ORIGINAL RESEARCH

Changes in Pulp and Roots of Deciduous Teeth during Different Stages of Physiologic Resorption: A Histologic Study

Prashanth Murthy[1], Nandlal Bhojraj[2], Usha Hegde[3]

Abstract

Background: Resorption of primary teeth and eruption of permanent teeth involves a complex series of changes. The cellular and histological changes occurring during the process of resorption vary stagewise. The knowledge of the changes occurring in the pulp of deciduous teeth would provide information about the resorptive process.

Aim: To evaluate the histologic changes of the pulp of deciduous teeth related to different stages of physiologic root resorption.

Study setting and design: To establish the cause and effect relationship, a contrived histologic study design was planned.

Materials and methods: A total of 60 extracted deciduous incisors, canines, and molars were included in the study. The remaining root length (RRL) was determined based on the standardized photographs. The teeth were then grouped into three based on the percentage of RRL. The teeth were subjected to decalcification with 5% nitric acid, following which histological processing was performed.

Statistical analysis: The present study being a qualitative study design, descriptively explains the histologic findings, and no statistical tests have been applied.

Results: During the initial stages of resorption, there were no histological alterations noted in the pulp, particularly in the cervical 3rd, with the cellular structure relatively maintained. As the resorption progresses, reversal lines were evident, indicating a process of repair occurring simultaneously during the process of resorption. With further advancement, the repair is overtaken by the resorption indicated by the presence of resorptive cells. Neovascularization and an increase in immune cells are also evident in advanced stages.

Conclusion: The pulp exhibits progressive changes as the resorption continues from stage I to stage III. The changes vary from a smaller number of immune cells and odontoclasts in stage I to increasing number of the same as resorption progresses.

Keywords: Decalcification, Deciduous teeth, Histological changes, Physiologic resorption, Remaining root length.

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Introduction

The histological findings in the tissues surrounding the deciduous tooth just before shedding have been studied and documented in the literature. These observations are enumerated as changes that happen during the eruption of permanent teeth.[1–3] Most of these studies are histological observations on sections taken from cadavers and timely sacrificed animal models. Few studies have been carried out on deciduous teeth after their exfoliation.[2] Some of the studies have been conducted on deciduous teeth, which were extracted just before their physiological exfoliation.[4–6] Certain studies describe the radiographic changes occurring during resorption in the primary teeth. Research carried out on rodents, felines, and canines has suggested that the process of resorption is not a single point event that would take place just before exfoliation; it is an elaborate phenomenon occurring much earlier and involving a larger cascade of biological events. The data available largely focuses on basic histological findings during and immediately following exfoliation.[4] The documentation of exfoliation through resorption, as it happens in various stages, is not clearly understood. Enumerating the cascade of the events that lead to the resorption process is a wide area of scope for scientific research. This encompasses the identification of the exact mechanism and sequential changes in all the involved tissues during the process of shedding by the process of "resorption." Hence this study was conducted to evaluate the histologic changes in the pulp of deciduous teeth related to different stages of physiologic root resorption.

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Conflict of interest: None

Materials and Methods

Ethical clearance was obtained from the Institutional Ethical Board for performing the study. Informed consent was obtained from the patients/parents prior to performing the extraction. A total of 87 deciduous teeth extracted for therapeutic reasons were collected for the purpose of the study. These teeth were indicated for extraction as a part of treatment procedures in interceptive orthodontics or traumatic injuries. The extracted teeth were then evaluated for the presence of internal resorption or fractures. A total of 60 teeth showing no radiographic evidence of internal resorption or no clinical evidence of fractures were included in the study.

Experimentation, Standardization of the Decalcification

The extracted teeth were stored in 10% formalin until further use. Each tooth sample was radiographed using radiovisiography to assess the internal anatomy of the pulp chamber and to also rule out any internal pathology like internal resorption. These images also served as the baseline for teeth prior to decalcification, as this process was repeated after decalcification to confirm the same. Hence the radiographic images were obtained using a custom-made standardized jig which was essential to reposition the tooth sample in a similar manner.

Standardized photographs of the teeth were then taken from all the aspects (mesial, distal, labial/buccal, and lingual/palatal) against a blue background. This was essential for the purposes of grouping the teeth. The teeth were grouped based on the RRL. The RRL is the distance between the trough of the cementoenamel junction (that is, the trough of the cervical line on each of the surfaces of the tooth) and the deepest point of resorption on that aspect of the root surface (Fig. 1).[5] The measurements were done on all the four surfaces (buccal, lingual, mesial, and distal) as the resorption is an irregular process, and the least RRL among the four measurements was considered.

Percentage RRL was determined from the following formula and the same was considered for grouping the deciduous teeth into stages of root resorption.[6]

$$\%\,\text{Remaining root length (\%RRL)} = \frac{\text{Remaining root length (RRL)} \times 100}{\text{Expected pre-resorption root length}}$$

(*Kramer and Ireland*)

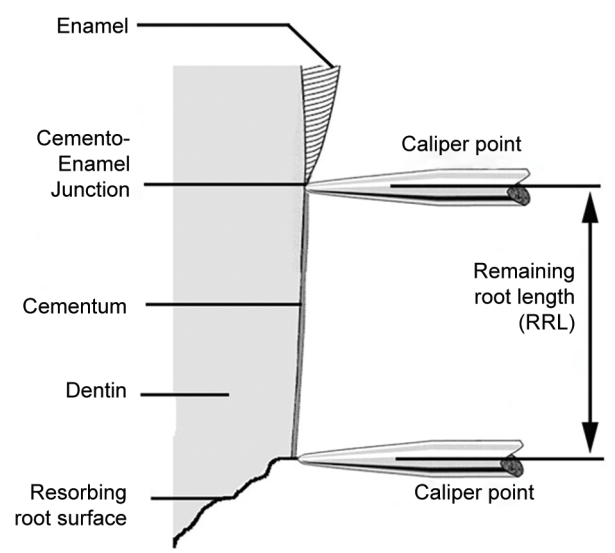


Fig. 1: Digital representation of measurement of RRL

The expected preresorption root length for primary teeth was obtained from Kramer and Ireland study.[5] The percentage RRL was used to group the deciduous teeth into stages of root resorption (Table 1).

Since the study was observational and qualitative, which aimed at reporting the histologic observations, saturation sampling was performed. In this method of sampling, the inclusion of samples in the group/subgroup is discontinued after a point beyond which no additional information can be gathered from the samples (Table 2).

The teeth were then subjected to a decalcification process. Prior to decalcification, predecal weight was determined for all the teeth. The teeth were then immersed in 15 mL of 5% nitric acid. Samples were weighed at intervals of 1, 2, 4, 6, 8, 10, 12, 24, 27, 30, 38, 40, and 66 hours. The time interval after which the weight remained constant for consecutive two readings was determined as the end point of decalcification. The end point was also tested with three other modalities, physical test, chemical test, and radiographic test. After the decalcification, the teeth were immersed in 15 mL of deionized water for 10 minutes to remove acid remnants. Samples were then stored in 10% formalin until further processing.

Tissue Processing, Staining, and Mounting

The samples were then dehydrated prior to paraffinization. Following these 4 µm thick cross-sections of the samples were obtained in the apical, middle, and cervical 3rd for stage I resorption, middle and cervical 3rd for stage II resorption, and cervical 3rd for stage III resorption. The sections were transferred to the water flotation bath and transferred onto albuminized glass slides. Slides were placed on the slide warmer for 10–15 minutes and deparaffinized using xylene. The sections were then stained by the hematoxylin and eosin method. Figure 2 describes the different sections made during each stage of resorption.

Results

Stage I of root resorption explains that the RRL was 67–100%. The following findings can be derived from the histological sections.

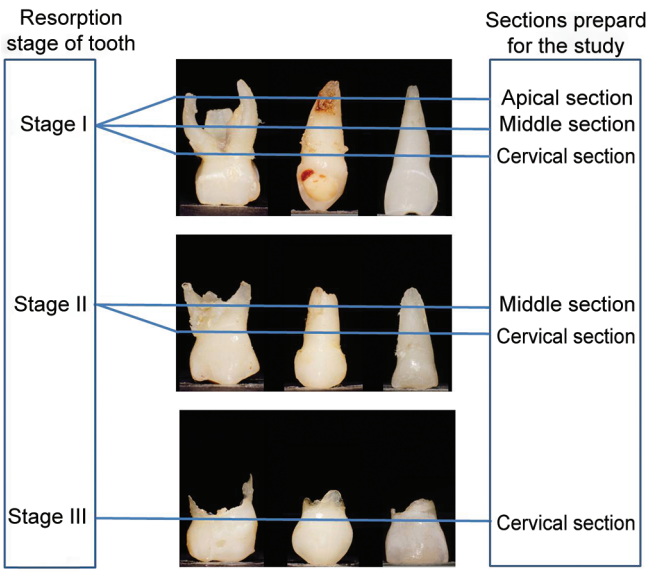


Fig. 2: Diagrammatic representation of the section made during each stage

Table 1: Criteria for grouping of teeth based on the RRL

	% RRL	*Stage of resorption*	*Clinical observation*
	67–100%	Stage I	Starting of resorption wherein resorption is seen only in the apical third
	34–66%	Stage II	Moderately resorbed root with resorbing front in the middle third of the root
	0–33%	Stage III	Severe resorption with resorption front in the cervical third of the root

Table 2: Distribution of samples across the stages

Groups	*Sample size (n)*
Stage I	20
Stage II	20
Stage III	20
Total number of samples studied (N)	**60**

60, Saturation sampling

Stage I: Cervical 3rd

The cross section of the root in the cervical 3rd at 10× magnification shows cementum, dentin, and pulp within the hard tissue. The continuous undisrupted thickness of cementum indicates a stage of normalcy unaffected by the resorption process with an essentially normal and intact pattern of dentinal tubules (Fig. 3A). The odontogenic zone surrounding the predentin contains the odontoblastic layer. This layer is well appreciated at 40× magnification by the presence of cells containing cuboidal nuclei stacked closely (Fig. 3B). The presence of crowded cells in this layer gives the pseudostratified appearance. The pulpal architecture, as seen in 40× magnification, appears normal with the presence of blood vessels and fibrillar intercellular substance which is known to support the cells and the vasculature. The pulpal architecture also shows the presence of a few capillary lumens characterized by the presence of endothelial cells lining the lumen walls. These capillaries appear thin walled. The similar findings can be appreciated at 100× magnification (Fig. 3C). A few occasional immune cells can be appreciated in the pulpal matrix.

Stage I: Middle 3rd

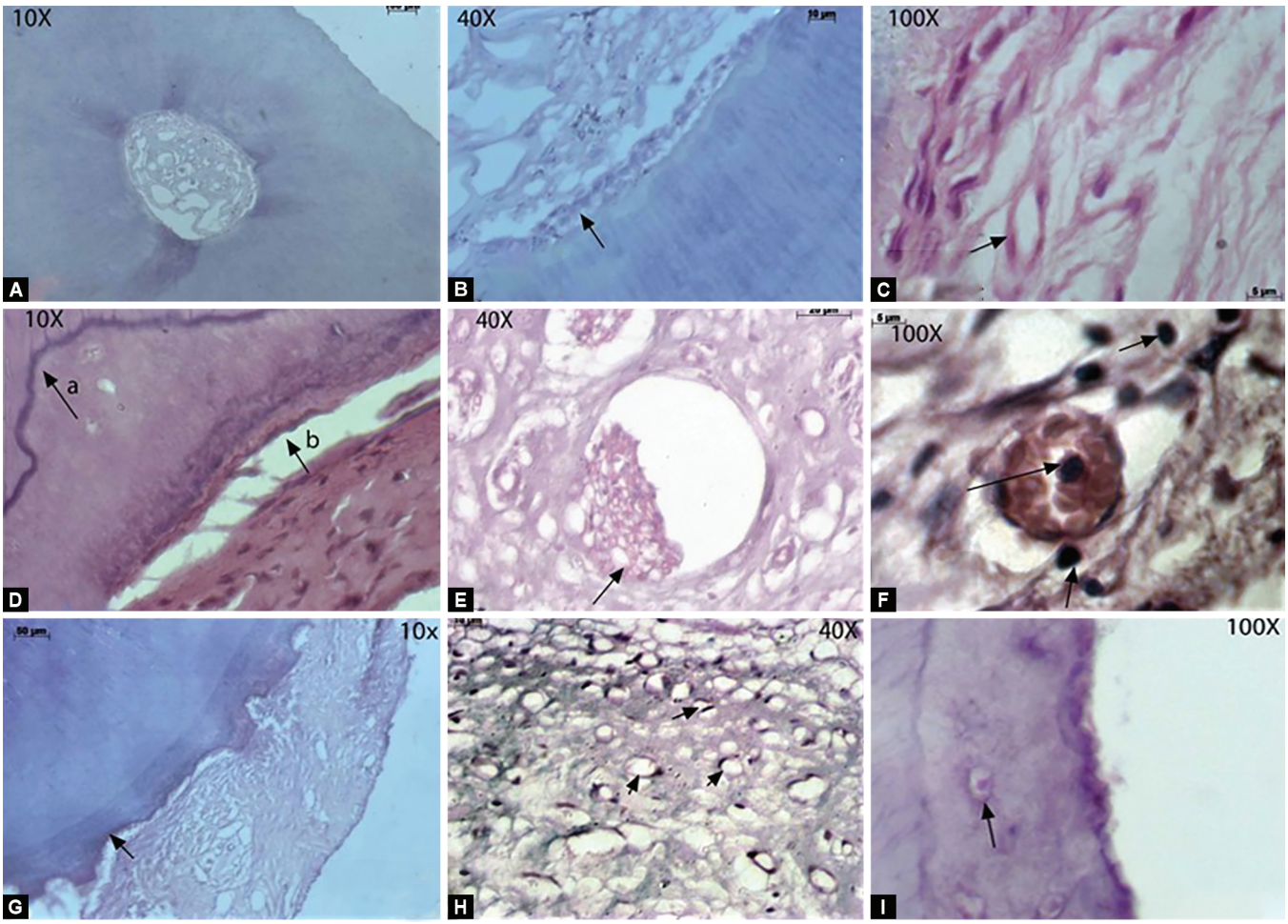
The sections in the middle 3rd at 10× magnification shows the presence of resorption bays or resorption lacunae (Fig. 3D). This section represents the reversal or repair stage of resorption; hence the predominantly acellular cementum-like tissue can be seen deposited in the resorption bays. As a result, the external surface appears essentially unaltered. The presence of reversal line can also be appreciated as irregular, accentuated, darkly stained lines formed between unresorbed cementum, and newly deposited acellular cementum demarcating the zone of active resorption and intermediate repair by deposition. In this stage, process of repair dominates the process of resorption, thereby maintaining the surface continuity (Fig. 3D). The sections at 40× magnification show the presence of capillaries with capillary lining containing endothelial cells. The endothelium of the blood vessels shows thickening with the margination of the cells toward the periphery of the lumen wall (Fig. 3E). At 100× magnification, it can be appreciated that there is an increase in the basophilic cells with a small round nucleus suggestive of an increase in the number of immune cells. The presence of basophilic immune cells can be well appreciated surrounding the capillaries with enlarged lumen, indicating perivascular infiltration of the immune cells (Fig. 3F).

Stage I: Apical 3rd

At 10× magnification, presence of caved, eroded areas can be identified along the external surface of the dental hard tissue indicating the presence of Howship's lacunae (Fig. 3G). The intertubular dentin appears more basophilic, indicating the formation of reparative or reactionary dentin to ward away the resorptive inflammation approaching the pulp. At 40× magnification, well-defined multinucleated giant cells representing the odontoclasts can be seen in these lacunae indicating the active process of resorption. These cells appear to follow a linear path along the external surface of the resorbing root. Multiple reversal lines can also be appreciated. The pulpal architecture shows a further increase in the number of tiny capillaries, indicating enhanced neovascularization (Fig. 3H). At 100× magnification, larger resorption bays are seen filled with cementum-like tissue. Cells are seen entrapped in the forming cementum (Fig. 3I). The 100× magnification shows pulp having predominantly many immune cells indicating the pulpal response to resorption.

Stage II: Cervical 3rd

Sections in the cervical 3rd with 10× magnification show areas of intact and undisrupted odontoblastic layer. The pulpal architecture shows an increase in the number of smaller blood vessels indicating neovascularization (Fig. 4A). Section at 40× magnification shows the presence of irregular cells representing cementoblasts in the Howship's resorption lacunae. These cells have numerous processes extending in resorbed surface and are in the process of deposition of cementum-like tissue (Fig. 4B). The older blood vessels show an increase in the red blood cells (RBCs). Basophilic cells representing the immune cells also populate in the blood vessels; however, the pulpal architecture appears normal. Sections at 100× magnification show presence of a large multinucleated basophilic cell, which is irregularly shaped measuring about 23.3 µm in size in the resorption lacuna (Fig. 4C). Presence of eosinophilic cytoplasm, multiple basophilic nuclei, and cytoplasmic vacuoles indicates that this cell is the odontoclast with appendages extending toward the resorbing tooth structure.



Figs 3A to I: Sections at stage I of physiologic root resorption of deciduous teeth at the cervical 3rd: (A) Section at 10× magnification shows intact cementum and dentinal tubules; (B) Section at 40× magnification shows intact odontoblastic layer; (C) Section at 100× magnification shows the presence of thin-walled capillaries. Sections at stage I of physiologic root resorption of deciduous teeth at the middle 3rd; (D) Section at 10× magnification shows reversal line and unaltered external surface of the root; (E) Section at 40× magnification shows margination of cells toward the periphery of lumen; (F) Section at 100× magnification shows presence of basophilic cells. Sections at stage I of physiologic root resorption of deciduous teeth at the apical 3rd; (G) Section at 10× magnification shows the presence of Howship's lacunae; (H) Section at 40× magnification shows neovascularization; (I) Section at 100× magnification shows the presence of cells entrapped in the cementum

Stage II: Middle 3rd

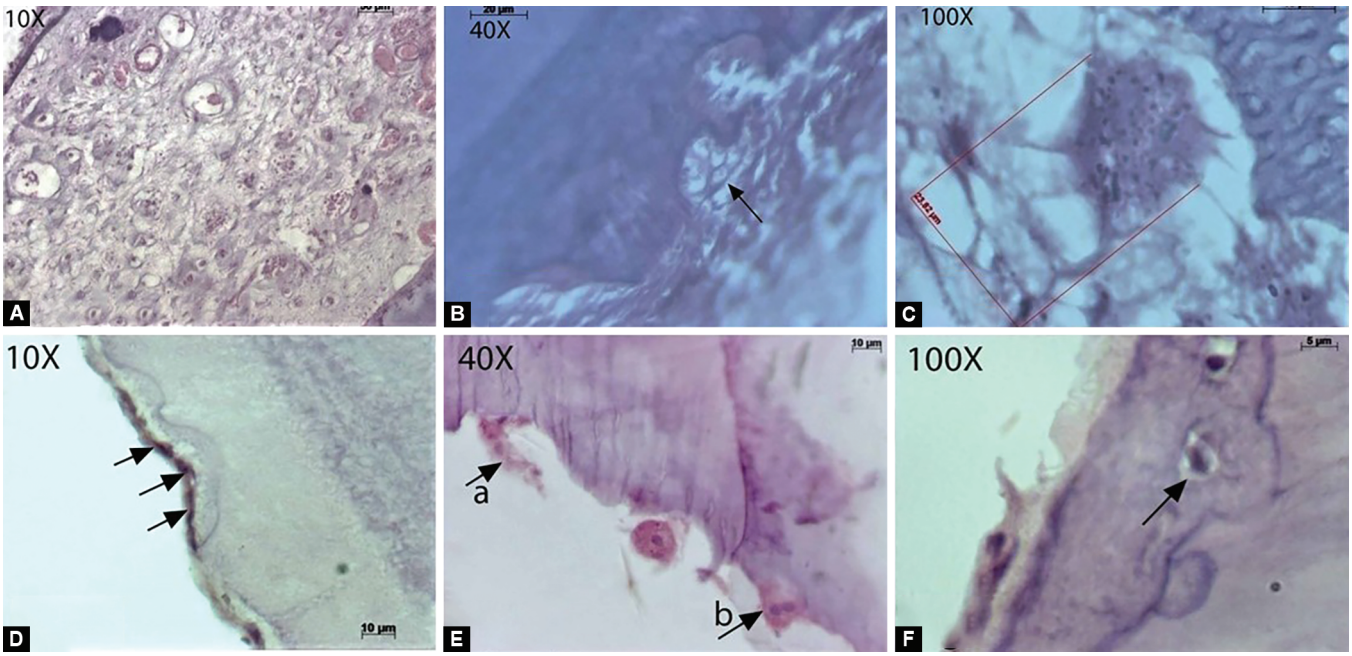
The section at 10× magnification shows the presence of numerous irregular wave-like patterns of Howship's lacunae indicating resorption being actively proceeding. The odontoclasts seem to follow a linear path of resorption, with the cells following each other rather than clustering around the area of resorption (Fig. 4D). At 40× magnification, the multinucleated giant odontoclasts can be seen in the resorption lacunae (Fig. 4E- a). This magnification also shows a reversal line with cementum-like deposition. Entrapment of odontoclasts can be seen in the deposited cementum-like tissue (Fig. 4E- b). At 100× magnification, the presence of cementocyte-like cells can be seen in the deposited cementum-like tissue (Fig. 4F).

Stage III: Cervical 3rd

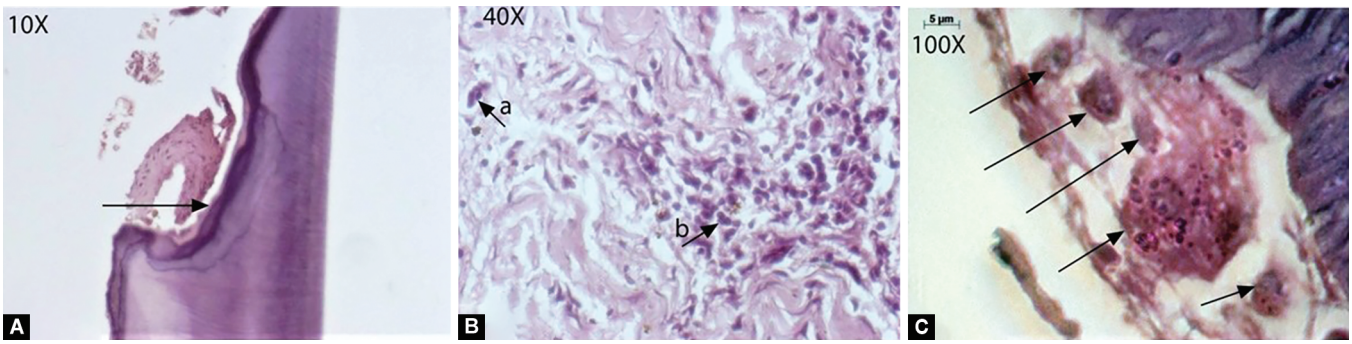
The section at 10× magnification shows the presence of a large denuded area of advanced resorption (Fig. 5A). Although the reversal lines are seen, the presence of deep resorption bays indicates the resorption has taken over the repair process and the cementum formation is no *longer* able to retain the external anatomy of the root structure. Around 40× magnifications show the presence of resorptive odontoclasts even in the pulpal tissue indicating the resorption from within (Fig. 5B- a). The normal architecture of the pulp also seems to have been lost with the absence of the gelatin matrix and the presence of only immune cells (Fig. 5B- b). The section at 100× magnification shows the presence of many multinucleated odontoclasts following a single path and bringing about resorption, indicating the resorption occurring in a linear manner (Fig. 5C). The odontoblastic layer now appears disrupted, and discontinuity can be appreciated at this magnification.

Discussion

The histological changes that happen during physiologic root resorption in deciduous teeth have been studied in various animal models. The topic, when extended to human studies, is limited to those that have been studied on cadavers. Isolated studies in clinical case scenarios have been published with an attempt to study the resorption process as an event rather than a continuous process, which leaves the scientific literature expectant with a study



Figs 4A to F: Sections at stage II of physiologic root resorption of deciduous teeth at the cervical 3rd: (A) Section at 10× magnification shows presence neovascularization; (B) Section at 40× magnification shows presence of irregular cells; (C) Section at 100× magnification shows large multinucleated basophilic cell. Sections at stage II of physiologic root resorption of deciduous teeth at the middle 3rd; (D) Section at 10× magnification shows the presence of resorptive cells following a linear path; (E- a) Section at 40× magnification shows presence of multinucleated giant cells in the resorptive lacunae; (E- b) entrapment of odontoblasts in the cementum; (F) Section at 100× magnification shows cementocyte-like cells in the cementum



Figs 5A to C: Sections at stage III of physiologic root resorption of deciduous teeth at the cervical 3rd: (A) Section at 10× magnification shows presence denuded areas of advanced resorption; (B- a) Section at 40× magnification shows the presence of resorptive odontoclasts in the pulpal tissue; (B- b) Shows presence of predominantly immune cells in pulp; (C) Section at 100× magnification shows multinucleated giant cells following linear path

that would comprehensively report the changes in the pulp of deciduous teeth at different levels of root resorption as progressive root resorption and the changes that happen in various stages of root resorption largely remains unexplored. This study is a part of the extensive study that has reported the pattern of resorption and ultrastructural changes in the hard tissues of the roots of resorbing deciduous teeth. This study describes the histological changes in the pulp and the roots of the resorbing deciduous teeth.[7,8]

Histological Findings of Stage I—67–100% of RRL—Cervical, Middle, and Apical 3rd Sections

Histologic findings of resorbing the tooth root at stage I (section in the cervical 3rd) of resorption showed the essential presence of intact pulpal architecture, dentinal pattern, and external anatomy of the tooth root. The pulp was characterized by the presence of uniformly distributed ground substance interspersed with spindle-shaped fibroblasts, which predominate the pulpal matrix. Similar findings were reported by Sasaki et al.[4]

Occasional immune cells are present in the ground matrix, indicating that the pulp in the cervical region of the tooth is nonresponsive to the resorptive process occurring in the apical 3rd of the tooth.

In the middle and apical 3rd of the root surface shows the presence of multiple small resorption bays. However, most of these denuded areas of resorption contain acellular cementum-like structure deposited, indicating that the process of resorption is not continuous but is intermittent with the phases of repair. The deeply stained, irregular line that demarcates between the resorption bays formed as a result of resorption and the cementum deposited as a result of the repair process is the reversal line. The

repair process generally tends to reestablish the former outline leading to what is described as Anatomic repair. Occasionally when the cementum deposition occurs at a faster pace, the resorptive cells get entrapped in the forming cementum, leading to the deposition of cellular cementum.[9,10] The pulpal tissue in the middle and apical 3rd, having advanced resorption showed the presence of multiple small blood capillaries with tiny lumen, indicating increased vascularity. The lumen walls appeared thickened as it was deeply stained, with the endothelial cells appearing compressed and flattened. Unlike the pulp in the cervical region exhibiting occasional immune cells, the pulp in the middle and apical 3rd of the root shows an increase in immune cell accumulation.[6]

Histological Findings of Stage II—34–66% of RRL—Cervical and Middle 3rd Sections

As the process of resorption continues and reaches stage II, apical 3rd of the root does not exist; hence at this stage, only two sections will be described, that is, the middle and cervical 3rd of the root. As the resorption progresses rapidly, a greater number of crater shaped, irregularly denuded areas of resorption can be appreciated on the external of the root. As the reparative mechanism continues more rapidly, the resorptive cells get entrapped in the newly formed cementum-like tissue giving a cellular cementum-like appearance.[11]

The pulp in the cervical and middle 3rd now responds to the process of ongoing resorption by showing an increase in the RBCs within the lumen of vessels. Neovascularization can also be appreciated. Similar findings were reported by Karayilmaz and Kirzioglu. They reported an increase in the blood flow to the resorbing deciduous tooth during the advanced stages of resorption by Laser Doppler flowmetry.[12]

Stage I showed the presence of relatively normal pulpal architecture in the cervical 3rd. However, stage II showed changes in the pulpal status. Along with increased vascularization, there was also an increase in the inflammatory cell infiltrate indicated by the increase in the number of basophilic immune cells. Similar findings were reported by Rajan et al.[13]

Histological Findings of Stage III—0–33% of RRL—Cervical 3rd Section

In this stage, only a single section of the cervical 3rd will be discussed as the process of resorption has progressed and involved the apical and middle 3rd. Externally larger areas of resorbing lacunae can be seen. As the process of resorption progresses rapidly, the process of repair lags and resorption continues at a faster pace. Hence areas of repair may not be evident uniformly in this stage. Occasional isolated areas of repair may be evident. The number of odontoclasts bringing about the resorption also appears to increase. The odontoclasts tend to follow each other in a linear path in the resorption bays rather than clustering around as a group. The odontoclasts can now be appreciated within the pulpal tissue as well causing disruption in the continuity of the odontoblastic layer. The gelatin matrix appears to have lost, and fibroblasts also cannot be appreciated within the pulpal tissue. These findings indicate that the pulpal tissue is affected the most in stage III of resorption.

Conclusion

Based on the findings observed in the study, the following conclusions can be drawn:

- During the initial stages of resorption, there were no essential histological alterations noted in the pulpal tissue, particularly in the cervical 3rd. Hence the cellular structure was relatively maintained in this stage of resorption, indicating that the pulpal tissue in this region is not affected by the process of resorption that has set in.
- The external root surface in the apical and middle 3rd showed the presence of resorption bays or lacunae, which were eventually filled with the acellular cementum, thereby maintaining the external surface outline. Hence it can be concluded that in the initial stages of resorption, the process of resorption and repair undergo simultaneously attempting to counteract the process.
- The abovementioned resorption bays and the eventual repair of the defects are characterized by the presence of deeply stained exaggerated reversal lines.
- In the initial stages of resorption, the pulpal changes are limited only to the pulp of apical 3rd.
- As the process of resorption advances, the odontoclasts increase in number.

Limitations of the Study

The stage I of the samples is the most difficult for collection. It requires a traumatic incidence or selected cases of early interceptive orthodontics for such sample collection. In traumatic incidences, there might be innate pulpal responses that may have been initiated in response to the event. Such a pulpal response may interfere with the findings of the normal resorptive process. However, the speed of such a response and the degree of its influence may require numerous time-based studies.

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