

A Case of Supernumerary Tooth Extraction in a Patient with Type 1 Glutaric Acidemia

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Abstract

The echidna incubates the egg for ten days in the pouch after it has developed in the uterus. The fetuses acquire an egg tooth and caruncle during this time to aid in hatching. Micro-CT, histology, and immunofluorescence were used to evaluate the development of the egg tooth and caruncle by utilizing rare and unprecedented access to limited echidna pre- and post-hatching tissues. The echidna egg tooth developed via evagination, like the first teeth of some reptiles and fish, in contrast to therian tooth germs, which develop via placode invagination. Instead of forming a tooth root with a ligamentous attachment like in other mammals, the egg tooth ankylosed to the premaxilla. The caruncle formed as a separate mineralization from the adjacent nasal capsule. This suggests that the monotreme egg tooth has been preserved from a common ancestor of mammals and reptiles because it shares many similarities with typical reptilian teeth.

Keywords: Tooth; Monotreme; Caruncle; Disease of the gums; Dementia; Disease of the Alzheimer; Oral wellness

Introduction

When the impacted canine mimics the natural eruption of the permanent tooth and penetrates the gingiva near the center of the alveolar ridge, with sufficient gingival tissues covering the lingual and buccal tooth surfaces, orthodontic surgical treatment of impacted canines is considered successful [1].

The absence of the attached gingiva, gingival recession, or the development of dehiscence or fenestration in the cortical bone may be associated with an impacted tooth erupting more buccally or lingually than the normal eruption position [2]. As a result, a number of authors have suggested selecting a force eruption technique that can mimic the natural eruption of the tooth.

The depth of the impaction, the location, and the anatomical structure of the soft tissue that covers the impacted canine determine which surgical exposure method is chosen, open or closed. Because it replicates the physiological tooth eruption, some authors have suggested that the closed eruption method improved periodontal outcomes, particularly in cases of deep alveolar bone impaction.

In order to compare the periodontal outcomes of the two primary surgical exposure methods, a number of studies have concluded that the soft tissues should be respected to the greatest extent possible.

Dalia and co. concluded that the open technique with free eruption produced comparable results when compared to the closed surgical method in terms of the periodontal status of palatally impacted canines and adjacent teeth. Periodontal conditions did not differ significantly between open and closed eruption methods, according to the findings of a recent systematic review. The systematic review revealed that the values of probing depth around the tracked canines were deeper than those of the canines on the opposite side with spontaneous eruption. The study's findings regarding the width of the keratinized tissue were inconsistent. However, there was no significant difference in the indices of plaque, bleeding, or gingival recession between treated and untreated canines.

The effectiveness of the minimally invasive corticomy-assisted orthodontic movement acceleration in a variety of malocclusion management procedures, such as the retraction of maxillary incisors

and the alignment of maxillary crowded teeth, has been the subject of a number of studies. According to these studies, the application of this acceleration technique had little effect on the periodontal tissues and did not significantly alter the condition of the teeth or gums [3]. A systematic review also found that the use of minimally invasive surgical techniques to accelerate orthodontic tooth movement did not have any negative effects on gingival indices. Lastly, a systematic review revealed contradictory results regarding the periodontal outcomes of using the PAOO (periodontally accelerated osteogenic orthodontics) technique to speed up orthodontic tooth movement.

Few studies on the acceleration of the impacted canine traction movement were found in the available medical literature. When comparing the intervention group to the conventional closed traction group, there were no significant differences in keratinized gingival width or gingival recession between the two groups in the study that used the vitamin C injection technique to accelerate the traction movement of impacted canines. However, despite the fact that the invasive surgical acceleration method used may have had a significant impact on the periodontal structures, they did not assess the canine and adjacent teeth's post-treatment periodontal status.

In Fischer's preliminary acceleration study, which is the only one to use assisted corticotomy to accelerate the traction movement of palatally impacted canines with a split-mouth design, some periodontal variables were evaluated. There were no tremendous contrasts in the periodontal examining and alveolar bone levels between the corticotomy-helped withdrawal of affected canines and their contralateral teeth lined up with the customary strategies. However, due to the small sample size (only six patients), this study did not evaluate the periodontal status

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of the adjacent teeth or other significant periodontal variables like the degree of gingival recession and the width of the keratinized tissue.

Therefore, the purpose of the current study was to compare the periodontal pockets, gingival recession, and width of keratinized tissue in the periodontal pockets of palatally impacted canines (PICs) and adjacent teeth when using conventional versus minimally invasive corticotomy-assisted canine traction [4]. The embryo transforms into a fetus during incubation, and the young hatches from the egg in just 10–10.5 days. The leathery, porous monotreme eggshell is made of loosely wound keratinous fibers. To escape from their egg, monotremes, in particular, develop both an egg tooth and a caruncle.

Reptiles and birds have either a caruncle or an egg tooth. A caruncle, a thickened, keratinized epithelium positioned above the nasal cartilages, is present in turtles, Rhynchocephalia, and crocodiles. Squamates, on the other hand, have a real tooth that can be single or paired. Indeed, even viviparous reptiles have an egg tooth, despite the fact that it is more modest and secret under a layer of connective tissue. The majority of birds also hatch from their eggs using an egg tooth. Be that as it may, the avian egg tooth is basically the same as the caruncle of turtles and crocodiles, comprising of a sharp, keratinized 'horn-like projection' instead of being a real tooth. It is interesting to note that, in contrast to birds and reptiles, the caruncle of monotremes is supported by an os caruncle, a bony protrusion. The question of whether the os caruncle is simply an extension of the premaxilla or an independent ossification that fused with the premaxilla has been debated regarding the relationship between the two structures.

During embryonic development, ligand-receptor interactions between the oral epithelium and mesenchyme kick off tooth development in therian mammals [5]. The actual tooth structures in various stages called the bud, cap, and ringer stages. Cytodifferentiation occurs during the bell stage, and odontoblasts and ameloblasts, which respectively produce dentine and enamel, are formed here.

Columnar cells known as odontoblasts surround the dental pulp cavity in a uniform layer. They are distinct from odontoblastic secretions from the dentine layer and the dental pulp. The majority of dentine is formed in layers laid down by odontoblasts. On the other hand, osteodentine, which gets its name from its resemblance to bone, is seen when dentine grows quickly and catches odontoblasts and other nearby cells. Ameloblasts mature on the outer surface of the dentine after the development of the dentine layer and secrete proteins like amelogenin, resulting in enamel formation between the ameloblasts and the dentine layer. The enamel that covers the tooth crown is the body's hardest substance.

Materials and Procedures

Cell isolation and ethics

Periodontal ligament tissue was manually extracted from extracted human wisdom teeth because it meets the minimum requirements for mesenchymal stromal cells and does not show any signs of inflammation or caries [6]. Up until passage 5, this study used human PDLSC from three male and three female donors between the ages of 18 and 45.

Prior to tooth donations, patients provided written and informed consent. The Ethics Committee of the Medical University of Vienna, Vienna, Austria, gave the study protocol its approval. The study's design adheres to the Medical University of Vienna's "Good Scientific Practice" guidelines and the Declaration of Helsinki's ethical guidelines for human subjects in medical research.

Treatment of cells under mechanical tension

A total of 300,000 cells per well were seeded onto collagen-type I-coated 6-well Culture Plates for the PDLSC treatment under mechanical tension [7]. Subsequent to cultivating, cells were left to append for the time being. Attached PDLSCs were inserted into a Tension System the following day to apply static mechanical tension, beginning with a maximum tension intensity of 10% for the first six hours and gradually decreasing to 3% over the following hours. PDLSC was subjected to mechanical tension for a total of 72 hours. Live-Dead staining was used to assess cell viability 72 hours later. In the treatment group, tension-treated PDLSC were used, in the negative control, untreated PDLSC, and in the positive control, both tension-treated and untreated PDLSC were incubated with pure DMSO for 15 minutes prior to the staining. All PDLSC samples were washed with PBS prior to staining [8]. The staining solution was then applied for 15 minutes at 37 °C in accordance with the manufacturer's protocol. Thereafter, pictures were taken with a fluorescence magnifying lens at 100-overlap amplification, catching Live-Dye™-positive cells at 470/40 nm excitation and 525/50 nm discharge frequency and PI-positive cells at 560/40 nm excitation and 605/70 nm outflow frequency. The experiment was carried out in two technical replicates, and PDLSC from three distinct donors were used for the staining. Micro-CT scanning and 3-D reconstructions

One of the fetuses was not able to be micro-CT (micro-computed tomography) scanned because it had already been collected and processed for histology prior to the start of this project. All excess echidna hatchlings and pocket youthful examples were filtered by miniature CT. Miniature CT filtering of the echidna babies and the d11 py was performed with a Phoenix nano tom m (Waygate Innovations, Huerth, Germany) worked utilizing xs control and Phoenix datos|x procurement programming (Waygate Innovations) [9]. The X-ray energy used was 300 A at 35–40 kV. Checks were directed utilizing a molybdenum focus to boost contrast from the delicate tissue examples. The specimen's size was taken into account when setting the voxel resolution, which ranged from 3.1 to 11.1 m. In a fast scan mode, scan times range from 5 to 15 minutes because the number of X-ray projections collected through a full 360-degree rotation was also tailored to the specimen's size. These projections ranged from 599 to 1798. For imaging and analysis, the data were exported as volume files with 16 bits. A Scanco micro-CT scanner was used to scan all of the remaining pouch young samples, and Amira was used to analyze the images.

Three babies were examined two times, first impeccable to identify mineralized tissues and also, after the hatchlings were stained with 1% iodine in 100 percent ethanol short-term to expand the difference of non-mineralized tissue [10]. After being stained with 1% iodine overnight in 100 percent ethanol, all other fetal samples were scanned once and cleared with sodium thiosulfate before being stored in 100 percent ethanol. After the babies were iodine and miniature CT examined, they were washed in 70% ethanol to eliminate any abundance iodine stain and afterward brooded in sodium thiosulfate to clear the iodine tinge before paraffin implanting and segment. Pocket youthful examples were examined two times. The MASSIVE platform was used to perform 3D image analysis and segmentation in Avizo v9.7.0 (ThermoFisher Scientific).

Histology

The fetuses were sectioned through the sagittal plane using a Rotary CUT 4060 microtome (Microtec Laborgerate, GmbH, Walldorf,

Germany) and dried overnight in an oven at 40 °C following the micro-CT scan. After de-waxing and rehydrating each third slide through a graded ethanol series to distilled water (dH₂O), the slides were stained with hematoxylin and eosin (H&E) according to standard procedures [11]. Every remaining slide was stained with a Picro-Sirius Red and Alcian blue trichrome stain in this area for a standard histological examination of bone, cartilage, and dentine. The tooth region was identified from the H&E-stained slides. The excess slides were saved for immunofluorescence. All fetal head histology images have been uploaded to Zenodo.

Immunofluorescence

Segments utilized for immunofluorescence were first de-waxed in histone and rehydrated as above, with a last wash in dH₂O [12]. To identify protein articulation of sonic hedgehog (SHH), collagen type II (Col2), cytokeratin 14 (K14), cytokeratin 8 (K8), and Loricrin, segments were first positioned in the antigen recovery cushion 0.01 M Sodium citrate pH10 (w/v) and brooded in a preheated water shower at 95 °C for 20 min prior to being passed on to cool at room temperature for 40 min. The slides were rinsed in 1X phosphate-buffered saline (PBS) containing 1.37 M NaCl, 27 mM KCl, 18 mM KH₂PO₄, and 100 mM Na₂HPO₄ after being cooled. Sections were washed for 5 minutes in 1X PBS after the 1x PBS wash, then incubated for 10 minutes in 0.3% Sudan Black in 70% ethanol before being briefly submerged in fresh 70% ethanol. Segments were then impeded with 10% (v/v) ordinary goat serum in 1x PBS for 1 h at room temperature. Subsequent to obstructing, the segments were brooded for the time being (16 h) in a sticky chamber at 4 °C with either bunny SHH immune response, mouse Col2 immunizer (1/50 weakening; II-II6B3; Formative Examinations Hybridoma Bank, Iowa City, IA, USA), mouse K8 immunizer, or co-named with mouse hostile to K14 and bunny Loricrin neutralizer. Segments were then washed multiple times in PBS containing 0.5% Tween 20 (v/v) and afterward hatched for 45 min with goat against hare Alexa Fluor In addition to 555 auxiliary counter acting agent at either 1:300 weakening (SHH), goat hostile to bunny weakening (Loricrin), or goat against mouse.

The slides were mounted with media and kept in the dark until visualization, and the nuclei were counterstained with 4',6-diamidino-2-phenylindole. Negative control areas were submitted to the very techniques with the exception of that the principal immune response was supplanted by the relative isotype control at a similar focus as the essential neutralizer. Staining in positive and negative control tissues, as well as non-staining in isotype controls, confirmed the specificity of all antibodies [13]. Using either a Zeiss Apotome fluorescence microscope at King's College London or a Nikon A1R spectral confocal microscope at the University of Melbourne, images were taken. Perception settings were at first upgraded to wipe out foundation utilizing the isotype control on each slide. The remaining positive sections of the slide were then visualized using these settings.

Results and Discussion

After oviposition, fetal samples were sectioned and imaged using micro-CT to follow the development of the egg tooth in the echidna. Remotely, no egg tooth was apparent in the d4 embryo however by d6 post-oviposition onwards, the egg tooth was plainly noticeable. Both micro-CT and the histology of the echidna fetal heads showed significant growth changes, particularly in the tooth region between days 4 and 7. In the d4 hatchling, the epithelium was thickened and communicated SHH, affirming the egg tooth was at the placode transformative phase. The mesenchyme beneath the placode had begun

to condense, but there was no evidence of the placode invading the underlying mesenchyme. In contrast to the invagination that occurs during therian mammalian dental development, the egg tooth began to project outwards at d6, resulting in the formation of a tooth by evagination [14]. A mineralized dentine layer had likewise started to frame which was affirmed with miniature CT yet the dental mash had just barely begun to separate, and the cells were thickly pressed. The evaginating epithelium of the d6 fetus continued to express SHH, and an additional positive domain was found in the oral epithelium further back in the mouth. By day 7, the inner surface of the dentine layer was clearly marked by odontoblasts, and the dental papilla cells appeared to be much less tightly packed.

From d6 to d7, polarized cells with ameloblast-like morphology formed at the tip of the epithelium around the egg tooth. There was an increase in the amount of dentine and a decrease in the number of epithelial cells at the tooth's tip from d8 to d9, one day before hatching. However, there was no evidence of an enamel layer at any of the stages analyzed. Mesenchymal cells were entrapped within the dentine matrix, and the egg tooth's smooth dentine layer at the tip gave way to osteodentine at the base [15,16]. This locale seemed constant with the premaxillary bone, as affirmed by Alizarin red staining. At any of the stages analyzed, there was no evidence of a root. The egg tooth is, accordingly, secured straightforwardly deep down.

Conclusion

Tissue regeneration engineering can be used to repair damaged teeth, which can be very beneficial to patients. because it can restore teeth's function and has improved cohesion and stability. Prosthetic dentistry and tooth structural defect repair are now possible thanks to current research successes, such as biomimetic remineralization and tissue engineering techniques. In the impending future, current drill-and-fill techniques will be supplanted by novel and imaginative procedures, including dentin-mash complicated or entire tooth recovery, biomimetic remineralization, and self-fixing even in huge holes. Future difficulties and perspectives.

Acknowledgement

None

Conflict of Interest

None

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