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Morphological Study of Teeth and Periodontal Structures after Extraction in Syrian Hamster

Ahmad Mohammad Khattab* **and Hikmat Yakub**

Department of Dentistry, Tishreen University, Lattakia City, Syria

Abstract

Background: Syrian Hamsters are considered a prime animal model to conduct histological examination in the oral cavity, having an exceptional ability to constantly regenerate their incisors. Syrian hamsters were also commonly used in experimentally induced cancerous growth studies.

Methods: 28 Syrian Hamsters have been distributed into six main groups in addition to control group. The animals of the control group have been sacrificed to get the normal morphological structure of the teeth and the periodontal structures. While the control group of animals have been sacrificed gradually, in order to study the stages of the restorative process, after the days that followed the extraction process.

Results: This research illustrates the morphology of the teeth in Syrian hamsters, mainly describing the depression area in the medial part of the root. It confirmed the presence of periodontal ligament tissue around these teeth. This Study confirmed in detail and with microscopic images the phases of the restorative process from the beginning of forming and organizing the thrombus till its Differentiation into the different dental and periodontal tissues.

Conclusions: The most significant achievement of this research its ability to detect focal regions of active cells which are able to differentiate into animal, dentin, and other different tissues.

Keywords: Experimental Pathology • Teeth extraction • Healing after Extraction • Syrian Hamster

Introduction

Studying the regenerative abilities of cells and tissues is considered a matter of high importance, Researchers frequently tend to use animal models where the studied specimens are sacrificed to closely monitor these processes exploring still-unknown terrain in the etiology and pathology in practice. Syrian hamsters were used in numerous experimental applications in malignant growth studies in the maxillary sinus using carcinogenic materials [1]. Hamsters were also used in oral leukoplakia studies and other premalignant lesions [2] and their relationship with the integrity of periodontal ligaments. Hamsters are a prime animal model to study experimentally induced cancers in the maxillary sinus in Syrian hamsters [3].

Aims and scopes

Reviewing the literature revealed a substantial lack of studies related to clarifying the histological of teeth and periodontal tissues in Syrian hamsters, there were also no studies regarding the significance of the blood clot after extraction in hamsters and its effect on the regenerative process as a whole. Based on the lack of sufficient information and experimental studies in this field, the scopes of this study arise as the following:

Studying the histological structure of the periodontal tissues in Syrian hamsters to determine whether a periodontal ligament exists or not.

Studying the regenerative properties in the alveolar socket in hamsters after extraction.

***Address for Correspondence:** Ahmad Mohammad Khattab, Department of Dentistry, Tishreen University, Lattakia City, Syria, Tel: +963- 947109206; E-mail: ahmadkattab777@gmail.com*

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Materials and Methods

28 three-month-old Syrian hamster specimens were divided into seven even groups with each containing four hamsters:

First group (Control group): Four hamsters inside this group were sacrificed in order to examine the histological structures of teeth and periodontal tissue to obtain a normal histological basis of the Syrian hamster after it has proven to be a model animal for this experiment.

First group (Control group): Includes four models, biopsies were taken one day after extracting the lower incisor to perform histological examination to monitor cell activity and differentiation in the extraction area one day after excision.

Second group: Includes four models, biopsies were taken two days after extracting the lower incisor to perform histological examination to monitor cell activity and differentiation in the extraction area two days after excision. This was replicated in the third, fourth, fifth, and sixth groups each including four hamsters that were sacrificed for examination, three, four, five, and six days after extraction respectively.

Biopsy method

Anesthesia was administered to hamsters with a cotton swab soaked with chloroform, and then the lower incisors were extracted using root forceps. Extracting teeth was notably accompanied by fracturing the roots in most cases. Hamster sacrifice mentioned earlier was carried out in a merciful manner with chloroform, biopsy was performed after five minutes from the desired region using a scalpel and bone rongeur. Biopsies were taken at 5mm thickness from both sides of the extraction, and at length of the mandible.

Biopsies taken go through these phases in order to conduct the histological examination

After surgical excision of the biopsy, it is directly placed in 10% formol for 24 hours, fixating the sample and preventing self-decomposition of the tissue caused by endoenzymes, its bacteriostatic qualities, and facilitating tissue staining. Samples are also permeated in nitric acid to facilitate bone resorption in the specimens. Specimens go through several clearing phases to increase translucency using alcohol and xylol, Then placed in molten paraffin wax baths

embedded specimens are left to cool and harden until it reaches a state ready for sectioning. A Microtome is used to create unified slices 7 microns in thickness, which are then placed on glass slides then the paraffin wax is removed. During the staining and fixation stages, specimens are stained with hematoxilin-iosine then coated with Canada Balsam then covered with another glass slide.

Results and Discussion

Tooth structure in the control group

Histological examination revealed high columnar enamel epithelium and their regular basal nuclei representing the inner enamel epithelium (Figure 1). These cells form a thin enamel layer while ameloblasts are located further peripherally (Away from center) and their nuclei are arranged peripherally too then the enamel is secreted from the apex of these cells while they remain encased with the dental follicle and surrounding bone, while the dentin arises internally (Figure 2). While examining a fully calcified section of the tooth, enamel had completely dissolved due to the effect of nitric acid while dentin was kept intact and comprised of dentinal tubules and intertubular dentin. The morphology of the tooth appeared to be comprised of a crown with dentinal horns and a cervical region where odontoblasts were arranged on the pulpal space surface (Figure 3). Periodontal ligament was clearly observed in the examined sections, it comprised juvenile dentinal tissue rich with Fibroblasts and some colloidal fiber tissue that is attached to the bone, while the other side is connected with a calcified material attached to the dentin. epithelial remnants were also observed in spherical-shaped groups with mast cells (Epithelial rests of Malassez) Multi sized vasculation was observed. As a newly formed bone structure (Figure 4). Periodontal ligament was observed during various phases of tooth bud growth, comprised of rich cells attached directly to the bone. This was accompanied by a thin dentin-like calcified layer, whereas in the late stages of periodontal formation, colloidal fiber tissues were observed to differentiate and lengthening fibroblast cells. Nuclei became more oval and elongated on the other side of the ligament, osteoblasts begin to differentiate. Osteoblast, large cells with extremely pigmented nuclei located peripherally on the forming alveolar bone. Newly formed bone was observed near

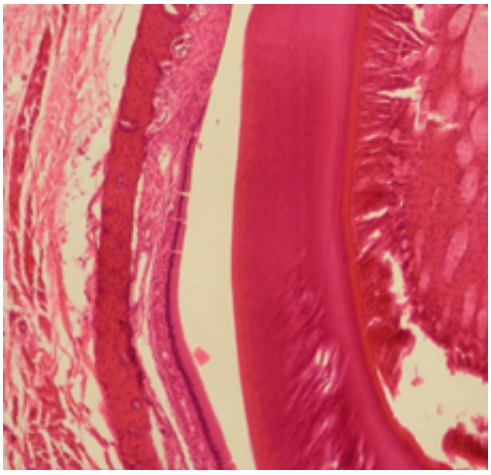

Figure 1. Inner nuclei epithelium.

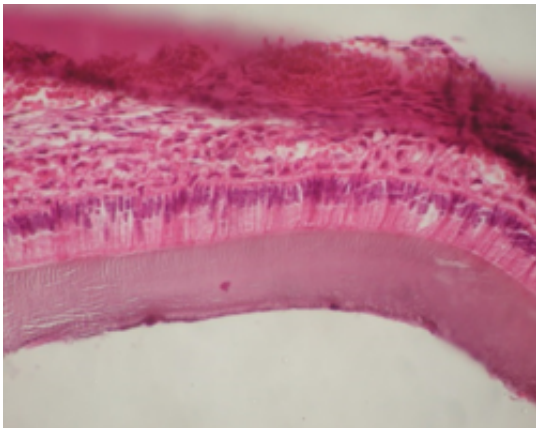

Figure 2. Ameloblasts and dentin.

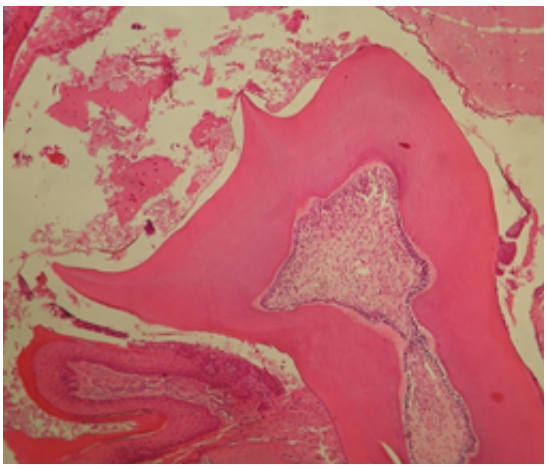

Figure 3. Odontoblasts arranged on the roof of the pulp periodontal tissue in the control group.

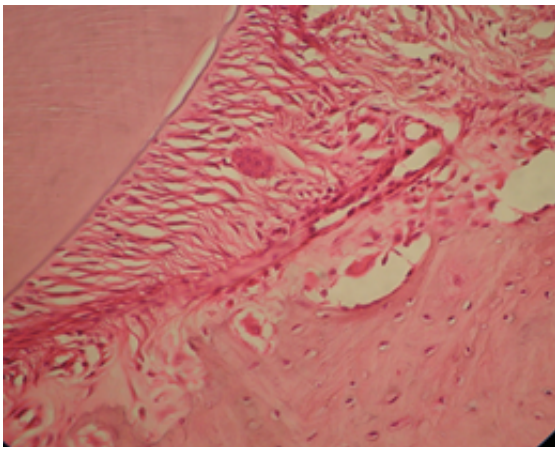

Figure 4. Juvenile periodontal ligaments and traces of the rests of malassez.

the wide ligamental space. Inside the bone tissue, smaller cells were observed with clear dark pigmented nuclei, those are Osteocytes. Some Mesanchyme cells differentiate into bigger-sized cells, spherical or cubical in shape, highly pigmented with hematoxylin, and probably are the cells that will form cementum (Figure 5). At the top of the alveolar process, active osteoblasts had formed high quantities of alveolar bone. Giant cells with several nuclei were observed in this region, their function was to refine excess bone formation, located in self-created bone cavities these are osteoclasts (Figure 6). Gingival tissue was composed of Stratified keratinized squamous epithelium and basal cells on the basal lamina. This epithelium lays on the side of the tooth forming an epithelial attachment. The gingival dermis consists of highly vasculated soft connective tissue and some fibroblasts the gingival dermis overlaps with the periodontal ligament connective tissue, and fibers from this tissue attach to the alveolar process (Figures 7 and Figure 8). The medial region of the root showcased a unique structure, there was considerable narrowing in tooth structures causing a narrow pulpal space to a high degree in some cases. Dentin thickness was also decreased in said area, meanwhile, dentin and pulp structures returned to normal thickness as they approached the apical region (Figure 9). A notable observation was made in the apical region, increased pulpal space and an increase in dentin thickness, newly formed enamel surrounded with ameloblasts a dental cyst. It appears as if the apex was attempting to recreate the crown structure (Figure 10). Histological changes after incisor extraction in hamsters. Day one: Laceration was observed in the extraction region, ligament tissue remnants, broken dentin and bone shards, and vast focal hemorrhages. White cells started infiltrating the region (Figures 11 and 12). Day two: increased white blood cells count and connective tissue originated cells like monocytes and fibroblasts, which indicates the start of clot formation (Figures 13 and 14). Quick formation of the lining of the socket on day two was observed. Epithelial spurs forming was observed in several regions of the socket, alongside focal hemorrhages and clotting. This epithelium consisted of one epithelial cell layer, or consecutive stratified layers, the most important of which are basal cells layering the basal lamina separating the former from the neighboring connective tissue. Some areas of the epithelium were more

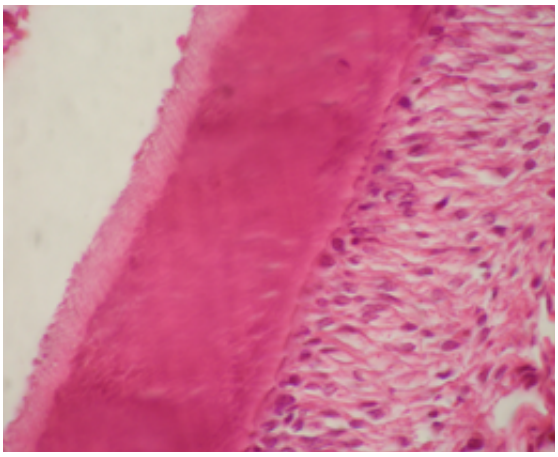

Figure 5. Ligament and cementoblast differentiation.

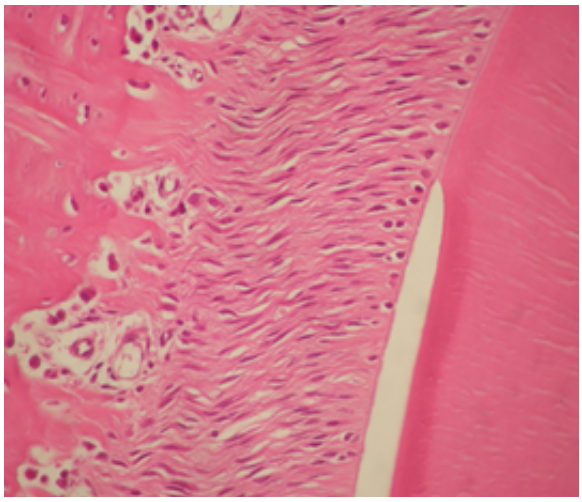

Figure 6. Ligament tissue development, osteoclasts present.

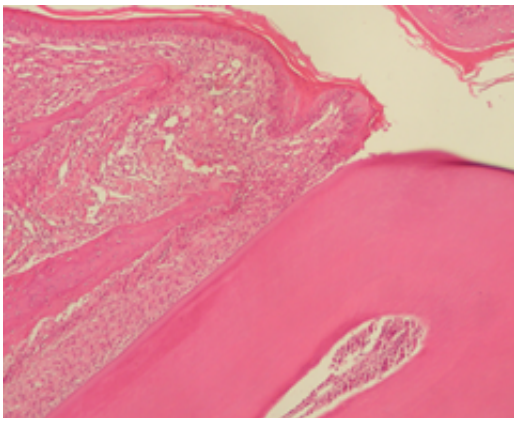

Figure 7. Junctional epithelium in gingiva.

differentiated, showing Malbiki cells and their keratenized layers (Figure 15). Day three: Clot organization on day three is described in the completion of epithelium formation. Connective tissue organization and differentiation offset of juvenile cartilaginous and bone tissue, with remnants of focal blood clots (Figures 16-19). Day Four: Fully formed clot, red blood cells dissipate and fibroblasts multiplied, bone trabeculae recently formed newly formed vacsculation was observed. Active ameloblasts were observed at the bottom of the alveolar socket, forming the shape of a crown or a cusp the epithelium consisted of high columnar cells with well pigmented nuclei. large numbers of congested blood vessels around it, which indicates an early activity of ameloblasts (Figure 20) Remnants of the extracted tooth due to the depressed region in the root led to continuous activity of ameloblasts and odontoblasts to create new enamel and dentin (Figure 21). Day Five:Quick development of the tooth bud was observed where the crown and

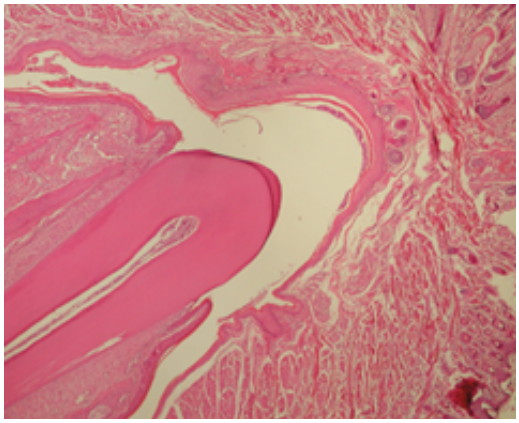

Figure 8. Junctional epithelium in gingiva (full scope).

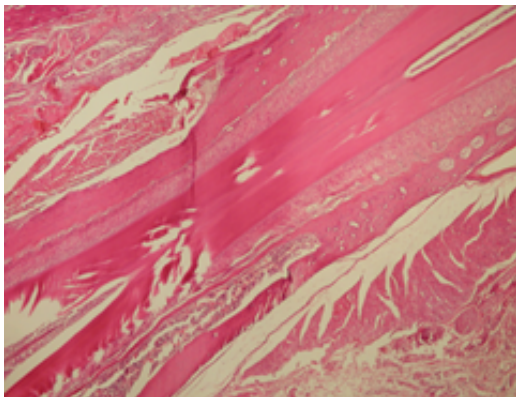

Figure 9. Tissue depression in the middle region of the root.

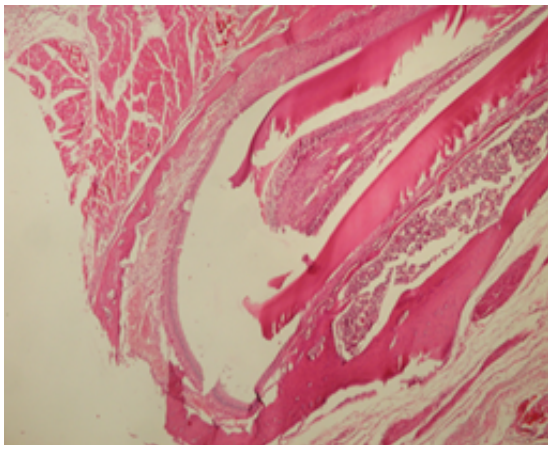

Figure 10. Apical region showing all types of dental tissues.

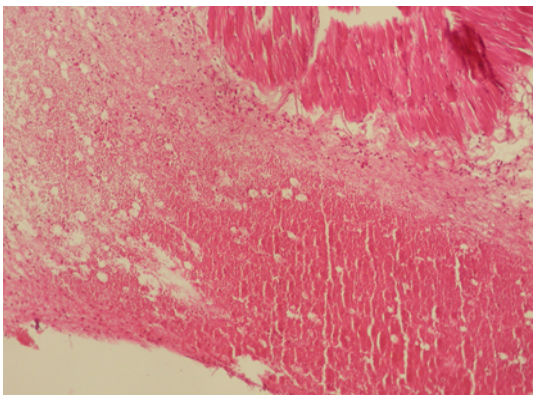

Figure 11. Vast focal hemorrhages in the extraction area.

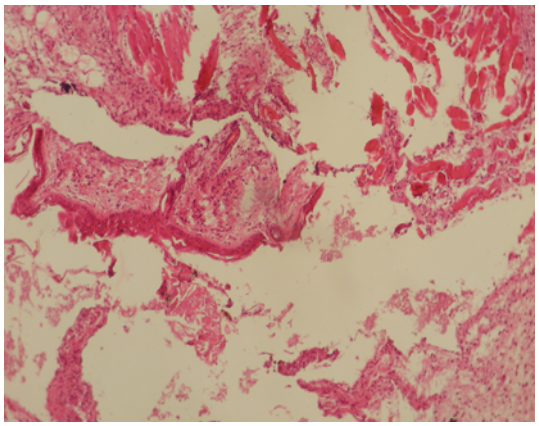

Figure 12. Tissue laceration area.

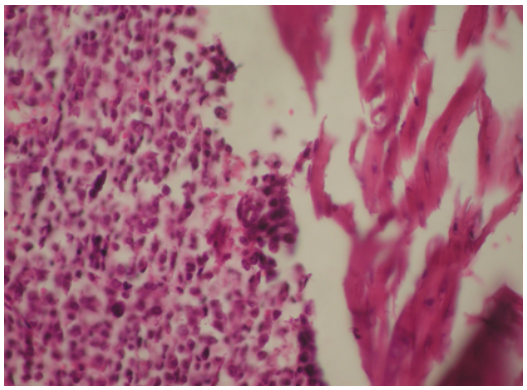

Figure 13. White blood cells infiltration.

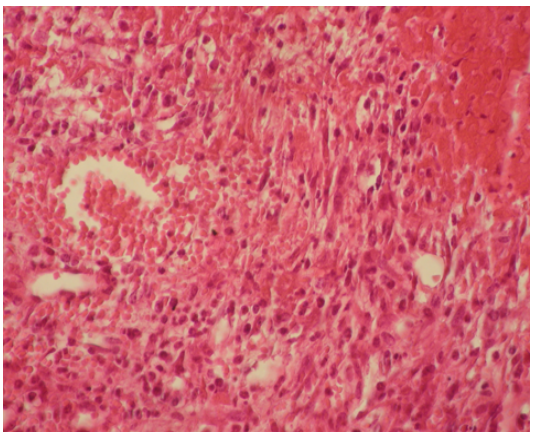

Figure 14. Connective tissue-originated inflammatory cell formation.

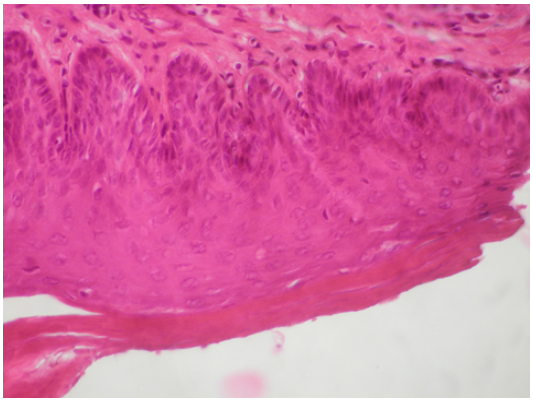

Figure 15. Formed epithelial layers in detail.

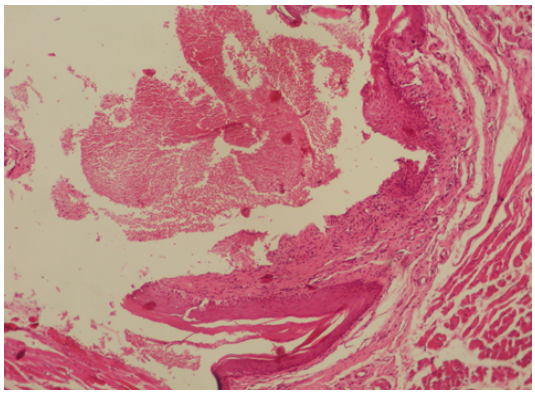

Figure 16. Formed epithelial layers lining the socket.

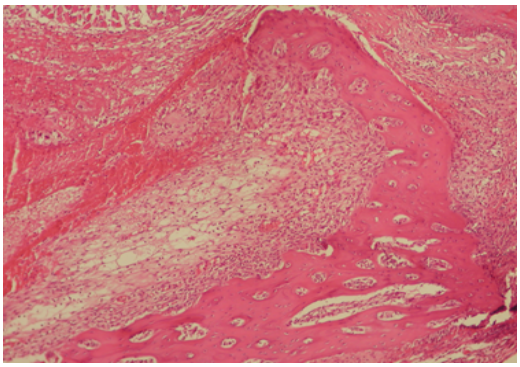

Figure 17. Fully formed epithelium.

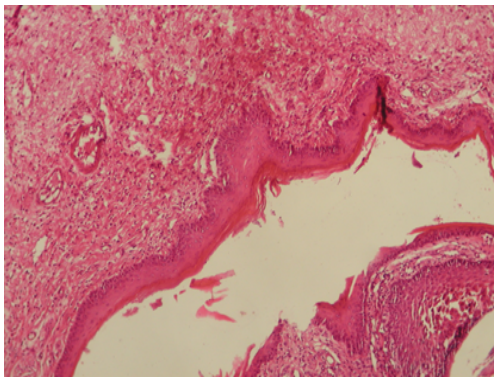

Figure 18. Organization of the connective tissue.

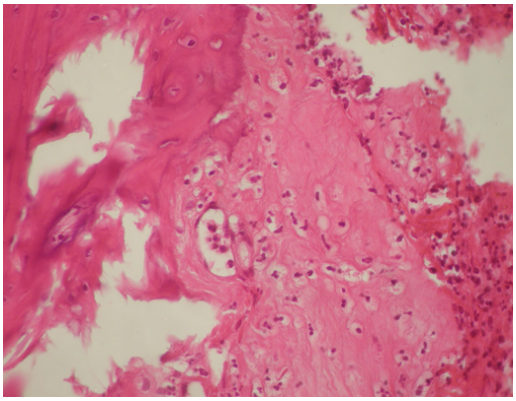

Figure 19. Differentiation of cartilaginous tissue.

the alveolar process became fully mature, periodontal space appeared to be full of juvenile cells, and early vasculation (Figures 22 and 23).

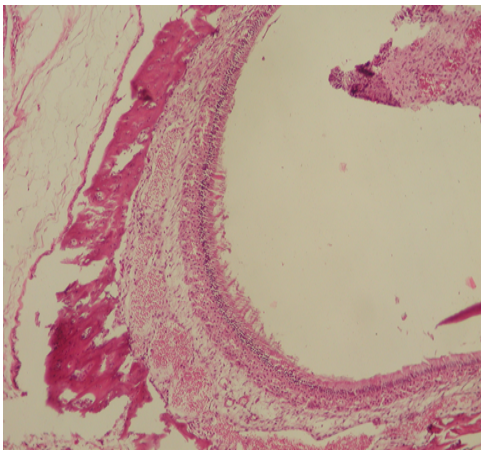

Figure 20. Early activity of ameloblasts in the extraction area.

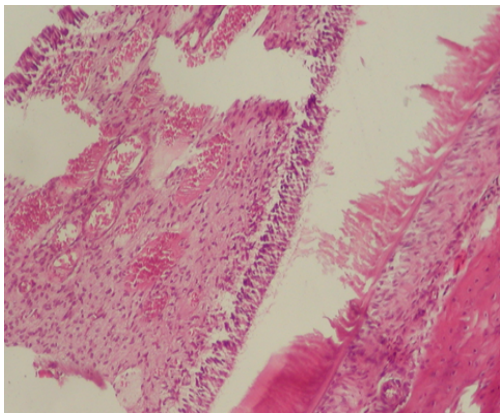

Figure 21. Forming of new enamel and dentin.

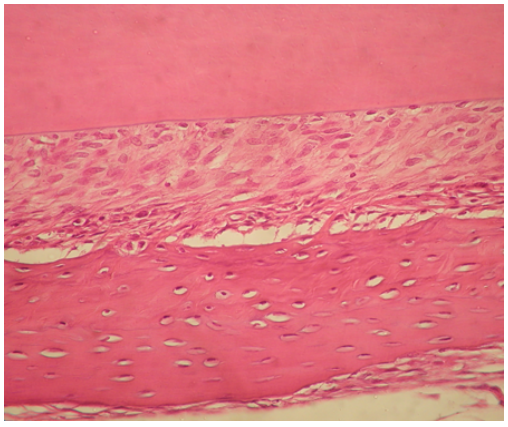

Figure 22. Ligamental space between bone and dentin (longitudinal section).

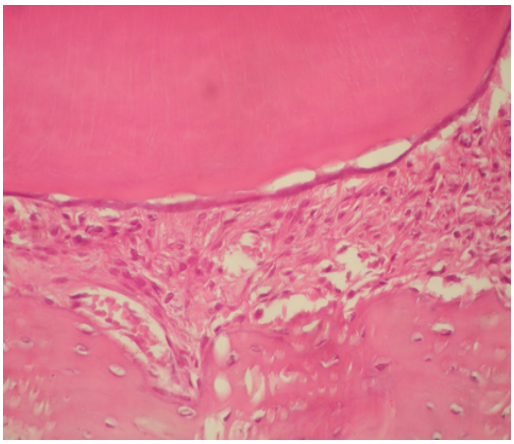

Figure 23. Ligamental space between bone and dentin (horizontal section).

Conclusion

Day Six: The alveolar socket is now filled with a new fully formed tooth showing newly formed pulp, dentin, and periodontal ligament. This study shows the possibility to use Syrian hamsters as an adequate and successful animal model to deepen our knowledge in various fields which could only be conducted with experimental methods. This was already conducted by (Bilal (4), Barakat (5), Muhareb (6), Shin(3) Wang(1),. in studying cancerous and premalignant growths. When reviewing the medical literature, no studies were found explaining the histological structure of teeth and periodontal tissues in hamsters, despite the numerous issues facing researchers in dental and oral medicine. This study managed to provide a clear understanding of the morphological and histological structures of hamster teeth and their surrounding tissues. This study prompted several intriguing results, as confirmed by the microscopic images of the presence of a periodontal ligament in hamsters, which paves the road to more advanced research in periodontology and studying the mobility of teeth. It's noteworthy to mention the constant regenerative ability of hamster teeth after extraction, where there were no extra tooth buds, but a unique structure was observed in the apical region, with active cells able to differentiate into ameloblasts and odontoblasts. These cells can be considered STEM CELLS although this assumption needs further investigation into the morphology and functionality of these cells. Knowing stem cells exist in the apical region, this study conceivably opens immense possibilities in activating these cells in order to utilize them in bone grafts and dental implants. The medial region of the root having such a unique form, where the pulpal space is depressed and dentin thickness too explains the frequent fractures of teeth while extracting, which helped preserve the odontoblasts to aid with the regeneration process after extraction. Regeneration process within the bone cavity happened fairly quickly in hamsters, incisor regeneration after extraction took less than one week, which provides adequate conditions to monitor the regenerative process within the cavity after extracting incisors in hamsters. This study explained in detail the different stages of the regeneration process: Clot formation stage, Clot organization stage, cartilaginous and bone tissue differentiation stage, and tooth bud development stage. Each stage was explained in detail with the necessary microscopic images.

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