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Histological Alterations in the Odontoblastic Layer of Dental Pulp After Tooth Preparation

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ABSTRACT

Objective: The dental pulp is a highly specialized tissue because of its capability to regenerate. The pulp shows inflammation in response to insult, and if untreated, it leads to pulp infection and pulp death. This study aims to explore the histological alterations observed in the odontoblastic layer of the dental pulp following tooth preparation.

Methods: The research was conducted from May 2022 to May 2023 in Lahore, adhering to World Medical Association ethical guidelines. Participants aged 18-25 undergoing orthodontic extractions of healthy premolars were included, excluding those with dental issues. Teeth were divided into control and experimental groups, with the latter undergoing a 2mm preparation. Local anesthesia and high-speed handpieces with water spray were used for preparation. Teeth were extracted, fixed in formalin, processed, and stained for microscopic examination. Between-group comparisons were performed using Fisher's exact test (SPSS 20), with significance set at $p < 0.05$.

Results: In this study, 40 individuals (23 males, 17 females) undergoing premolar extraction for orthodontics were divided into control and experimental groups. All 20 control samples showed normal odontoblast layers and nuclei in the coronal regions, while the experimental group had consistent abnormalities and absence of nuclei, with a significant p-value (<0.001). In the radicular area, the control group was normal, but the experimental group showed abnormalities in 11 samples, with nuclei absent in 8, yielding a non-significant p-value (≤0.100).

Conclusion: The findings shed light on the impact of injury on healthy dental pulp. Histological analysis following a 2mm coronal tooth preparation revealed normal radicular pulp, with histological alterations observed in the coronal pulp. These observations contribute to the existing literature on pulp responses to coronal tooth preparation.

Keywords: Dental Pulp; Odontoblasts; Tooth Preparation, Prosthodontic; Histology; Bicuspid; Dentin

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Introduction

The intricate biology of the dental pulp orchestrates a delicate balance between protection and responsiveness to external stimuli. The dental pulp is a vital soft tissue located within the pulp chamber and root canals of teeth. The primary function of the pulp is the formation, induction, and nourishment of the developing tooth germ. In an adult tooth, it becomes reparative and protective. [1] The unique nature of dental pulp is due to its low compliance environment, the occurrence of more sensory nerves to stimulate, and the massive circulation of blood in microvasculature. [2]

Histologically, dental pulp embodies a dynamic microenvironment comprising diverse cell populations such as odontoblasts responsible for dentin formation, fibroblasts maintaining the extracellular matrix, and mesenchymal cells with regenerative potential. Immune cells, including macrophages and lymphocytes, contribute to immune surveillance. The extracellular matrix, rich in collagen fibers and ground substance, provides structural support and fills interstitial spaces. Vascular elements, including arterioles and capillaries, ensure nutrient and oxygen delivery, while sensory nerves transmit pain and sensory stimuli. This intricate network serves not only as a repository of sensory nerves but also as a vital contributor to dentin formation and maintenance. [3]

Throughout life, the dental pulp can undergo a spectrum of changes in response to various insults, ranging from microbial invasion in cases of caries to mechanical trauma due to injury. These insults provoke adaptive responses within the pulp tissue, aimed at preserving its integrity and functionality. [4] Caries, a prevalent dental pathology, poses a significant threat to the structural integrity of the tooth and the vitality of its pulp. In response to cariogenic bacteria and their byproducts, the pulp initiates a series of defensive mechanisms, including the recruitment of immune cells and the deposition of reparative dentin. While these processes aim to contain the infection and restore homeostasis, they can also lead to histological alterations within the odontoblastic layer, influencing its responsiveness to subsequent stimuli. [5]

Similarly, traumatic injuries to the tooth, whether due to accidents or iatrogenic factors, can elicit profound changes in the pulp tissue. The severity of the trauma dictates the extent of pulpal damage, ranging from reversible inflammation to irreversible necrosis. [6] Such insults not only disrupt the delicate architecture of the odontoblastic layer but also compromise its regenerative capacity, posing clinical challenges for subsequent dental interventions. In the realm of restorative dentistry, procedures like tooth preparation for fillings, crowns, or bridges represent pivotal moments where the dental pulp is directly impacted. The mechanical and thermal stimuli associated with these procedures can trigger a cascade of cellular responses within the pulp tissue, leading to histological alterations in the odontoblastic layer. [7] Studies have demonstrated various alterations in the odontoblastic layer following tooth preparation like odontoblast degeneration, disorganization of odontoblastic processes, and the formation of reparative dentin. Odontoblasts may exhibit morphological abnormalities, such as cytoplasmic vacuolization, nuclear pyknosis, and loss of cellular polarity. [8] These odontoblasts may form either reparative dentin or reactionary dentin, depending upon the extent of the injury. Pre-existing odontoblasts are responsible for the formation of reactionary dentin, whereas reparative dentin is laid down by newly differentiated odontoblasts derived from the undifferentiated mesenchymal cells. Additionally, inflammatory responses characterized by the infiltration of immune cells, such as macrophages and lymphocytes, are commonly observed in the vicinity of injured odontoblasts. The release of inflammatory mediators and cytokines further contributes to pulpal inflammation and tissue remodeling processes. This study aims to explore the histological alterations observed in the odontoblastic layer of the dental pulp following tooth preparation. By understanding the underlying mechanisms driving these changes, we can gain valuable insights into the dynamic interplay between dental interventions and pulp biology, paving the way for improved clinical practices and patient care.

Materials and Methods

This experimental research was conducted in the Histology laboratory within the Anatomy Department of the Postgraduate Medical Institute in Lahore, as well as at de 'Montmorency College of Dentistry, also in Lahore, spanning from May 2022 to May 2023. It adhered strictly to the ethical guidelines set forth by the World Medical Association (WMA - The World Medical Association-WMA International Code of Medical Ethics, n.d.). The study enrolled participants aged between 18 to 25, all of whom were scheduled for orthodontic extraction of permanent premolars. Each participant provided written consent, and the institutional review board approved the study (ERB/No-5001/15). Specifically selected were fully erupted premolars, in proper occlusion with opposing teeth, while those with caries, cracks, incomplete roots, periapical infections, or artificial crowns were excluded. The teeth were divided into two groups, control and experimental, each consisting of 20 teeth (total n=40). The sample size was determined based on a prior study by Ahmed et al., which reported histological alterations in 85% of prepared teeth versus 0% in controls. Using a two-sided Fisher's exact test with $\alpha = 0.05$ and 90% power, a minimum of 8 specimens per group was

required; 20 per group were enrolled to account for potential processing failures and to improve precision of effect estimates.

The control group remained untouched, while the experimental group underwent tooth preparation up to 2mm. The processing of the specimens was overseen by a clinical supervisor.

Tooth Preparation Procedure

The dental procedure began with the administration of local anesthesia (lignocaine-5mg/kg body weight) using a fine needle to numb the premolar designated for orthodontic preparation and extraction. A specialized diamond-tipped bur (No. 021 by ISO standards) was employed alongside an NSK high-speed handpiece (with speeds of up to 400,000 rpm and air pressure set at 2.2 bar), coupled with a water spray, to initiate the process by breaking the inter-proximal contact. Subsequently, 2.0 mm guiding grooves were meticulously crafted for axial reduction using a diamond-depth cutting bur (ISO No. 834. FG). [9] These grooves were then connected to achieve complete axial preparation using a standard diamond grit, via a tapering fissure bur (ISO No. 846R.FG). A parallel procedure was followed on the occlusal surface, where similar guiding grooves were etched and subsequently connected to accomplish occlusal reduction, employing the same bur. Following completion of the preparation, teeth were extracted immediately within 5 mins of the preparation procedure. This interval was kept consistent across all experimental specimens to standardize the duration of pulp exposure to iatrogenic stimulus.

Tooth Extraction Procedure

In each cohort, the dental team conducted a tooth removal procedure. They carefully manipulated the gum tissue using a periosteal elevator, ensuring a clean separation on both the outer and inner sides of the tooth. Employing universal forceps, they applied controlled pressure to extract the tooth intact, without any breakage. Following extraction, the socket underwent thorough irrigation with normal saline, followed by gentle digital compression to promote clotting. Sterile gauze soaked in saline was then delicately positioned within the socket, and the patient was instructed to apply gentle pressure by biting down. Comprehensive post-extraction guidance was provided before the patient was discharged from care.

Tissue Processing

Following tooth extraction, the teeth underwent immediate immersion in a solution of 10% formalin for 72 hours to ensure complete fixation of the dental pulp. After fixation, specimens were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) solution at pH 7.4 for approximately 6–8 weeks, with the decalcifying solution changed every week. Completion of decalcification was confirmed by radiographic examination. After decalcification, meticulous cleaning ensued, followed by a gradual dehydration process utilizing escalating concentrations of alcohol. Ultimately, the specimen was encased in paraffin wax. Sections of both coronal and radicular pulp, each measuring 4µm in thickness, were longitudinally sliced. Employing Haematoxylin-Eosin (HE) staining, the specimen was prepared for observation under a light microscope (Nikon C1 eclipse) at magnifications of 100x, 200x, and 400x, facilitating the examination of histological attributes within the dental pulp. [10]

Histological Assessment Criteria

Slides were examined under a light microscope at 100×, 200×, and 400× magnification. The odontoblast layer was classified as "normal" when a continuous, single-layered, columnar arrangement with well-defined cell boundaries was observed, and as "abnormal" when one or more of the following features were present: loss of cellular polarity, disruption or discontinuity of the columnar layer, cytoplasmic vacuolization, or cell detachment from the predentin surface. Odontoblast nuclei were classified as "present" when clearly identifiable basophilic nuclei were visible within the cell bodies, and "absent" when nuclei could not be identified despite intact or partially intact cell outlines. All histological slides were independently assessed by two observers (SS and AC) who were blinded to group allocation. In cases of disagreement, a consensus was reached by joint review.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Odontoblast layer status (normal/abnormal) and nuclei presence (present/absent) were treated as categorical variables and summarized as frequencies and percentages. Between-group comparisons (control vs experimental) were

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performed using Fisher's exact test (two-sided), which was selected because several expected cell frequencies were below 5. A significance level of $p < 0.05$ was adopted. P-values below 0.001 are reported as $p < 0.001$.

Results

In this investigation, a cohort of 40 individuals, comprising 23 males and 17 females, necessitated the extraction of their premolar teeth for orthodontic purposes. All participants were randomly divided into two groups, the control group and the experimental group.

Regarding the odontoblast cell layer, observations revealed normalcy in the coronal regions across all 20 samples in the control group, while abnormalities were consistently noted in the experimental group's coronal regions. Fisher's exact test yielded a highly significant result ($p < 0.001$), as depicted in Table 1.

Examining the radicular area, the control group exhibited normal odontoblast cell layers across all 20 specimens. In the experimental group, 9 specimens displayed normalcy in this area, while abnormalities were detected in 11 specimens. Fisher's exact test did not yield a statistically significant result ($p = 0.241$; Table 1).

Nuclei of odontoblasts

In the control group, the coronal area of all 20 specimens exhibited the presence of odontoblast nuclei, whereas in the experimental group, these nuclei were notably absent in the same area across all specimens. This discrepancy yielded a statistically significant result with a p-value below 0.001 (Table 2). Moving to the radicular region, the control group displayed odontoblast cell nuclei in all 20 specimens, while in the experimental group, they were found in 12 out of 20 specimens.

However, in eight specimens of the experimental group, these nuclei were absent in the radicular area. Fisher's exact test yielded a non-significant result ($p = 0.100$; Table 2).

Table 1: Odontoblastic cell layers in different groups

	Groups	Normal N (%)	Abnormal N (%)	p-value
Coronal pulp	Experimental group	0 (0)	20 (100)	<0.001*
	Control group	20 (100)	0 (0)	
Radicular pulp	Experimental group	9 (45)	11 (55)	0.241
	Control group	20 (100)	0 (0)	

† Comparisons between control and experimental groups were performed using Fisher's exact test (two-sided). * Indicates statistical significance at $p < 0.05$. p-values below 0.001 are reported as $p < 0.001$. N = number of specimens per group (n = 20 per group).

Table 2: The Number of Odontoblastic Nuclei in different groups

	Groups	Present N (%)	Absent N (%)	p-value
Coronal pulp	Experimental group	0 (0)	20 (100)	<0.001*
	Control group	20 (100)	0 (0)	
Radicular pulp	Experimental group	12 (60)	8 (40)	0.100
	Control group	20 (100)	0 (0)	

† Comparisons between control and experimental groups were performed using Fisher's exact test (two-sided). * Indicates statistical significance at $p < 0.05$. p-values below 0.001 are reported as $p < 0.001$. N = number of specimens per group (n = 20 per group).

Discussion

In the present study, the researchers examined the morphological changes occurring in the odontoblastic layer of the dental pulp tissue, comparing the coronal and radicular regions between control and experimental groups following a 2mm depth preparation. This iatrogenic intervention eventually led to clinical symptoms manifesting over time. The control group slides displayed normal dental pulp morphology, highlighting each of the four distinct zones along

with their respective cells. A continuous layer of columnar odontoblastic cells with prominent nuclei was evident, characteristic of healthy dental pulp. Conversely, the experimental group showed significant alterations in the odontoblastic cell layer, marked by the presence of vacuolization and the absence of nuclei in the odontoblastic cells. The results of the current study are important because it adds information to the current literature.

The morphological changes observed in this study are consistent with the findings from a previous investigation, which reported abrupt degenerative alterations in dental pulp following various tooth preparation procedures. [11] However, the earlier study did not provide any explanations for the histological changes observed in the odontoblastic layer. Given that odontoblastic cells possess the potential to induce a formative response in the underlying undifferentiated ectomesenchymal cells, this ability could account for the regenerative capacity of the pulp tissue. [12] Despite this, the reason behind the absence or reduced number of odontogenic cells in the experimental group remains unclear. Further research is needed to elucidate the factors contributing to this phenomenon and to better understand the mechanisms underlying the histological changes in the odontoblastic layer observed after dental procedures.

A comprehensive study was previously conducted to evaluate the immediate changes occurring in the pulp-dentin complex resulting from crown preparation, specifically examining the correlation between the thickness of the remaining dentin and the preparation technique used, whether with or without water spray cooling. The most severe changes were observed following deep preparation without the use of water-cooling. In the group that received water spray as a coolant, the odontoblastic cells were still affected, and there were notable vascular reactions in the pulp core. [13] The study claimed that 9% of people who underwent crown preparations would develop pulpal diseases. Additionally, another study revealed that histological changes in the pulp and dentin occurred following complete crown preparation, even when an adequate preparation technique was employed. [14] These findings are in agreement with the results of the current study. Further research is required to investigate the cause of this reactionary response from pulpal cells.

Research conducted by Ahmed et al. aimed to determine the immediate changes under standardized tooth preparation procedures that could lead to pulp necrosis. Their results indicated that acute inflammatory infiltration and necrosis were absent in all specimens from the experimental group. [15] However, they observed vacuolated odontoblasts and the absence of nuclei. This suggests that while acute inflammatory responses were not evident, significant morphological changes still occurred in the odontoblastic layer. These findings underscore the importance of preparation techniques and the potential for pulp damage even under controlled conditions.

In the present study, none of the dental pulp specimens exhibited signs of irritation or evidence of severe heat injury. This finding suggests that the crown preparation technique, when limited to a depth of 2 mm, falls within a safe range, thereby avoiding both reversible and irreversible damage to the pulp. The study focused specifically on the immediate changes occurring in the dental pulp post-preparation. It is important to note, however, that while immediate alterations were observed, the possibility of acute inflammatory infiltration emerging within 24 hours cannot be entirely ruled out, although it is unlikely to develop immediately following the crown preparation. This implies that while the immediate safety of the procedure has been confirmed, further observations are necessary to understand the potential delayed inflammatory responses that might arise within a day after the crown preparation. The study reinforces the notion that careful technique and controlled depth in dental procedures are critical to preventing long-term damage to the dental pulp.

This study has a few limitations that should be acknowledged. Firstly, the specimens used were exclusively healthy premolars, which were collected from patients undergoing orthodontic extractions. This narrow focus limits the generalizability of the findings to a broader range of dental conditions. Future research should include teeth affected by caries, periodontitis, trauma, and other pathological conditions. Investigating a more diverse array of dental health scenarios will provide a clearer and more comprehensive understanding of how pulp cells respond to various forms of trauma and injury. Such studies would be instrumental in revealing the different regenerative capacities and defensive mechanisms of dental pulp under varied clinical circumstances. This expanded scope of research could also inform more effective and tailored approaches to dental treatments, ensuring better outcomes for patients with diverse dental health backgrounds.

Conclusion

In conclusion, the findings shed light on the impact of injury on healthy dental pulp. Histological analysis following a 2mm coronal tooth preparation revealed normal radicular pulp, with histological alterations observed in the coronal pulp. These observations contribute to the existing literature on pulp responses to coronal tooth preparation.

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Author Contribution

SS conceived the study, performed the tooth preparations and extractions, conducted histological analysis, and drafted the manuscript. AC supervised the histological processing, reviewed the data, and critically revised the manuscript. Both authors approved the final version.

Data Availability Statement

All relevant data are within the manuscript. Additional data supporting this study are available from the corresponding author upon reasonable request.

Ethical Considerations

This study was approved by the Ethics Review Board of the Postgraduate Medical Institute, Lahore (ERB/No-5001/15). All participants provided written informed consent prior to enrolment. The study was conducted in accordance with the ethical guidelines of the World Medical Association Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflicts of interest.

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