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Histoanalysis for Epithelial and Mezanchyme Stem Cells Responsible for Continuous Regeneration of Incisors in Syrian Hamsters, an Experimental Study to Monitor Reproductive and Differentiation Properties after Extraction

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Abstract

Background: Syrian Hamsters are considered prime animal models used in numerous experimental research and applications especially in the field of dentistry. Studies on Syrian hamsters have shown their exceptional regenerative abilities of incisors after extraction. Isolating and identifying cells responsible for the constant regeneration of the incisors, determining their unique properties, and confirming their ability to differentiate into ectoderm and mesoderm derived cells. Exploring their capabilities to construct cellular cultures in sufficient numbers and identifying the proper cellular markers is an extremely important issue.

Methods: 15 Syrian Hamsters were distributed evenly into three Main groups plus a control group. Control group was sacrificed in order to obtain a sound Morphological structure in the incisor region. Specimens in Main groups were gradually sacrificed to monitor the progress of the regeneration process within the next days following extraction on the first, fifth, and tenth day respectively.

Results: Results confirmed the presence of lymphoblastic lengthening encased inside the bone cavity consisting of active homogenous cell cultures. These cells can proliferate and differentiate forming the pulp, dentin, cementum, and periodontal ligaments. This was confirmed with immunohistochemical markers proving these cells are in fact adult stem cells in the periodontal tissues.

Conclusion: 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.

Keywords: Extraction • Post-extraction healing • Syrian hamster • Stem cells

Introduction

Although the vast majority of mammals have lost the ability to regenerate their teeth, there are a few exceptions to this rule, constantly regenerating fully functional teeth throughout their life cycle [1]. Rodents are a prime example of this exceptional ability to regenerate teeth, and it's most prominently obvious in mice [2]. These teeth samples have provided an optimal model to study stem cells [2]. Constantly regenerating incisors in mice are still considered the most optimal model to conduct research on epithelial stem cells, stem cells of incisors are considered semi-differentiated epithelial stem cells [2]. Numerous Laboratories across the world are currently conducting extensive research to harvest the merits of mice DNA, which expresses the importance of studying epithelial progenitor and stem cells with quick and sustainable regenerative capabilities (respectively) in dentistry [3]. Determining the characteristics of epithelial progenitor and stem cells, and isolating the ability to regenerate fully functional teeth\enamel is considered a major aim of future studies [3]. Dentition in most rodents including mice is compiled of a constantly regenerating incisor and three molars spaced out from the incisors with edentulous spaces in each quadrant [4]. Asymmetric central incisor is composed of Enamel (The hardest Tissue in the tooth) that covers the labial surface of the tooth while the lingual surface consists of softer dentin [4]. Selective abrasion caused by the asymmetry of the enamel affects only the lingual surface, which in addition to maintaining constant growth of the tooth, keeps the incisor sharp [5]. Incisors are comprised of a crown and a symmetrical root [5].

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Materials and Methods

12 three-month-old Syrian hamster specimens were divided into four even groups with each containing three hamsters.

First group (Control group)

Includes three models, biopsies were taken from the incisal region before excision, and longitudinal and horizontal sections were prepared for histological examination.

Second group

Includes three models, biopsies were taken one day after excising the lower incisor to perform a histological examination to monitor cell activity and differentiation in the excision area one day after excision.

Third group

Includes three models, biopsies were taken five days after excising the lower incisor to perform histological examination to monitor cell activity and differentiation in the excision area two days after excision.

Fourth group

Includes three models, biopsies were taken ten days after excising the lower incisor to perform histological examination to monitor cell activity and differentiation in the excision area two days after excision.

Biopsy method

Anesthesia was administered to hamsters with a cotton swab soaked with chloroform, and then the lower incisors are excised using root forceps. Extracting teeth was notably accompanied by fracturing the roots in most cases. The hamster sacrifice mentioned earlier was carried out mercifully 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 (Figures 1-12).

Results and Conclusion

Results confirmed the presence of lymphoblastic lengthening encased inside the alveolar cavity consisting of active homogenous cell cultures. these cells can reproduce and differentiate forming the pulp, dentin, cementum, and periodontal ligaments. This was confirmed with immunohistochemical markers proving these cells are in fact adult stem cells in the periodontal tissue.

First group (Control group)

Histological examination of longitudinal and horizontal sections in the control group specimens showed highly vasculated pulp-like, under-differentiated mesenchyme tissue, with a plethora of embryonic cells. In addition, Dentinoblasts, predentin, ameloblasts, these formations are asymmetrical due to the proliferation of odontoblasts and ameloblasts on the labial side but only odontoblasts form on the lingual side (Figures 1 and 2). The medial region of the root showcased a unique structure, there was considerable depression in tooth

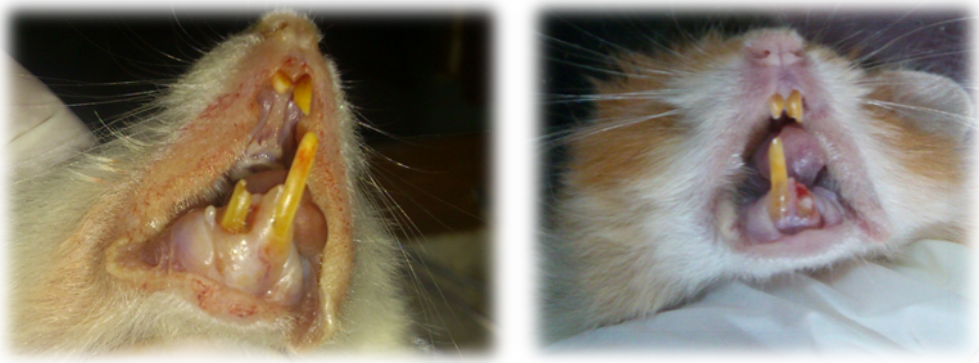

Figure 1. Images of the syrian hamster showing quick regeneration of incisors.

Figure 2. Image details obtaining the incisor and aroma from the hamster.

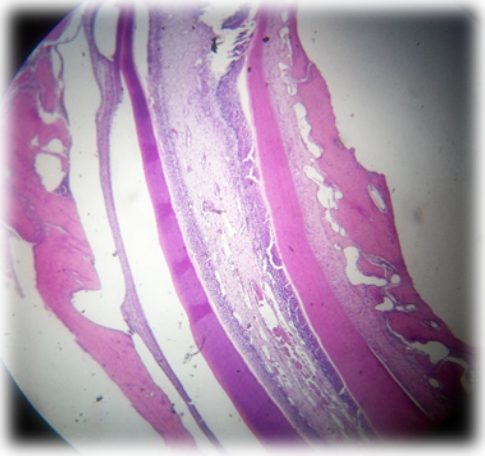

Figure 3. Longitudial section showing aromic tissue encased with the alveolar socket with underdeveloped predentin and amelogenic cells.

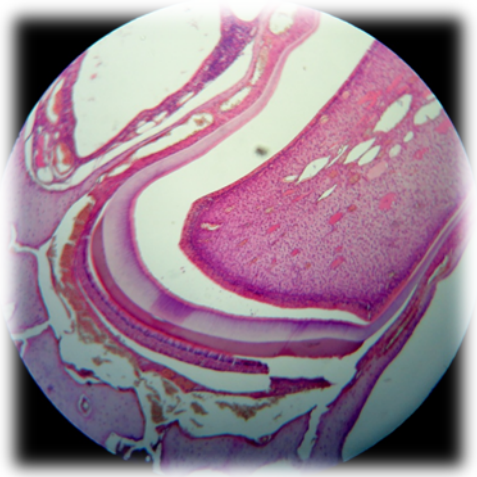

Figure 4. Highly vasculated Pulp-like Mesanchyme tissue with no fibroblasts. Dentinoblasts and under developed predentin are present alongside with inner and outer enamel epithelium.

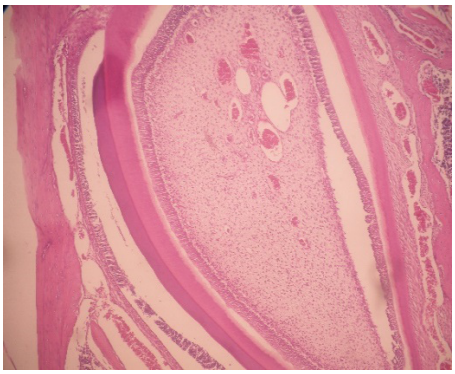

Figure 5. Horizontal sections shows only dentinoblast formation on the lingual side, while enamelblasts and dentinoblasts are present on the labial side.

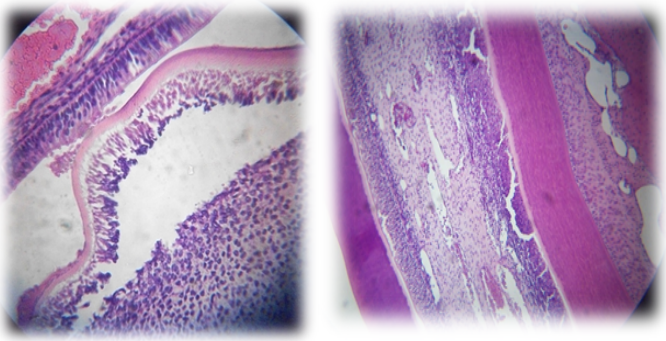

Figure 6. Mesenchyme tissue surrounded by odontoblasts, while ameloblasts are present on top (60X Magnification).

structures causing a narrow pulpal space to a high degree in some cases (Figures 3 and 4). Dentin thickness was also decreased in said area, meanwhile, dentin and pulp structures returned to normal thickness as it approached apical region. Histological examination of specimens harvested from models of this group were sacrificed one day after extraction. Evidence of remnant dentin were observed in the extraction area surrounded with inflammatory cells bone splinters, early onset mitotic activity of ameloblasts, and early stage juvenile enamel despite the inflammatory process. Histological examination of specimens harvested from models of this group were sacrificed five days after extraction. Extraction region contained mitotic activity of odontoblasts and ameloblasts (Figures 5 and 6). Predentin and juvenile enamel were present. Histological examination of specimens harvested from models of this group were sacrificed ten days after extraction. Quick development of the tooth bud was observed where the crown and the alveolar process became fully mature, periodontal space appeared to be rich with juvenile cells and early vasculation. 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 (Figures 7 and 8). This study managed to provide a clear understanding of the morphological and histological structures of hamster teeth and their surrounding tissues. This study resulted in various interesting aspects, microscopic images, and context confirmed the presence of aromatic lengthening from the pulp-like mesenchyme tissue without the presence of fibroblasts and fiber tissue, it was also highly vasculated. star and square form embryonic cells, cervical nodules composed of active epithelial basal cells proliferating and differentiating towards the newly formed apex into odontoblasts and ameloblast cells on the labial side, and odontoblasts and bone-like which could pave the way for advanced experimental studies to isolate these cells, culture it and use markers to identify it (Figures 9 and 10). 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 noticed in the apical

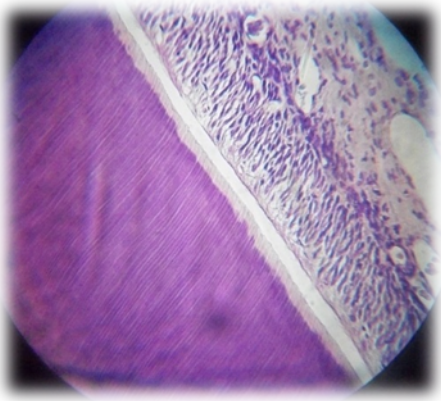

Figure 7. Longitudinal section 40X magnification.

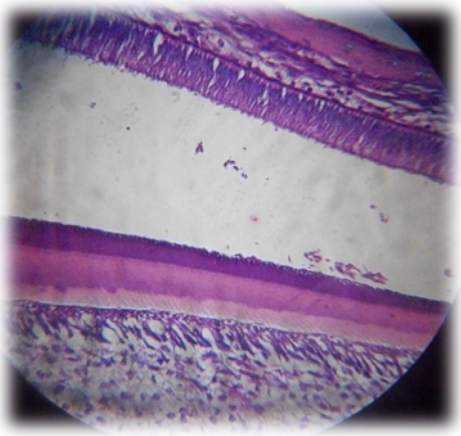

Figure 8. Longitudinal section 40X Magnification.

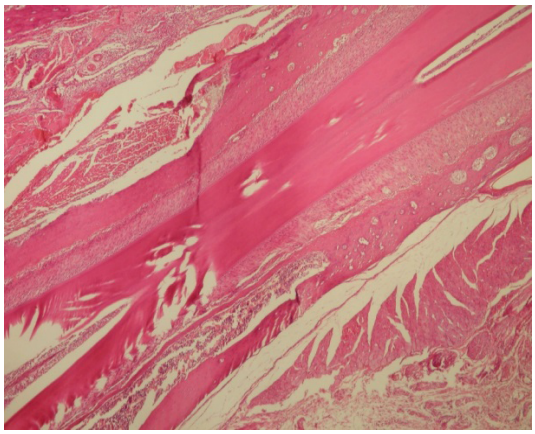

Figure 9. The medial region of the root showcased a unique structure.

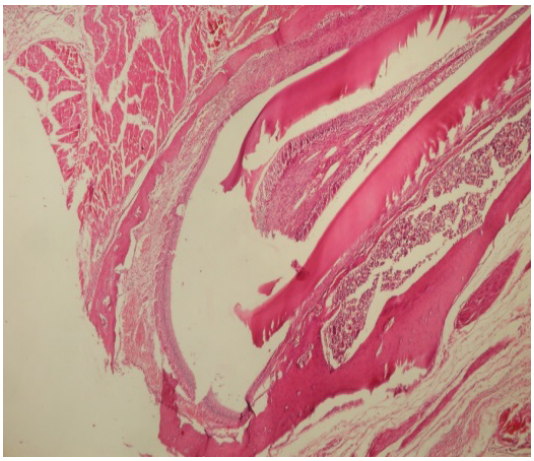

Figure 10. Histological examination of specimens harvested from models of this group were sacrificed one day after extraction.

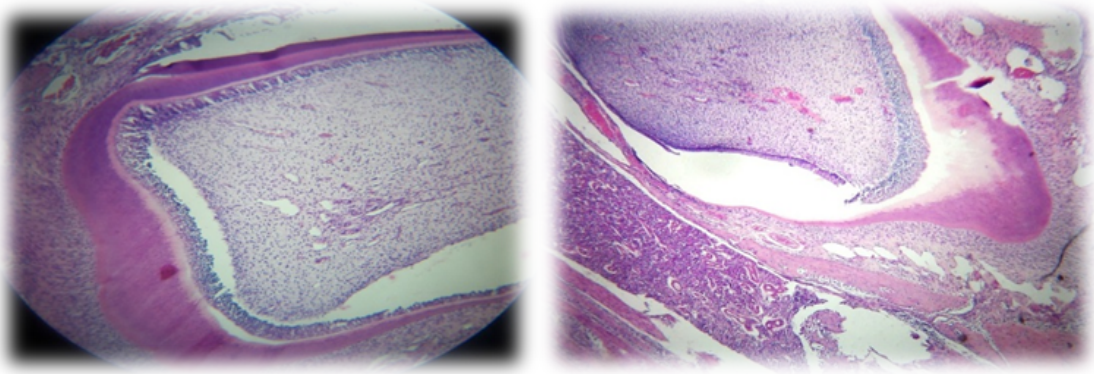

Figure 11. Histological examination of specimens harvested from models of this group were sacrificed five days after extraction. Extraction region contained mitotic activity of odontoblasts and ameloblasts. Predentin and juvenile enamel were present.

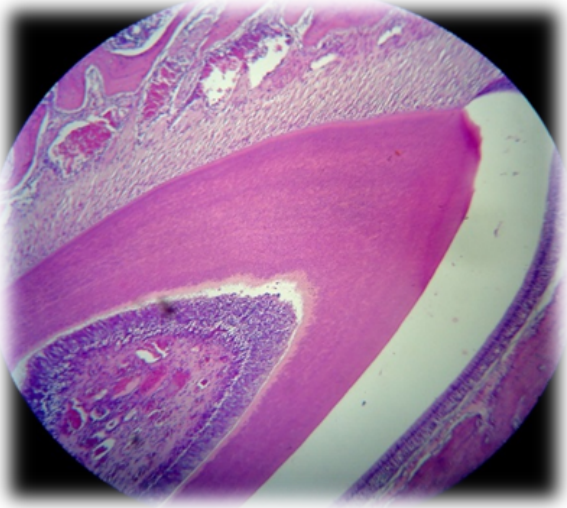

Figure 12. Fully formed dentin and enamel, enamel mineralized tissue was dissolved due to treatment with acid.

region, with active cells able to differentiate into ameloblasts and odontoblasts. The medial region of the root having such a unique form, where the pulpal space is narrowed and dentin thickness too explains the frequent fracture of teeth while extracting, which helped preserve the odontoblasts to perform regeneration 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. Constantly regenerating incisors in mice are still considered the most optimal model to conduct research on epithelial stem cells. Stem cells of incisors are considered semi-differentiated epithelial stem cells numerous Laboratories across the world are currently conducting extensive research to harvest the merits of these cells (Figures 11 and 12).

Recommendations

It is recommended to conduct further advanced research to deepen the understanding of these cells and to determine the effect of various factors which could create a basis of human morbidity and periodontal tissues, also the possibility of adding it to bone grafts to induce bone regenerative qualities in bone

grafts and possibly taking advantage of its differentiating capabilities into different tissue structures and in tissue reprogramming research and in regenerative medicine.

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