Fully Guided Tooth Bud Ablation in Pigs Results in Complete Tooth Bud Removal and Molar Agenesis

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Purpose: Fully guided microwave tooth bud ablation has the potential to become a minimally invasive means for managing third molars in adolescent patients. If developed, this new modality could provide improved outcomes and reduced complications compared to traditional third molar management strategies. The purpose of this 28-day longitudinal characterization study was to determine if the healing response following fully guided microwave ablation of second molar tooth buds in juvenile pigs would result in the complete removal of targeted tooth bud tissues, molar agenesis, and no significant collateral tissue damage.

Methods: Investigators performed fully guided microwave ablation on 24 mandibular second molar tooth buds (#18 and #31) in seven-week-old pigs. Postablation healing assessment consisted of radiographic and histological evaluation of 3 subcohorts (consisting of 4 animals each) at 7-, 14- and 28-days post ablation. Controls were untreated, opposing maxillary second molar tooth buds. Neurological assessment was performed to determine if there was any detectible loss of inferior alveolar nerve function.

Results: Healing processes were nearly complete at 28 days post ablation. While one tooth bud was identified as partially ablated at 14 days post treatment, all treated tooth bud tissues were replaced with trabecular new bone formation by the end of this study. There was no detectible loss of inferior alveolar nerve function. The thermal dosing strategy used in this study appears to deliver prescribed ablation volumes and–within the context of this animal model–there was no detected collateral tissue damage.

Conclusions: The results of this study confirm the hypothesis that healing processes following fully guided tooth bud ablation resulted in removal of targeted tooth bud tissues, complete molar agenesis, and trabecular new bone growth at 28-days post treatment.

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Removal of third molars (3M) is an invasive procedure with considerable surgical risk. The healing response following 3M removal is highly variable and adversely affected by uncontrollable conditions, such as age, degree of impaction, pathology associated with teeth to be extracted, patient health at the time of surgery, and noncompliance following surgery. Surgical extraction of 3Ms can lead to a wide range of complications, including protracted reductions in patient quality of life and chronic problems that can be difficult and expensive to resolve.

To reduce problems following 3M surgery, extensive clinical research has been largely focused on mitigating the degree of trauma associated with removing these

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Conflict of Interest Disclosures: Leigh E. Colby and David P. Watson have relevant financial relationship(s) with a commercial interest.

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teeth. While improved surgical technique or the use of autographs and pharmaceutical adjuvants appear to result in more predictable outcomes, a wide range of complications persist. Regardless of the long-standing controversy surrounding prophylactic removal of 3Ms, recent studies continue to demonstrate that 3M removal at a young age–especially before problems occur–remains the most effective approach to reduce surgical risk, reduce the severity of short-term complications, and improve long-term outcomes.[1-3]

Those few individuals who do not develop 3Ms have an improved long-term oral health profile compared to those who form these teeth. Development of a minimally invasive surgical technique that predictably induces 3M agenesis at an early age could provide patients with the same improved health profile. Such a preventative treatment will have significantly less surgical risk and will eliminate postoperative discomfort and complications associated with traditional 3M management strategies.

Previous research demonstrates that fully guided microwave ablation of entire tooth bud volumes is possible. Gross visual assessment suggests that thermonecrosis by this method appears to be limited to the bony crypt of treated tooth buds.[4] Further refinement of this treatment modality will determine whether tooth bud tissue thermonecrosis will result in a predictable healing response. Research that includes histologic evaluation will also determine whether eradication of targeted tooth bud tissues can be achieved with no adverse effects on collateral tissues.

The purpose of this 28-day longitudinal characterization study was to test the hypothesis that the healing response to fully-guided microwave ablation of second molar tooth buds in juvenile pigs would result in removal of targeted tooth bud tissues, molar agenesis, and no significant collateral tissue damage. Radiographic and histologic assessments were used to address the specific aims of this study.

The specific aims of this study were to

1) determine the efficacy of fully guided tooth bud ablation (TBA) for inducing molar agenesis;
2). determine whether the postablation healing process resulted in complete tooth bud tissue removal; and
3). determine whether the thermal dosing strategy employed resulted in ablation volumes that appeared to be consistent with prescribed ablation volumes.

Materials and Methods

STUDY DESIGN/SAMPLE

To address the research purpose, investigators designed and implemented a split-mouth animal study that compared mandibular second molar TBAs against unablated maxillary second molar tooth bud controls.

The study sample consisted of 12 juvenile Yorkshire-Cross domestic swine, sourced from Oak Hill Genetics, Ewing, IL. Enrolled subjects met prespecified inclusion and exclusion criteria based on age, sex, and health status. Subjects also had to pass an inferior alveolar nerve assessment. All subjects were 7 weeks of age at the time of treatment and were in good health. Each subject was 10 to 15 kg in weight. The study used female subjects exclusively because they are less aggressive to one another, and they are safer for staff to handle under laboratory conditions.

The investigators chose pigs as test subjects because the animals' tooth bud development resembles that of humans, both architecturally and histologically.[5] Given their developmental, size, and shape similarities with human molar formation, the porcine 2nd molar mandibular tooth bud model was used. In humans, third molar TBA (3TBA) would likely be performed at 8 to 10 years of age, before mineralized tooth structure is formed and complete bony encapsulation occurs.

This study took place in Fort Collins, CO, at CARE Research, LLC., in accordance with federal regulations for Good Laboratory Practices (GLP) 21CFR58 (Title 21 Code of Federal Regulations, Part 58). CARE is a USDA-approved preclinical research laboratory. The investigators and CARE technicians conducted this GLP study with oversight from an independent quality manager. CARE Research obtained Institutional Animal Care and Use Committee (IACUC) review and approval prior to commencing this study. All subjects were housed, cared for, treated, and euthanized at CARE Research facilities as part of Study Protocol TA-1805 (CARE Research IACUC Number: 1837).

VARIABLES

The independent variable used in this study had 2 levels, which were used to compare an experimental treatment with a control treatment using a split-mouth model. The experimental level was fully guided TBA treatment applied to tissues inside radiographically observed bony crypts of mandibular tooth buds. The control level was no treatment to maxillary tooth buds.

The following were the study's 3 dependent variables:

1) The characterization of post treatment healing responses;
2) The level of formation of mineralized tooth structure following treatment; and
3) The observation for signs of collateral tissue damage.

Investigators used the following 4 criteria (coded using a scale of 0 to 3) to characterize the healing response of treated mandibular 2nd molar tooth buds and immediately adjacent tissues. Unablated upper arches served as controls.

0 = No Bony crypt; bone infill appears complete; no evidence of thermal damage of immediate adjacent tissues.

1 = Bony crypt reduced in size; bone infill progressing; no evidence of thermal damage of immediate adjacent tissues.

2 = Bony crypt unchanged; no bone infill evident; no evidence of thermal damage of immediate adjacent tissues.

3 = Bony crypt increased in size; no bone infill evident; evidence of thermal damage of immediate adjacent tissues.

Investigators used the following 4 evaluation criteria (coded using a scale of 0 to 3) to characterize the efficacy of cessation of mineralized tooth structure formation in the treated 2nd molar tooth buds. Unablated upper arches served as controls.

0 = No radiographic evidence of 2nd molar mineralized tooth structure formation.

1 = Minimal or trace level of detectable radiographic evidence of 2nd molar mineralized tooth structure formation.

2 = Moderate or intermediate level of detectable radiographic evidence of 2nd molar mineralized tooth structure formation with crown formation to be less than 50% complete.

3 = Advanced level of detectable radiographic evidence of 2nd molar mineralized tooth structure formation with enamel formation estimated to be 50% or more complete.

Ancillary variables, including sex, age, and weight of the subjects were held to a minimum. In addition, the design of all TBA guides and delivery of all treatments were limited to one operator.

DATA COLLECTION METHODS

Data collection was performed in accordance with recognized GLP standards. Treated mandibular tooth buds (n = 24) and untreated maxillary tooth buds (n = 24) were subjected to a peer-reviewed radiographic evaluation and an independent histological evaluation.

Healing response and/or the formation of mineralized tooth structure following ablation was characterized through radiographic and histological evaluation at 7-, 14-, and 28-days post treatment. Collateral tissue damage was characterized through radiographic, histological, and neurological evaluation at the same time intervals.

Computed tomography (CT) scan evaluation forms were used to code the evaluation criteria outlined above. Evaluation forms and final reports were audited by the study quality manager.

DATA ANALYSES

This study included a peer review of CT images in parallel with an independent histopathology evaluation. The principal investigator and an independent dental reviewer conducted a radiographic characterization of treated and control sites using images from axial, coronal, and sagittal planes. The reviewers did not have access to the histology report until after radiographic assessments were complete.

As a characterization study (where the results were dichotomous, ie, tooth formation or no tooth formation), no formal statistical evaluation was performed other than to summarize findings in the form of average scores for assessment criteria scoring values. Where relevant, percentage values were reported to further summarize or highlight assessment scale scoring differences between study animals or study scientists.

STUDY PROCEDURES

This study began 1 day prior to TBA treatment. All the 12 test subjects received a neurological evaluation and cone-beam computed tomography (CBCT) imaging. CBCT scans were used to manufacture surgical guides for each animal in preparation for TBA procedures planned for the following day.

On the preoperation day, laboratory staff randomly assigned and attached animal identification ear tags to each test subject. Each animal was anesthetized with intramuscular (IM) Telazol (5.0 mg/kg), ketamine (2.0 mg/kg), and xylazine (1.0 mg/kg). Technicians administered approximately half of the IM anesthesia cocktail dose to prepare the animals for inferior alveolar nerve assessment. The sedative was delivered via IM injection into the subjects' gluteal muscle mass.

Approximately 3 to 5 minutes after receiving anesthesia, the subjects appeared calm and physically manageable. At this time, each study animal underwent a comparative neurological evaluation of their inferior alveolar nerve function. The upper and lower quadrants of the animals' lips were assessed bilaterally, with upper lips serving as a control. A sterile 27-gauge needle was used to superficially pierce the right and left sides of the lower lip. Similar pricks to the right and left sides of the upper lip were performed to check for any neurological asymmetries or deficits between the quadrants of the subject animals' lips. Any animal with a demonstrable sensory asymmetry or deficit would have been excluded from the study.

Upon completing the inferior alveolar nerve assessment, the remaining half-dose of IM anesthesia was administered, and each animal was intubated approximately 10 minutes later. Sedation was complemented with inhalation agents as needed. Once each animal was sufficiently sedated, CBCT scans of the mandible and maxilla were obtained to image 2nd molar tooth buds (#2, #15, #18, and #31). Mandibular quadrant dental impressions were then obtained, which included the right and left posterior deciduous teeth and retromolar soft tissues over the anterior aspect of the ramus. Fast-set polyvinylsiloxane dental impression material was used. The study animals were then placed into recovery and returned to holding pens once they adequately recovered.

Q6 The CT scans and polyvinyl siloxanedental impressions were used to design tooth-supported, quadrant surgical guides using CoDiagnostiX (Dental Wings, Inc) at a regional dental lab. Surgical guides were 3D printed overnight for the TBA surgery the next day.

THERMAL DOSING

This study used a specific thermal dosing strategy. The maximum diameters of the bony crypts of #18 and #31 were measured orthogonally in the rostral/caudal, dorsal/ventral, and right/left dimensions using Blue Sky Plan. Investigators used these dimensions to determine the microwave energy dose for each targeted tooth bud. Each predetermined energy dose was expected to deliver a spherical zone of ablation that would extend 1 mm beyond the maximum observed diameter of each targeted tooth bud. TBA surgical guide reports generated by the dental lab recorded the TBA probe total insertion depth necessary to reach the center of ablation, and the length of the surgical guide pathway inside the TBA surgical guide.

TBA PROCEDURES

The following day, all 12 study animals were anesthetized using the same anesthesiology and nerve assessment protocols described above. Preoperative photos were obtained prior to performing each TBA procedure. Using the mandibular left and right TBA surgical guides produced the previous day, the investigators created an osteotomy using a sterile 2 mm orthopedic bone drill. The bone drill extended from the anterior aspect of the ramus of the mandible through the proximal wall of the targeted 2nd molar tooth bud. After the bone drill was withdrawn, any bone chips or other tissue debris was removed from the TBA surgical guide. A TBA probe that was 2 mm in diameter was immediately inserted into the TBA guide to a depth that placed the center of ablation of the TBA probe into the center of the targeted 2nd molar tooth bud.

With the TBA probe fully seated in the surgical guide, the principal investigator delivered the prescribed microwave energy dose for each tooth bud that was expected to induce complete tooth bud thermonecrosis. In this study, 12 GHz microwave energy was applied using a proprietary microwave generator. Upon completion of mandibular left and right TBA procedures, intraoral photos of the TBA probe insertion sites were obtained and each animal was then placed in recovery and returned to holding pens once adequately recovered. Even though it was anticipated that test subjects would not experience significant postprocedure pain, all subjects received analgesics (meloxicam, 0.4 mg/kg) IM for 3 days post ablation.

CLINICAL OBSERVATIONS PROTOCOL

As specified in the clinical observations protocol for this study, each animal was checked daily for facial abnormalities including swelling or bruising. Clinical observations included taking each subject's temperature daily and weight weekly.

PROCEDURES AT 7-, 14-, AND 28-DAYS POST ABLATION

At 7-, 14-, and 28-days post ablation, 4 randomly selected study animals received the same inferior alveolar nerve assessment described above. As before, upper and lower lips were tested bilaterally to determine the presence of any ablation-induced inferior alveolar nerve impairment. After completing nerve function assessments, the study animals were euthanized. Immediately following euthanasia, CBCT scans were obtained for radiographic assessment.

Once CBCT scans were obtained, each pig's head was resected at the base of the skull and immersed in formalin for approximately 15 minutes to prepare the samples for shipping to a histopathology lab for evaluation. Care was taken to ensure that the oral cavity surfaces did not dry or become over hydrated. Each head was placed in separate sealed plastic bags and then into a Styrofoam container with frozen gel ice packs to keep the specimens cool without freezing them. The specimens were shipped via overnight first morning delivery to West Virginia University Pathology Laboratory for Translational Medicine, Morgantown. The histopathology assessment was also conducted in accordance with GLP regulations. Complete documentation was included with the shipments to preserve the chain of custody upon receipt by the West Virginia University pathologist.

Results

INFERIOR ALVEOLAR NERVE ASSESSMENT

During this study, 12 pigs were screened for eligibility based on age, sex, and health status. The final sample was composed of 12 female subjects that were all 7 weeks old at the time of treatment.

All subjects received a comparative neurological evaluation of nerve function on the day impressions and CBCT scans were obtained. This evaluation was repeated on the day of treatment, and again on the days each subcohort of 4 animals was euthanized. No test subjects (*n* = 12) demonstrated a reduced or differential lip sensitivity between mandibular and maxillary lip quadrants at any time point during the study. In addition, there were no adverse clinical observations noted with respect to weight loss, facial swelling, or signs of distress.

HISTOLOGICAL ASSESSMENT

This study found no histological evidence of mineralized tooth structure initiation or formation associated with treated 2nd molar tooth buds (#18 and #31) at any time point. This finding of complete molar agenesis was consistent for all study animals (*n* = 12) and all treated sites (*n* = 24).

At 7 days post ablation, histological assessment of treated mandibular molar tooth buds (*n* = 8, teeth #18 and #31) demonstrated no viable tooth bud tissue inside the targeted bony crypt. Complete tooth bud necrosis was observed. The presence of nonvital tooth bud tissues within the bony crypt included predentin, enamel organ, inner epithelium, dental papilla, pulp and/or dental lamina tissues. The thermally affected sites were characterized by adjacent trabecular and transmural cortical bone necrosis with early stage fibroproliferative healing, trabecular bone healing, and/or reparative marrow changes. Minimal neovascularization was involved with the thermocoagulated treatment zone at this early stage. Healing changes extended variably outside the bony crypt of the tooth bud into tissues that were closely associated with the thin wall of the bony crypt. These immediately adjacent tissues were variably affected and included the transmural cortical bone of the bony crypt, the mandibular canal intima, distal mandibular foramen, inferior alveolar nerve, lingual nerve and/or adjacent skeletal muscle. The overlying oral mucosa appeared unaffected. Representative 7-day post ablation histology is shown in Figure 1.

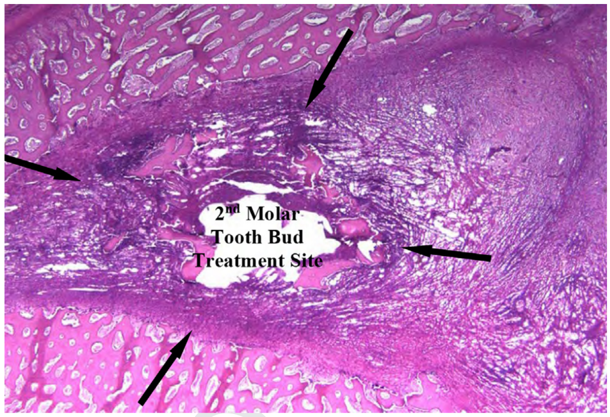

FIGURE 1. Representative 7-day post ablation of treated mandibular 2nd molar #31. Histology shows complete hyperthermic tissue necrosis of the tooth bud within the boney crypt (between *arrows*). Hematoxylin and eosin staining; 20x magnification. Photo credit: TriAgenics, Inc.

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At 14 days post ablation, histological assessment of treated mandibular 2nd molar tooth buds revealed one tooth bud (subject #1507, tooth #31) that appeared to have been partially ablated. This tooth bud had viable tooth bud follicle, enamel organ, dental papilla and pulpal tissues that constituted approximately 30% of original tooth bud volume. The remaining 70% of this tooth bud showed treatment-associated necrosis similar to other 14-day post treatment sites.

All other treated tooth buds at 14 days post ablation (n = 7) showed residual tooth bud necrosis. Nonvital remnant tooth bud structures included enamel organ, inner epithelium, dental papilla, pulp, and/or dental lamina within the bony crypt. Residual necrotic tissue without identifiable remnant tooth bud tissues was also observed. Bony crypt periosteal healing changes were present around the treated tooth bud with focal cortical bone thinning evident. Treated tooth bud sites were surrounded by primary stage fibroproliferative healing with variable myxoid features, adjacent trabecular bone necrosis, and new trabecular bone formation with reparative marrow changes and neovascularization. The zone of thermally affected tissue showed active healing extending outside the targeted bony crypt. This included tissues in the immediately adjacent mandibular canal, mandibular foramen, transmural cortical bone necrosis or defects, lingual nerve, and/or immediately adjacent lateral skeletal muscle. Portions of the adjacent inferior alveolar and lingual nerves showed evidence of hyperthermic exposure, while the overlying oral mucosa appeared unaffected. Representative 14-day post ablation histology is shown in Figure 2.

At 28 days post ablation, histological assessment of treated mandibular sites (n = 8, teeth #18 and #31) revealed no identifiable 2nd molar mandibular tooth bud tissues. Two treatment sites had small amounts of residual necrotic tissue without identifiable tooth bud remnant tissues. All treatment sites exhibited primary to secondary stage fibroproliferative healing. All treatment sites were surrounded by maturing trabecular

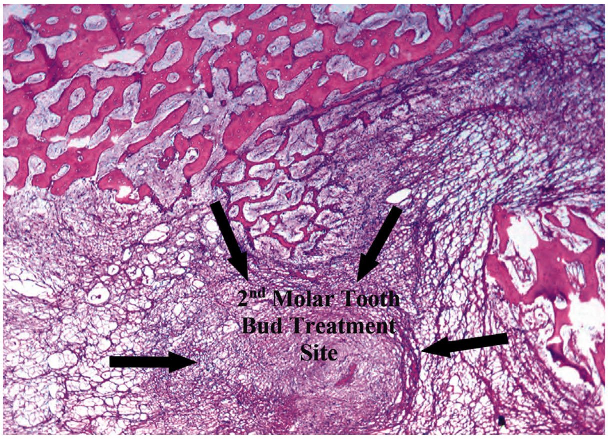

FIGURE 2. Representative 14-day post ablation of treated mandibular 2nd molar #31. Histology shows complete hyperthermic tissue necrosis of the tooth bud and adjacent tissue within the boney crypt (between *arrows*). Hematoxylin and eosin staining; 20x magnification. Photo credit: TriAgenics, Inc.

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new bone formation and reparative marrow. Minimal to mild chronic inflammation was present in the healing tissues. No acute inflammation or neovascularization was identified. The zone of active healing extended outside of the treated bony crypt. This included the immediately adjacent mandibular canal, transmural cortical bone, and/or inferior alveolar nerve. Associated periosteal healing changes were present, adjacent to the treated tooth bud site. Portions of the adjacent inferior alveolar nerves showed residual thermal effects alteration, while the overlying oral mucosa appeared unaffected. Representative 28-day mandibular post ablation histology is shown in Figure 3.

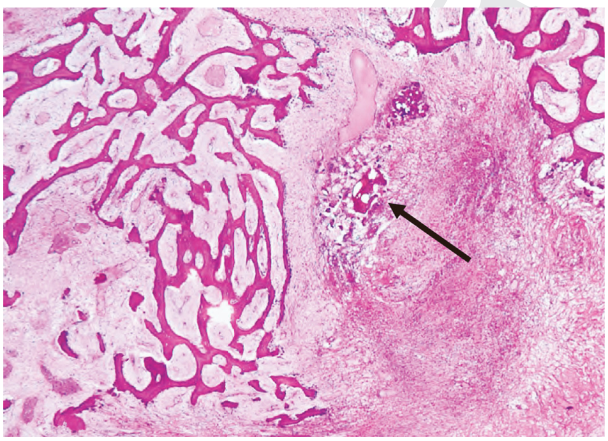

FIGURE 3. Representative 28-day post ablation of treated mandibular 2nd molar #31. Histology shows molar agenesis with complete removal of thermo-necrotic tissue (*arrow*) with nearly complete trabecular new bone formation encapsulating the former treatment site. Hematoxylin and eosin staining; 20x magnification. Photo credit: TriAgenics, Inc.

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RADIOGRAPHIC ASSESSMENT OF HEALING

This study found no radiographic evidence of mineralized tooth structure initiation or formation associated with treated second molar tooth buds #18 and #31 (n = 24) at any time point. Radiographic healing assessment scores are graphically summarized in Figure 4.

Radiographic evidence of treatment effects was pronounced at 7 days post ablation. However, by 14 days post treatment, radiographic evidence of treatment was minimal. At 28-days post ablation, radiographic evidence of treatment effects was nonexistent. Healing assessment scores indicate active bone regeneration into the bony crypt of treated sites at 14 days post ablation and nearly complete bony ingrowth by 28 days post ablation. The absence of the bony crypt of targeted tooth buds was the only evidence of treatment at 28-days post ablation.

The radiographic healing assessment score at 7-days post ablation was 2.3 (n = 8), indicating that healing inside the bony crypt was minimal at this early stage. There were significant changes radiographically evident in the adjacent bone surrounding the tooth bud. Changes included radiolucent circumscribed rings approximately 1 mm from the bony crypt of the targeted tooth buds and several instances of bony sequestrum evident in axial, coronal, and sagittal views.

At 14 days post oblation, the composite radiographic healing score was 2.1, indicating active bone regeneration. There were no radiographically evident bony sequestrations or other indications of thermal damage. As noted above, one tooth bud (subject #1507, tooth #31) was identified as partially ablated during histologic assessment at 14-days post treatment. This tooth bud was radiographically indistinguishable from other treatment sites at this time point.

The aggregate radiographic healing assessment score at 28 days was 0.6, indicating that healing appeared complete. Radiographic imaging at 28-days post ablation showed little to no trace of the original bony crypt of the targeted tooth bud, as bone regeneration appeared to be complete or nearing completion. Representative radiographic preoperative imaging and the subsequent healing responses at 7-, 14-, and 28-days post ablation are shown in Figures 5-8 respectively.

In comparison, unablated maxillary control tooth buds at 28-days post ablation showed rapid expansion of the tooth bud bony crypt diameter with rapidly progressing mineralized tooth structure formation. Representative radiographic imaging of maxillary control tooth bud formation is shown in Figure 9.

Discussion

The purpose of this 28-day longitudinal characterization study was to determine if the healing response

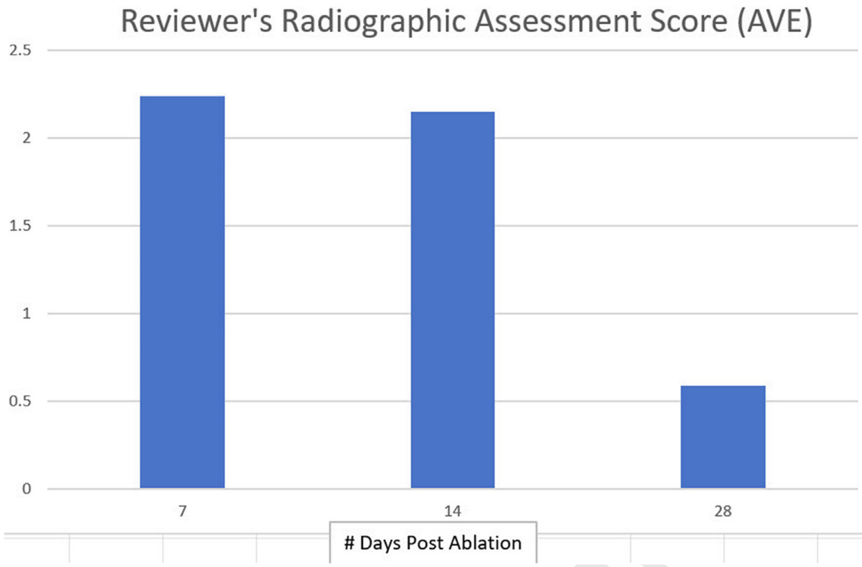

FIGURE 4. Radiographic peer review assessment scoring at 7-, 14- and 28-days post ablation. A radiographic assessment score of 0 indicates no bony crypt is present and bone regeneration appears complete with no evidence of thermal damage of immediate adjacent tissues.

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following fully guided microwave ablation of 2nd molar tooth buds in juvenile pigs would result in the complete removal of targeted tooth bud tissues, molar agenesis, and no significant collateral tissue damage would be observed.

The specific aims of this characterization study were *1)* to determine the efficacy of fully guided TBA for inducing molar agenesis; *2)* to determine whether the postablation healing process resulted in complete tooth bud tissue removal; and *3)* to determine whether the thermal dosing strategy employed resulted in ablation volumes that appeared to be consistent with prescribed ablation volumes.

The study sample size was carefully determined. This dichotomous study compared 24 maxillary unablated tooth buds to 24 mandibular treated tooth buds. The control demonstrated a 100% incidence (95% confidence level) of tooth formation compared to 24 mandibular ablated sites that had 0% tooth formation. The resulting post hoc statistical power is greater than 99% for detecting a statistically significant difference in outcomes between the ablated tooth buds and unablated control tooth buds. Doubling or tripling the sample size would not have changed the findings of this study and would have been criticized as being unnecessarily large when undergoing the IACUC review.

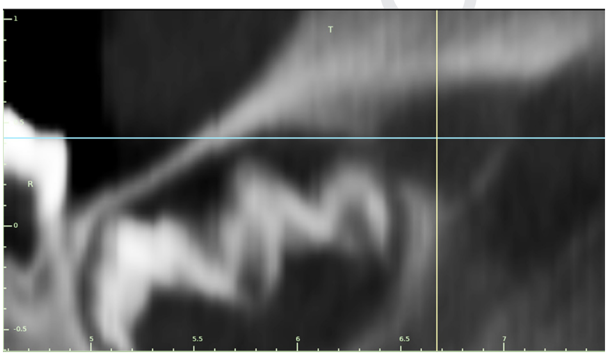

FIGURE 5. Representative 1-day preoperative sagittal view of tooth bud #18 (at crosshairs). Note the close proximity of the distal margin of tooth #19's developing crown and the bony separation of less than 1 mm from the mandibular canal in the pig model. Photo credit: TriAgenics, Inc.

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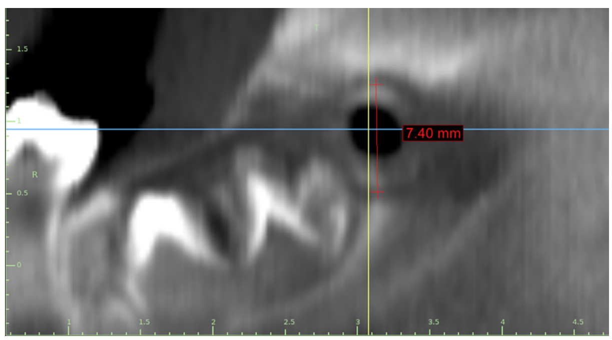

FIGURE 6. Radiographic view of treated tooth bud #18 (at crosshairs) showing the zone of thermonecrosis at 7-days post ablation (subject animal #1504). The ablation zone diameter was 7.4 mm compared to a prescribed ablation zone of 7.2 mm. Note that the distal marginal ridge of the developing crown of tooth #19 is in contact with the zone of thermonecrosis. In humans, 2nd molar crown formation is completed prior to radiographic detection of third molar tooth buds. Photo credit: TriAgenics, Inc.

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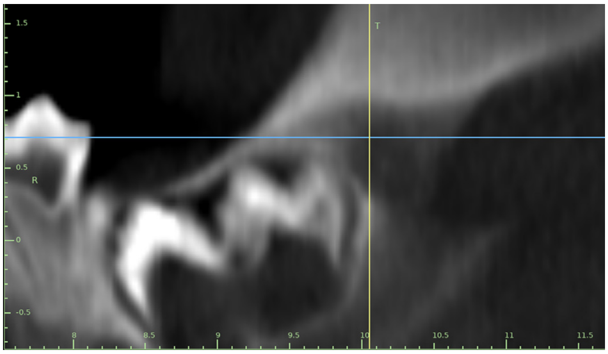

FIGURE 7. Radiographic view of tooth bud #18 (at crosshairs) at 14-days post ablation. Treatment site shows active bone regeneration. Photo credit: TriAgenics, Inc.

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KEY RESULTS

The results of this study confirm the hypothesis that fully guided TBA is effective at inducing complete molar agenesis. When thermal ablation of the entire tooth bud volume occurs, postablation healing processes appeared complete at 28 days post. Treated tooth bud tissues were resorbed and trabecular new bone formation was present in all treated sites. The ablation volumes prescribed for each subject were based on the maximum diameter of the bony crypt measured on CBCT scans. This thermal dosing strategy appeared to deliver targeted ablation volumes. Within the context of this animal model, there was no meaningful collateral tissue damage detected beyond the bony crypt of targeted tooth buds at 28 days.

This study found no radiographic or histological evidence of mineralized tooth structure formation associated with treated second molar tooth buds #18 and #31 (n = 24) at any time point.

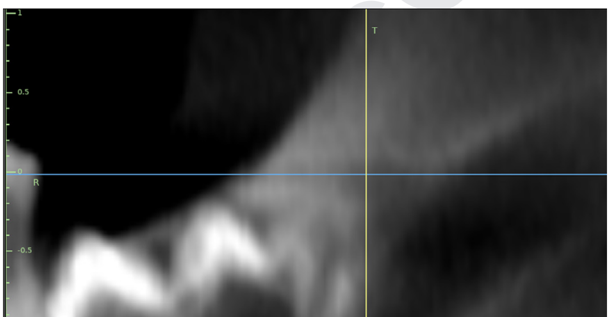

FIGURE 8. Representative 28-day postablation radiograph of tooth bud #18 (at crosshairs). Bone regeneration appears nearly complete. Photo credit: TriAgenics, Inc.

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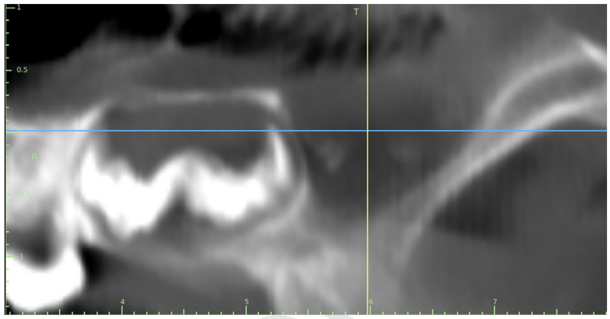

FIGURE 9. Representative 28-day postoperative sagittal view of control tooth bud #15 (at crosshairs). The diameter of the rapidly developing bony crypt of the tooth bud is approximately 80% of the diameter of tooth #14. Photo credit: TriAgenics, Inc.

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At 28 days post ablation, radiographic and histological assessment of treated mandibular sites revealed no identifiable 2nd molar mandibular tooth bud tissues.

This longitudinal study utilized a split-mouth study design involving a single cohort of 12 pigs that were randomly assigned to 3 subcohorts. Each subcohort consisted of 4 animals. All study animals had mandibular second molar tooth buds #18 and #31 treated at 7 weeks of age. A refined version of the fully guided microwave ablation technique, described in the principal investigator's previously published ablation study, was used to perform the guided ablations in this study.[4]

Characterization of postablation healing processes was conducted by obtaining radiographic and histological characterization of treated mandibular tooth bud sites at 7, 14, and 28 days. Untreated, opposing maxillary 2nd molar tooth buds served as radiographic and histological controls.

The animal model developed for this research appeared to be well-suited for this investigation. The variety of pig used is known for its rapid growth. The predictable timing for development of the pigs' 2nd molar mineralized tooth structure greatly facilitated this longitudinal study. Investigators chose to ablate seven-week-old pigs because mineralized tooth structure for maxillary and mandibular 2nd molars first become radiographically detectable in the animals at 8 weeks of age.

The investigators used an energy dosing strategy that was verified in a separate GLP-level animal study. This energy dose strategy was verified to be the minimum necessary energy dose to result in complete removal of targeted tooth buds. This thermal dosing strategy is intended to create a zone of thermonecrosis approximately 1 mm beyond the maximum diameter of the bony crypt. The radiographic presence of circumscribed radiolucent rings at 7 days post ablation correlated well with the planned diameter of each zone of ablation. Circumscribed radiolucent rings noted in axial, coronal, and sagittal views at 7 days post ablation indicate accurate positioning of the center of ablation in the center of the targeted tooth bud prior to delivering the energy dose. For example, when evaluating study animal 1504 (tooth bud #18), the maximum measured postablation bony crypt diameter was 5.2 mm, and the observed circumscribed radiolucent rings were approximately 7 mm in diameter at 7 days postoperation, as shown in Figure 6. The targeted tooth bud #18 was treated for 30 seconds, which is an energy dose that is expected to result in a 7.2 mm diameter zone of ablation.

Figure 10 shows TBA probe positioning in a surgical guide report. Figure 11 shows the 2 mm diameter TBA microablation probe completely seated into a TBA surgical guide used to ablate tooth bud #18. Figure 12 shows a subject's oral cavity with a small treatment wound immediately post ablation. There was no visual evidence of the treatment site at 7-days post ablation.

PRIOR STUDIES

Given the success of CT-guided microwave tumor ablation for treatment of patients with cancer, it is surprising to note how little research exists on guided TBA. Aside from the principal investigator's previously published TBA research and the present study, there are no other reports in the literature that explore this approach.

The use of CT-guided microwave tumor ablation supports the utility of the CT-guided approach used in this study. For example, Qi et al reported that 131 chest cavity tumors that were less than 5 mm from the diaphragm were treated using CT-guided microwave ablation, with a 1-year post treatment recurrence rate of 15% and no reported instances of severe complications.[6]

The overall histologic and radiographic findings of this study describe the expected course and timeline of tissue thermonecrosis induced through the process of thermocoagulation, where tissue temperatures do not exceed 100 °C. This is confusingly referred to as "cold ablation" and "low-temperature thermocoagulation,"

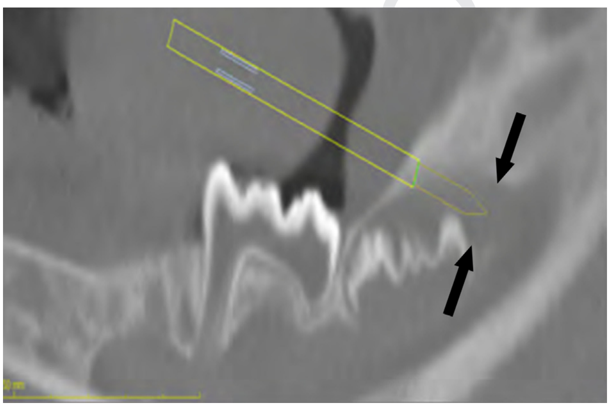

FIGURE 10. Representative surgical guide report showing the planned tooth bud ablation probe surgical path. Note that the probe extends approximately 20 mm beyond the surgical guide to reach tooth bud #18 (between *arrows*). Photo credit: TriAgenics, Inc.

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FIGURE 11. Intraoperative view of the tooth-supported surgical guide with the tooth bud ablation probe seated to its mechanical stop. Photo credit: TriAgenics, Inc.

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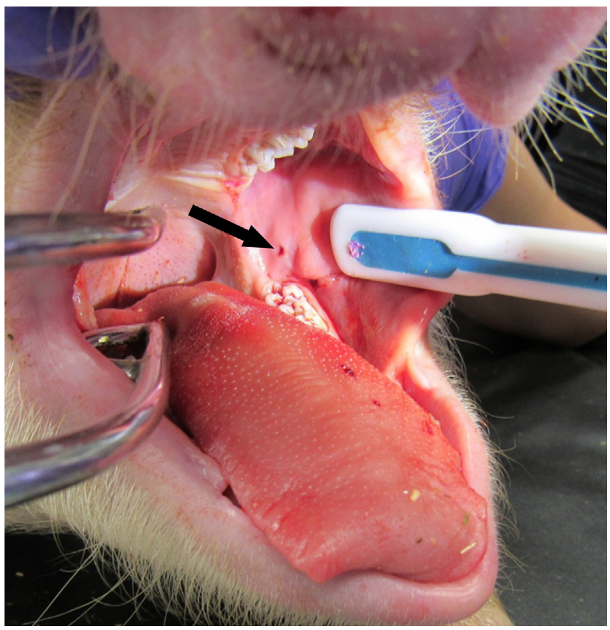

FIGURE 12. Immediate postoperative view of the 2 mm puncture wound (*arrow*) created by the TBA procedure. No puncture sites were detectable 7 days following TBA. Photo credit: TriAgenics, Inc. TBA, tooth bud ablation.

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which describes the hyperthermia treatment strategy commonly used to remove cervical precancer lesions.[7] The World health Organization recommends use of the phrase ‘thermal ablation’ to describe hyperthermia treatments that do not exceed 100 °C.[8] In preparation for this study, great care went into developing thermal ablation technology and an energy dosing strategy that limited tissue temperatures to less than 100 °C. Doing so eliminates the possibility of tissue carbonization (ie, charring), which occurs when tissue temperatures exceed 100 °C and water is physically driven out of tissues.

The unique technology used in this study was designed to promote diffuse microwave energy tissue penetration in a spherical pattern without exceeding 100 °C around the ablation probe. The thermal dosing strategy employed a low-temperature thermal ablation approach with prescribed outer margins of the ablation zone that were planned to reach temperatures of approximately 50 to 55 °C to achieve cell death through thermocoagulation. The resulting microvascular thermocoagulation in the outermost zone of ablation cuts off nutrient and oxygen supply to cells, resulting in a delayed necrosis. Further increasing tissue temperature results in undesirable physical changes, such as water vaporization that leads to tissue desiccation and tissue carbonization (ie, charring). These heat-induced physical changes do not enhance cell death directly, but have the potential to interfere with energy distribution and post ablation healing responses to the charred tissue remnants.[9]

LIMITATIONS

Regardless of the many physiological similarities pigs share with humans, differences in anatomy present important limitations when using a pig model. For example, the surgical pathway to a 7-week-old porcine tooth buds is substantially longer compared to human 3M tooth buds. To reach the center of targeted 2nd molar tooth buds, the investigators chose surgical access through the anterior of the ramus. While this was manageable, the probe extended 15 millimeters or more beyond the base of the surgical guide.

As a result of this extended surgical path, the histology report at 14-days post ablation showed one treatment site had 30% of its tooth bud tissue remaining vital, even though ablation volumes were prescribed to extend 1 mm beyond the bony crypt of the tooth bud. No mineralized tooth bud structure was identified, but vital tooth bud tissue was present and this was likely introduced by positioning error. As would be expected, the farther the ablation probe extends beyond the TBA guide, the greater the potential for positioning variance. Based on measurements obtained in mock TBA human clinical trials, investigators found that microablation probes extended no more than 6 mm beyond the surgical guide to reach third molar tooth buds #17 and #32 in human subjects aged 8 to 12. The surgical guide report in Figure 10 shows the TBA probe extending nearly 4 times farther (20 mm) beyond the TBA guide to reach the targeted 2nd molar.

Another important limitation to this animal model has to do with the small physical separation between the bony crypt of targeted tooth buds and the subjects’ adjacent tissue structures. Structure separation is extremely compact in 7-week-old pigs that weigh just 20 to 25 pounds at the time of treatment. Adjacent structures in pigs are within 1 to 2 mm of the bony crypt of the tooth bud compared to 5 mm or more in adolescent human subjects.

The energy dosing strategy used in this study was to deliver an energy dose that would result in a zone of ablation that would extend beyond the maximum measured diameter of the targeted bony crypt. Porcine tooth buds have an oval shape that is maximally elongated in the anterior/posterior dimension. That means that tissue structures adjacent to the narrowest portion of the oval tooth bud will be included in the prescribed zone of ablation. In pigs at this age, the walls of the 2nd molar bony crypt can be a millimeter or less in thickness. Because of the close proximity to these thin walls of the bony crypt, the mandibular foramen, the mandibular canal, the lingual nerve, and the lateral muscle attachments in this animal model were peripherally affected at the narrowest part of the targeted tooth bud. In contrast to humans, there is generally 5 mm or more of separation between the inferior border of the bony crypt of the third molar tooth bud and the superior aspect of the mandibular canal, making it physically impossible to induce nerve damage using the thermal dosing strategy used in this study.

Drawing conclusions based on interspecies testing carries a degree of uncertainty when extrapolating effects in humans. This model enabled a detailed description of the post ablation healing cascade that can be reasonably expected in humans. Pigs have been validated as a good comparative burn/healing model to human burn and healing. The postburn healing process in pigs and humans occur through physiologically similar phases (inflammation, proliferation, and remodelling) and timeframes.[10]

The fully guided TBA system deployed in this study was used in a fashion that will be consistent with its intended use in humans. The maximum diameters of targeted mandibular 2nd molar tooth buds in this pig model ranged from 6 to 7 mm. This diameter is similar to the maximum diameter of 7 to 8 mm found in human mandibular third molars at age 9 years, based on an internal study by the investigators that included more than 1,100 CBCT scans obtained for orthodontic purposes.

A notable weakness of this study is that there was no attempt to obtain sequential CBCT images of the same subject animals in the 14- and 28-day subcohorts. Interim imaging was not obtained because of concerns associated with adverse anesthesia-related events, such as bronchial and pulmonary infections, which are secondary to repeated intubation of these study animals. A study that collects longitudinal imaging at 7-, 14-, and 28-day intervals on the same subject animals could provide more information on the postablation healing sequelae.

The sample size of each subcohort was small (4 animals with 8 treated tooth buds).

Conclusion

The histological and radiographic findings of this study confirm the hypothesis that fully guided TBA is effective at inducing complete molar agenesis. When thermal ablation of the entire targeted tooth bud volume occurs, the postablation healing process results in tooth bud agenesis and trabecular new bone formation within 28 days. The thermal dosing strategy used in this study appears to deliver the prescribed ablation volumes and–within the context of this animal model–resulted in no meaningful collateral tissue damage.

The investigators have completed mock 3TBA human clinical trials and are in the process of seeking approval for first in-human 3TBA feasibility trials.

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