histological structure of the hard mineralized tissue deposited on the coronal dentine in retained deciduous teeth.

1-B- Histomorphometric analysis: The data were obtained by using Leica Qwin 500 image analyzer system (England). After staining the sections with haematoxylin and eosin, sections were examined by a light microscope and the clearest areas of the sections were selected and photographed at x 400 magnification. Images were transmitted from the microscope to a computer monitor and width of the

pale bands was measured with the help of image analysis Leica Qwin 500 software. The maximum, minimum and mean width of the bands within each section was recorded.

1-C-Immunohistochemical staining: using primary antibody osteonectin**.**

Immunohistochemical procedure:

Sections were cut longitudinal sections or transverse sections (L.S &T.S) into 6 μm and mounted on positively charged glass slides. The sections were deparaffinized, hydrated in graded ethanol and phosphate buffer saline (PBS). The deparaffinized sections were incubated in 3% hydrogen peroxide at room temperature for 10 min to inhibit the activity of endogenous peroxidase, wash with PBS, and non specific protein binding sites blocked with 10% rabbit serum at 37°C for 10 min. The primary antibody used was rabbit anti- bovine osteonectin (Dako, Catalog. MO737). The tissue sections were incubated with the primary antibody overnight in moist chamber at4°C then rinsed with PBS, 3times, 2min each. The sections were labeled with a streptavidin- biotin method using Dako- LAB vision (Catalog CA 94539). After washing with PBS, the tissue sections were visualized with a freshly prepared 0.02% w/v 3, 3 diaminobenzidine -HCl (DAB) until optimal staining was obtained. The slides were washed in three changes of PBS for 10 min each. Then slides were immersed in a bath of Mayer's haematoxylin for 1-5 min, slides were rinsed, dehydrated, cleared and examined under the light microscope.

II- Four canines and four second molars were embedded in a quick setting transparent blocks (bioplast). The specimens were cut into mesio-distal sections parallel to their long axis by using Bronwill sectioning machine having diamond disc under water spray. The obtained ground sections were about 1mm thick. Further thinning was carried out by Carborundum abrasive paper with water proof adhesive grit no. 320& 400 **(Frost, 1958).** Ground sections of about 100 um in thickness were mounted on clean glass slides. All sections were examined under light microscopy.

3. Results:

Histological results:

Most of the decalcified sections showed eosinophilic homogenous structureless calcified band deposited along the pulpal surface of dentin which could be termed cementum -like tissue. The thickness of this cementum - like tissue bands varied from tooth to tooth and also within the same tooth. Scalloping of the previous resorped pulpal surface of

dentine was observed in most of the specimens along the whole surface or at certain sites. The amplitude and depth of scallops were varied. In the pulp chamber the resorption had often occurred followed by cementum-like repair tissue. The pulp was highly cellular and contained many widely dilated blood vessels (Fig.1). Cementum- like tissue was deposited on the resorped dentine surface. At some areas the deposited cementum- like tissue changed the scalloped dentin surface into relatively flat. The pulp revealed sign of fibrosis and mild inflammatory cells infiltration (Fig.2). Cementum- like tissue containing lacunae with cellular elements was detected. The dentine and the repair tissue were separated by a haematoxyphilic zone, which was markedly obvious at some specimens. A thin layer of cementoid - like tissue was often present on the cementum-tissue surface, which stained lighter than the remaining tissue but sometimes was absent. The pulp was appeared normal and vital with mild inflammatory cell infiltration   (Fig.3). Also the deposited tissue showed a stratified structure, the cellular cementum -like tissue and the acellular cementum -like tissue appeared in alternating layers. Cementum-like tissue was deposited in an irregular rhythm resulting in unevenly spaced incremental lines (Fig.4).Sometimes the cementum like- tissue occupied nearly the whole pulp chamber in deciduous canine. Mineralization seemed to start in the incisal region and proceed inward. The central part of the pulp was the last part to be obliterated in some canine specimens (Fig.5). The cementum like - tissue in canine had various structures consisting of abundant collagen fibrils, granular tissues and some relatively homogeneous areas. Also some defects in the structure of the cementum- like tissue in the form of minute spaces were obvious observed. The structure of the deposited cementum like-tissue was different from physiological cementum found on the roots of normally shedding deciduous canine and molars (Fig.6).

Examination of the ground sections showed irregular type of mineralized tissue (cementum- like tissue) with a varied structure in the pulp chamber and root canal. Deposition of the mineralized tissue often started in the occlusal region and had a lace-like appearance. In some specimens the pulp chamber and part of the root canal were mostly obliterated by mineralized tissue. In addition the pulp chamber of the deciduous canine and second molar at some areas appeared unmineralized while the remaining part contains mineralized tissue with dark spaces (Fig.7).

At higher magnification of the cementum- like tissue deposited on the resorped dentin of pulp chamber showed structural variations. Various types of cementum- like tissue were found on the resorped

dentin surface of pulp chamber of the same deciduous tooth. In some specimens cementum- like tissue adjacent to the border of the resorbed pulp chamber wall was appeared homogenous and similar to a cellular cementum (Figs.8A&B). Moreover granular structure was also obvious at the innermost layer separating the repair tissue from overlying dentine at some sections. Numerous cementocyte-lacunae like spaces were observed distributed throughout the repair tissue in the coronal portion and the root canal (Figs.9&10).

Morphometric results: All specimens of the retained deciduous teeth examined and revealed that the apposition of cementum-like tissue was occurred in two types of layers with different staining properties that can be observed by light microscopy. The layers undulate and are of variable width. Narrow dark staining incremental lines are separated by wider bands of pale staining cementum (Fig.11). The mean of the widths for the pale bands were ranged between 6.98um to 8.23 um with standard deviation about ±1.82 to ±2.52μm. However the maximum widths of the pale bands were ranged between 9.53μm to12.94 μm and the minimum widths range between3.5μm to 3.48 μm in most examined sections (Table 1), i.e. So the widths of the pale cementum bands were variable. The narrow pale bands may be developed at the period of low activity while the wide pale bands developed at the active period of the cells.

Immunohistochemical results: In longitudinal and transverse sections of demineralized deciduous teeth a moderate to strong immunoreactivity for osteonectin was observed in dentine, dentinal tubules, cementocyte-like cells and pulp cells. Moderate reaction at cement lines (incremental lines) and markedly strong reaction at the reversal line between resorbed surface of dentine and deposited cementum. Also strong reaction at the reversal line was observed in cementum- like tissue itself between old and new formed cementum. Cementum resorption was observed in some specimens and was repaired by new cementum deposition creating reversal lines between old and new deposited cementum -like tissue. Osteonectin staining in the pulp was seen predominantly within cells, with lower staining in the tissue matrix (Fig.12). Cementocyte -like cells were appeared as osteonectin positive while the cementoid matrix itself remained osteonectin negative (Fig.13).

Fig. (1): A photomicrograph of deciduous second molar showing eosinophilic structure less band of calcified tissue along the whole dentine surface similar to cementum (C) scalloping of resorped dentine surface (arrows) and pulp (P). (T.S, H&E x40)
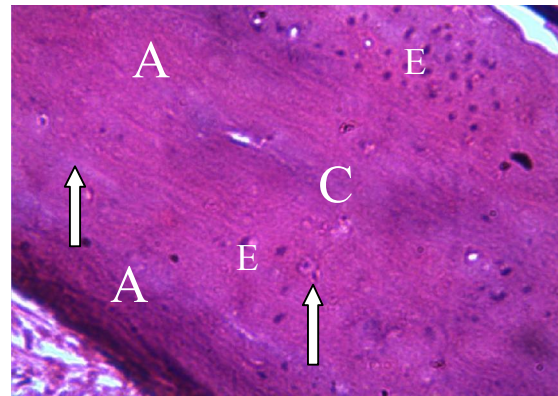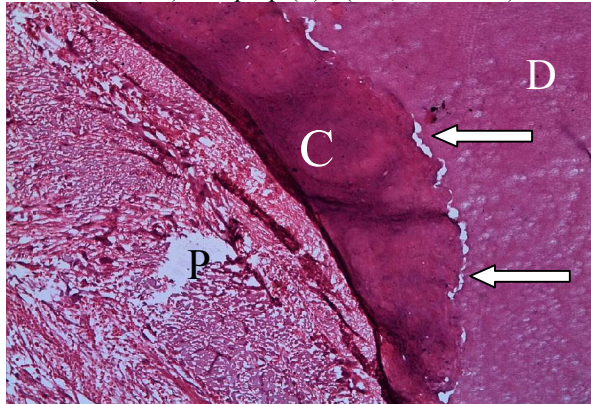

Fig. (2): Higher magnification of the previous figure showing the calcified band of the cementum- like tissue (C) dentine (D), scalloping of the resorped dentine surface (arrows)and pulp with signs of fibrosis (P). (T.S, H&E x100)
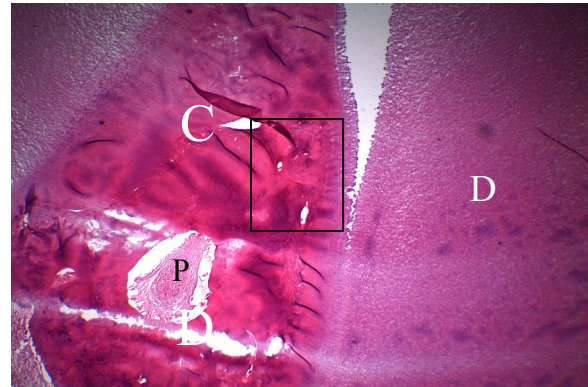

Fig.(3)A photomicrograph of deciduous second molar showing numerous minute spaces in the cementum like- tissue (arrows) haematoxyphilic line (white arrow), cementoid tissue (T) cementoblast- like cells (C) and pulp with mild inflammatory cells(P). (T.S, H&E x 200)

Fig.(4):A photomicrograph of deciduous second molar showing cementum- like tissue (C), alternating layers of cellular (E) & acellular cementum-like tissue(A) and cementocyte-like cells (arrows). (T.S, H&E x400)

Fig.(5):A photomicrograph of deciduous canine showing large mass of cementum-like tissue (C) occupying the pulp chamber with remnant of pulp tissue inside the calcified mass (P) and dentin (D) (L.S, H&E x40)
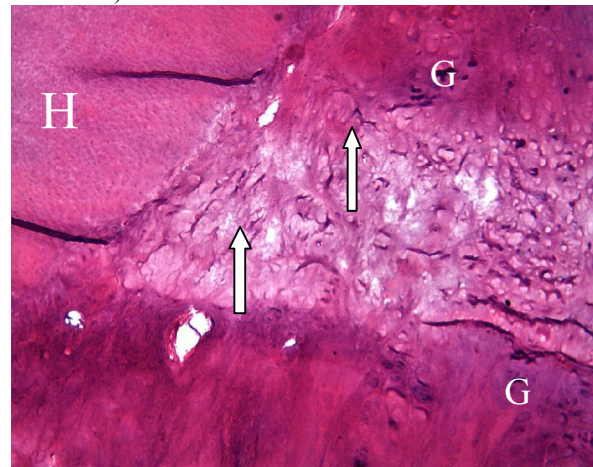

Fig.(6): Higher magnification of the square area in the previous figure showing cementum- like tissue with numerous fibers (arrows), granular materials(G), homogeneous areas (H) with some defects(arrow heads).(L.S,H&E x200)
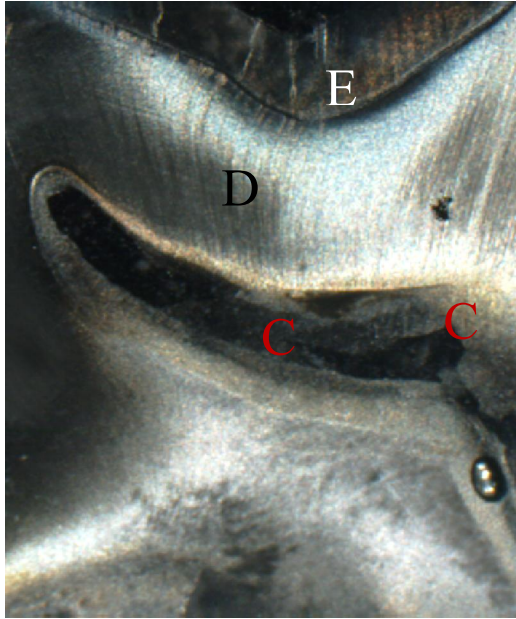
Fig.(7):A photomicrograph of deciduous second molar showing enamel (E), dentine (D)and cementum-like tissue(C) deposited on the wall of the pulp chamber and root canal. (L.G.S. x20).
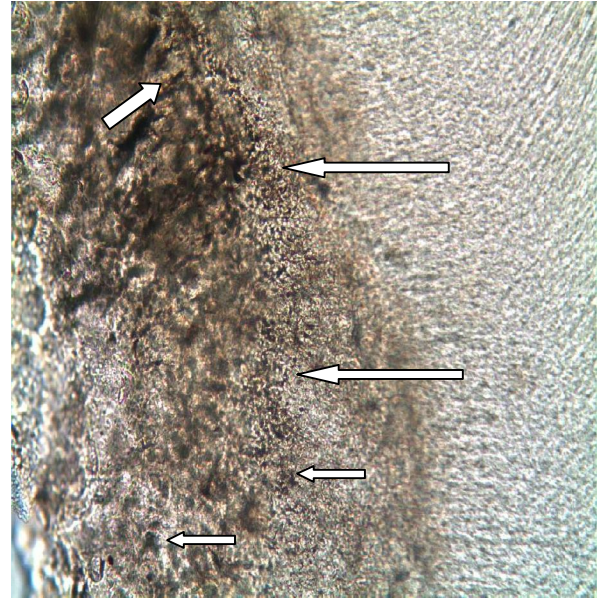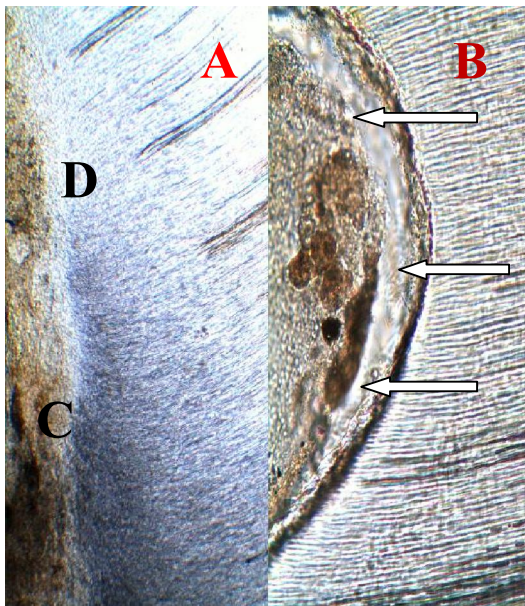

Fig.(9):A photomicrograph of deciduous second molar showing the calcified tissue contained several lacunae -like spaces (short arrows) and granular structure similar tomes granular layer) long arrows (L.G.S. x40)

Figs.(8A&B): A photomicrographs of deciduous second molar showing;(A) reversal line separating repair tissue from overlying dentine, dentinal tubules (D) and cementum- like tissue (C) (L.G.S. x40)& higher magnification of (A) showing the innermost layer of cementum- like tissue appeared homogenous (arrows)(L.G.S. x200).
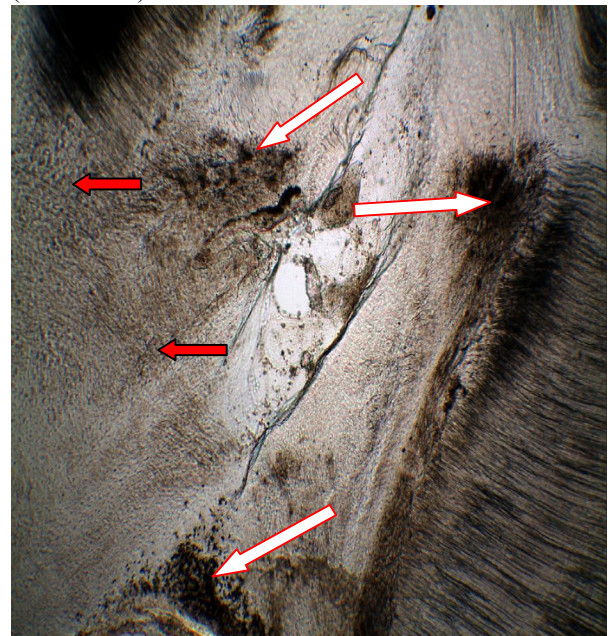

Fig.(10):A photomicrograph of deciduous canine showing the calcified tissue contained numerous ce-mentocyte lacunae -like spaces(long arrows) and granular structure at root portion (red arrows) (L.G.S x40).
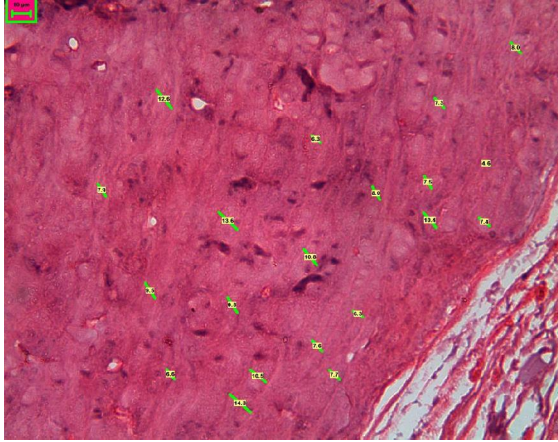
Fig. (11):A photomicrograph of deciduous second molar showing the measurement of the width of the pale bands in the cementum- like tissue. (T.S, H&E x400)
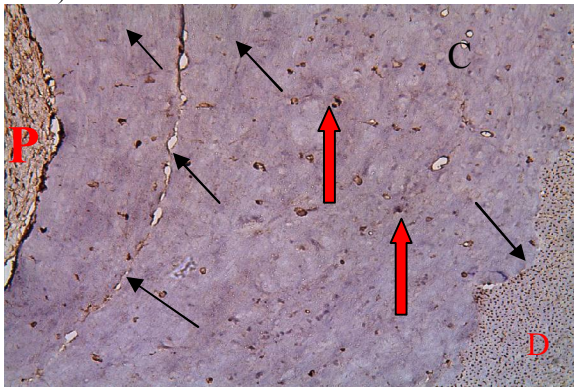

Fig.(12):A photomicrograph of deciduous second molar showing strong immunoreactivity for osteonectin in dentinal tubules (D), cementocyte-like cells(red arrows)and pulp cells (P), moderate reaction at in cement line(incremental line and reversal line) (black arrows) cementum-like tissue (C)(T.S, LAB-SA. x200).
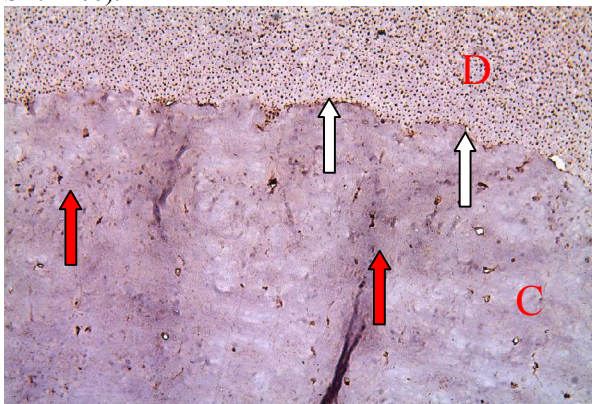

Fig.(13): A photomicrograph of deciduous second molar showing strong immunoreactivity at the reversal line (white arrow), dentinal tubules(D) and cementocytes- like cells(red arrows) cementum-like tissue (C)((T.S, LAB-SA. x200)

Table 1: the pale bands width of cementum -like tissue in two different specimens.

Object 1	Distance	Object 2	Distance
1	8.55	1	3.48
2	7.3	2	10.8
3	3.5	3	9.42
4	8.71	4	8.09
5	4.66	5	7.56
6	5.35	6	12.94
7	7.69	7	6.79
8	7.56	8	8.99
9	6.7	9	8.73
10	9.53	10	5.51
Total	69.75	Total	82.32
Mean	6.98	Mean	8.23
Std Dev	1.82	Std Dev	2.52
Max	9.53	Max	12.94
Min	3.5	Min	3.48

4. Discussion

   Lindqvist (1980) reported that the retained tooth interferes with the proper eruption of the permanent teeth. The permanent teeth can erupt too medially and hit on the sensitive soft tissues of the hard palate, causing sores or fistulas. More commonly, the retained baby teeth trap food and debris, and promote plaque and calculus formation. **Robinson & Chan (2009)** suggested that there are undoubtedly indications for extraction of retained primary teeth. These include increasing mobility, clinical symptoms, pathology, unfavorable position and poor aesthetics. In the present study the retained deciduous teeth were extracted for orthodontic treatment.

   Histological examination of the retained deciduous teeth in this investigation revealed that odontoclastic resorption occurred at the pulpal surface of the coronal dentin. The resorped surface was scalloped and irregular. However, this resorption was not continuous and the resorped dentin was repaired by deposition of eosinophilic calcified band which termed cementum -like tissue.The location and thickness of the deposited newly formed cementum-like tissue on the resorbed dentin varied from tooth to tooth. These findings are in agreement with that of **Marks & Walker(1981)** suggested that the odontoclasts are believed to arise from monocytes-like cells transported to the pulp by the blood vessels during dentin resorption under the effect of certain signals resident in the pulp tissue. Also **Sahara et al., (1993)** reported that when resorption of dentin occurs, the pulp tissue may react by differentiation of hard-tissue-forming cells from undifferentiated ectomesenchymal cells and deposition of cementum-like tissue occurred. **Sahara and Ozawa (2004)** demonstrated that numerous mononuclear cells were ob-

served along the resorped enamel surface and play an important role in the repair process. Cementum-like tissue formed and increased in width, and appeared to undergo mineralization as time progressed. Tooth repair may be coupled to tooth resorption.

In the present study, resorption of the coronal and radicular dentine of the retained deciduous teeth was occurred and repair of the resorped lacunae by deposition of a cementum-like tissue was noted. Resorption of the coronal dentin was occurred in many specimens however long parts of their roots still present. These findings are in agreement with that of **Randi, (2001)** found that resorption at the pulpal surface of the coronal dentine occurred even if large parts of the roots were unresorped.

In the present study the formation of mineralized tissue in the pulp of the retained primary teeth varies from thin band to thick band which could be obliterate the whole pulp chamber and the root canal. Also various morphological types of the cementum, cellular and acellular cementum-like tissue were observed in the pulp of the retained primary teeth. These findings are in accordance with that of **Jones (1981)** showed compositional variation of the newly formed cementum- like tissue. These variations of this hard mineralized tissue seem to indicate that hard tissue is more likely to be cementum than bone.

In the present work examination of the inner most layer of the cementum- like tissue of the ground sections appeared homogenous and resemble the hyaline layer of Hopewell Smith which could be found to seal the dentinal tubules. These findings are in accordance with that of **Ho et al. (2004)** reported that there is 100 to 200 um translucent region between cementum and dentin was observed by using light microscopy. Authors added that the microindentation and nanoindentation results indicated a gradual transition in hardness from dentin to cementum over a width ranging from 100 to 200 μm**.**

In the present investigation the origin of the cementoblasts like cells may be arise from the undifferentiated mesenchymal cells of the pulp of the retained deciduous tooth which appeared normal and vital during the repair period. This finding is in agreement with that of **MacNeil& Somerman (1993)** they recorded that a strong experimental evidence for the dental ectomesenchymal cells residing within the dental papilla and dental follicle regions to be the source of cells giving rise to cementoblasts. This hypothesis is supported by a series of studies in which tooth germ development has been artificially manipulated in an attempt to determine the important of various component tissues in cementogenesis. Also **Tenorio et al. (1993)** demonstrate that osteoblasts and cementoblasts of cellular cementum share many phenotypic characteristics. The authors added that there

may be phenotypic differences between cementoblasts associated with cellular and acellular cementum. Moreover **Ten Cate (1998b)** reported that the cells responsible for the formation of cellular cementum are claimed to come from the osteoblasts lineage. The author added that the progenitor cells may also migrate from the periodontal tissues into the pulp chamber**. Hosoya et al. (2007)** indicated that pulp cells possess the ability to form mineralized matrices as bone- or cementum-like matrix.

Herein a distinct haematoxyphilic reversal line was seen between the dentin and the cementum-like tissue which indicating where resorption had stopped and repair had begun. This finding agrees with that of **Baron et al. (1980)** in studying bone remodeling, they reported a reversal line components somehow was responsible for recruiting osteoblasts to form new bone on the resorbed bone surface. It is possible that a similar mechanism is active in the initiation of cementum-like tissue formation on the resorbed surface of dentin. Also **Sasaki et al. (1990) Tanaka et al. (1990) Bosshardt and Schroeder (1994)** . reported that in the case of tooth resorption a variety of mononuclear cell types, cementoblast - like cells or macrophage - like cells or fibroblasts- like cells and a special class of mononuclear cells were seen lining the resorption surface prior to the onset of repair cementum- like tissue formation**.**

There was continuous increased in thickness of the cementum-like tissue on the wall of the pulp chamber which resulted in narrowing of the pulp space. Similar results were also obtained by **Saad (1997)** who reported reduction in the pulp size, abnormal odontoblastic pattern, , pulp degeneration, pulp stones formation, accelerated formation of secondary dentin, and hypercementosis were detected in retained deciduous teeth.

Herein the histological examination revealed that the cementum like- tissue in many specimens formed continuously until the pulp chamber was completely obliterated. The cementum- like tissue seemed to start in the incisal region and the central part of the pulp was the last part to be obliterated. These findings are in agreement with that of **Lieberman (1994)** who stated that cementum is a bone like connective tissue that grows in concentric bands around the roots of teeth. Unlike other dental tissues cementum is deposited continuously throughout the life of the tooth. Also **Randi (2001)** reported that the central part of the pulp with its large blood vessels appeared to be the last part of the pulp to mineralize. The presence of canals in the microradiographs and haematoxyline and eosin sections reflects the presence of blood vessels and shows that circulation persists, thus making it possible for minerals to be transported to the mineralization sites.

In the present work resorption of cementum - like tissue was observed in many specimens which may be active for some period and then stopped for cementum – like tissue deposition again creating reversal lines. This finding is in agree with that of **Konx & Aukhil (1988)** They demonstrated that the traditional concept of cementum as a static or terminal tissue will be undoubtedly replaced by another one which is more accurately, cementum is biologically active and responsive. However at this time the factors or stimuli which regulate cementum activity during development, maintenance, repair and regeneration of the periodontium remain ill defined and a common link between these separate but potentially related processes has not yet been established

In mammals, the deposition of cementum forms a pattern of light and dark bands, the pairings of which are annual. Each light and dark band is a pair of increments together forming an annulation's. Deposition of cementum throughout the year is not at a constant rate. The thicker increments are seen as being representative of periods of growth while the thinner ones are deposited during periods of rest or slower growth. **Grue & Jensen (1979)** and **Monks (1981**) introduced descriptive terms such as translucent and opaque are dependent on the type of light being used to examine cementum. **Lieberman (1994)** suggested that the dark lines are rest phases of mineralization during continual growth of the fibroblasts, leading to a change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands. The dark lines have been referred to as incremental lines and the cementum between each two lines as incremental bands. **Renz, and Radlanski (2006)** further complication at attempts to explain the occurrence of increments is the doubling phenomenon: the deposition of two sets of increments within one year. This phenomenon is widely reported and occurrences are documented in both humans and mammals alike. This phenomenon is not understood and has not been specifically studied.

In the present study cementum- like tissue is deposited in an irregular rhythm resulting in unevenly spaced incremental line. The means widths of the cementum- like tissue pale bands were ranging from 6.98 to 8.23 um. This finding is in agreement with that of **Charles et al. (1989)** The authors found that bands were reported in both cellular and acellular cementum. Cellular cementum increments are characterized by large poorly mineralized bands separated by darker thin highly mineralized bands. Bands in cellular cementum are uneven in size and distribution and vary in their widths. **Phillipson et al. (1990)** recorded translucent bands in cellular cementum result from periods of rapid cementum growth with variations in the size of cellular cementum bands which probably reflect changes in the rate of tooth eruption. **Lieberman and Meadow (1992)** stated that variation in cementum microstructure causes the optical phenomenon of these bands. They concluded that two primary factors that result in cementum bands are variation in relative mineralization and variation in collagen orientation **Lieberman (1993)** demonstrated that bands in cementum in mammals were different in width at different seasons; in some animals translucent bands laid down in the summer at a rate of 4.66um/ month and opaque bands at the winter at 2.55um/ month. However **Pooja et al. (2008)** reported that unlike most mammals; human have relatively small teeth and relatively long lifespan, making counting of the incremental lines difficult. However **Yamamoto et al. (2010) suggested** that cellular cementum generally consists of alternate lamellar layers, in which intensely and weakly stained lamellae each about 2.5 um thickness. The alternate lamellar pattern results from periodic changes of the intrinsic fiber arrangement and collagen fibrils rotate regularly in the same direction to form two alternating types of lamellae.

In the present study osteonectin was detected in dentine, cementum and pulp tissues which is in accordance with findings of **Reichert et al.** (**1992**) using immunohistochemical techniques, they reported that osteonectin is first seen after disruption of Hertwig's epithelium root sheath and with early primary cementum formation. Also **D'Errico et al. (1997)** reported that the osteocalcin, osteonectin, bone sialoprotein and Collagen type I are the common markers found in cementum and cementoblasts. And **Amar et al. (1997)** found that during cementogenesis, osteonectin was synthesized by cement-producing fibroblasts, cementoblasts, and cementocytes. The expression of osteonectin during dentinogenesis and cementogenesis is closely related to the development of the calcified tissue. Moreover **Papagerakis et al. (2002)** found that human osteocalcin and osteonectin mRNAs were detected by reverse transcription-polymerase chain reaction in primary cell cultures of dental pulp. In addition, osteocalcin, osteonectin, and dentin sialophospho-proteins were localized in forming human mineralized tissues using immuno-histochemistry.

Corresponding author
Zoba H. Ali
Oral Biology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
rawya_h2a@yahoo.com

Reference

1. Amar S, Chung K.M, Nam S.H, Karatzas S, Myokai F, Dyke T.E. Markers of bone and cementum formation accumulate in tissues regenerated in periodontal defect treated with expanded polytrafluoroethylene membranes. J Periodontal Research, 1997; 32(2):148-158.

2. Barberia LE, Suarez MC, Saavedra, D. Ectopic eruption of the maxillary first permanent molar: characteristics and occurrence in growing children. Angle Orthod., 2005; 75(4):610-5.

3. Baron R, Vignery A, Tran V. The significance of lacunae erosion without osteoclasts. Studies on reversal phase of the remodeling sequence. Metab Bone Dis Relat Res., 1980; 2: 35-40.

4. Bosshardt DD, Schroeder HE. How repair cementum becomes attached to the resorbed roots of human permanent teeth. Acta Anat., 1994; 150: 253-266.

5. Charles DK, Condon K, Cheverud JM, Buikstra, JE. "Estimating Age at Death from Growth Layer Groups in Cementum." IN Iscan and Kennedy (ed.) Age Markers in the Human Skeleton, Springfield: Charles C.T., 277316 ;1989.

6. D'Errico, J.A., MacNeil, R.L., Takata, T., Berry, J., Strayhorn, C. and Somerman, M.JExpression of bone associated markers by tooth root lining cells, *in situ* and *in vitro*. Bone., 1997; 20, 117–126

7. Frost H.M. Preparation of thin undecalcified bone sections by rapid manual method. Stain Techn.,1958; 33: 273- 277.

8. Grue H, Jensen B Review of the formation of incremental lines in tooth cementum of terrestrial mammals. Danish Review of Game Biology, 1979; 11:1-48.

9. Ho SP, Balooch M, Marshall SJ, Marshall GW. Local properties of a functionally graded interphase between cementum and dentin. J. Biomedical material Res. Part A. 2004; 1;70(3):480-9.

10. Hosoya A, Nakamura H, Ninomiya T, Hoshi K, Yoshiba K, Yoshiba N, Takahashi M, Sahara N ,and Ozawa H. Hard tissue formation in subcutaneously transplanted rat dental pulp. J Dent Res., 2007; 86(5):469-74.

11. Jones SJ. Cement in Osborn JW (ed). Dental Anatomy and Embryology Oxford Blackwell Scientific., 1981;193-204:286-294.

12. Knox B, Aukhil I. Ultrastructural study of experimental cementum regeneration in rats. J Periodont Res., 1988; 23: 60-67.

13. Kurol J, Thailander B. Infra-occlusion of primary molars with aplasia of permanent successors a longitudinal study. Angle Orthod., 2000; 54: 283-94.

14. Lieberman D.E. Mobility and Strain the Biology of Cementogenesis and its Application to the Evolution. Archaeological papers of the American Anthropological Association, 1993; 207-219.

15. Lieberman DE. The biological basis for seasonal increments in dental cementum and their application to archaeological research. J. Archaeol Sci., 1994; 21:525-539.

16. Lieberman, D. & Meadow, R.H. The biology of cementum increments (an archaeological perspective). Mammal Review, 1992; 22:57-77.

17. Lindqvist B. Extraction of the deciduous second molar in hypodontia. European Journal of Orthodontics,1980;2:173-181.

18. MacNeil RL, Somerman MJ. Molecular factors regulating development and regeneration of cementum. J Periodont Res., 1993 ;28: 550-559

19. Marimo C. Delayed exfoliation of primary teeth due to second pathosis case series study. Med. J. of zambia.2009; vol. 36 (2). 54-63.

20. Marks SC Jr, Walker DG. The hematogenous origin of steoclasts: experimental evidence from osteopetrotic (microphthalmic) mice treated with spleen cells from beige mouse donors. *AmJAnat.,* 1981;16:1-10.

21. Monks GG. Seasonality studies. Advances in Archaeological Method and Theory. 1981;4: 177-240.

22. Papagerakis P, Berdal A, Mesbah M, Malaval L, Nydegger J, Macdougall M. Investigation of osteocalcin, osteonectin, and dentin sialophosphoprotein in developing human teeth. Officinal J. Int. Bone and Mineral Society, 2002; 20: 377-385.

23. Phillipson S, Lindskog S, Flock A. Mineralization of and cementoblast attachment to original and reparative cementum. Scand. J. Dent Res., 1990; 98: 295-300.

24. Pooja A, Susmita S, Puja B. Incremental lines in root cementum of human teeth: An approach to their role in age estimation using polarizing microscopy. Indian J of Den. Res., 2008; 19 (4): 326-330

25. Randi F.K. Further Observations on Tertiary Dentin in Human Deciduous Teeth. Adv Dent Res., 2001;15:76-79.

26. Reichert T, Störkel S, Becker K, Fisher, L. The role of osteonectin in human tooth development: an immunohistological study. Calcif Tissue Int. 1992; 50(5):468-72.

27. Renz H, Radlanski J. Incremental lines in root cementum of human teeth--a reliable age marker? *Homo,* 2006*;* 57(1):29-50.

28. Robinson S, Chan Y. New teeth from old: treatment options for retained primary teeth . British Den. J. 2009; 207(7)315-320.

29. Saad AY. Regressive changes of the dental pulp complex in retained primary molars with congenitally missing successor teeth. J Clin Pediatr Dent., 1997; 22(1):63-7.

30. Sabri R. Management of over-retained mandibular deciduous second molars with and without permanent successors. World J. Ortho., 2008; 9: 209-219.

31. Sahara N, Okafuji N, Toyoki A, Ashizawa Y, Yagasaki H, Deguchi T, Suzuki K. A histological study of the exfoliation of human deciduous teeth. . J Dent Res., 1993; 72(3):634-40.

32. Sahara N, Okafuji N, ToyokiA, Suzuki I, Deguchi T, Suzuki K. Odontoclastic resorption at the pulpal surface of coronal dentin prior to shedding of human deciduous teeth. Arch Histol Cytol., 1992;55:273-285.

33. Sahara N, Ozawa H. Cementum-like tissue deposition on the resorbed enamel surface of human deciduous teeth prior to shedding. The Anatomical Record., 2004; 279:779–791.

34. Sasaki T, Watanabe C, Shimizu T, Debari K, Segawa K. Possible role of cementoblasts in the resorpent organ of human deciduous teeth during root resorption. J Periodont Res., 1990 ; 69:67-74.

35. Shapira Y, Kuftinec MN. Early diagnosis and interception of potential maxillary canine impaction. *J Am Dent Assoc*., 1998; 129:1450-4.

36. Sletten DW, Smith BH, Southard KA, Casko JS, Southard TE. Retained deciduous mandibular molars in adults: a radiographic study of long term changes. Am J Orthod Dentofacial Orthop., 2003;124:625-630

37. Suprabha BS, Pai SM. Ankylosis of primary molar along with congenitally missing first permanent molar. J of Indian Society and Pedodontics. 2006; 24 (5): 35-37.

38. Tanaka T, Morioka N, Ayasaka N, Iijima T, Kondo T. Endocytosis in odontoclasts and osteoclasts using microperoxidase as a tracer. J Dent Res., 1990; 69: 883-889.

39. Ten Cate AR. Development of the periodontium. In:Oral histology. Development, structure and function. 5th ed. Ten Cate AR, editor. St. Louis: Mosby, 1998b; 236-252.

40. Tenorio D, Cruchley A, Hughes FJ. Immunocytochemical investigation of the rat cementoblast phenotype. J Periodontal Res., 1993;28(6):411-19.

41. Yamamoto T, Li M, Liu Z, Guo Y, Hasegawa T, Masuki H, Suzuki R, Amizuka N. Histological review of the human cellular cementum with special reference to an alternating lamellar pattern. Odontology, 2010 :98(2):102-9.

42. Yasutaka Y, Masakazu O, Akemi A, Rie N, Haruhisa O. Histological study of deposited cementum in human deciduous teeth with pathological root resorption. Annals of anat. 2005; 185(4):335-41.

1/5/2012