Fully Guided Third Molar Tooth Bud Ablation in Pigs

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Purpose: The purpose of this study was to selectively ablate the entire volume of targeted third molar (3M) tooth buds in a pig model. This study demonstrates the potential for a less-invasive alternative to contemporary surgical techniques for removal of 3Ms.

Methods: The investigator developed a mandibular split-mouth animal model study design. The model used pigs because the animals' 3M tooth buds are dimensionally similar to those of humans. The study sample consisted of 5 female Yorkshire-cross pigs at 20 weeks of age. The investigator delivered microwave energy thermal doses to thermocoagulate tooth bud tissues inside the bony crypts of targeted 3M tooth buds. Based on the bony crypt dimensions obtained from computed tomography scans of each subject, the microwave thermal dose was predetermined. The mandibles were dissected to visually compare thermocoagulated right-side 3M tooth bud tissues to left-side untreated controls.

Results: All 5 study animals were successfully treated. All 5 fully guided third molar tooth bud ablation (3TBA) procedures resulted in thermocoagulation of the entire volume of targeted 3M tooth bud tissues, with no visual evidence of damage to structures beyond the bony crypt.

Conclusions: The animal model developed for this study enabled the demonstration of a fully guided 3TBA protocol. The animal model and 3TBA procedure employed in this study appear to be appropriate for use in future long-term animal studies designed to demonstrate the efficacy of 3TBA for inducing molar agenesis.

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At the time of this study and at the submission of this report, there were no prior studies published that described partially guided or fully guided molar tooth bud ablation by any ablation means. Investigators searched bibliographic databases, including MEDLINE (https://www.nlm.nih.gov/medline/index.html), Google Scholar (http://scholar.google.com/), and PubMed (PubMed.gov). This search used the following keywords with no restriction on date range: microwave ablation swine, third molar tooth bud ablation, third molar tooth bud surgery, and third molar agenesis. No alternatives for the in vivo procedures used in this study were identified.

The purpose of this study was to implement a fully guided surgical technique that selectively ablates third molar (3M) tooth bud tissues without damaging structures outside the boney crypt of the targeted tooth bud in a pig model. The investigator's hypothesis was that the fully guided third molar tooth bud ablation (3TBA) procedure used in this study would result in thermocoagulation of the entire volume of targeted 3M tooth bud tissues, with no visual evidence of damage to structures beyond the bony crypt. This research is the first step in developing fully guided 3TBA as a practical and effective means of clinically inducing 3M agenesis in humans. The clinical goal of 3TBA is to minimize surgical risk and improve lifelong clinical outcomes compared to established 3M management strategies. This animal model will be used in future studies designed to establish 3TBA procedure safety and efficacy.

Private Practitioner and President & CEO of TriAgenics, Inc, TriAgenics, Inc, Redmond, OR.

Conflict of Interest Disclosures: Research performed in this Phase I animal trial was personally funded by Leigh E. Colby. Following completion of the research work reported herein, Leigh E. Colby incorporated TriAgenics, Inc, which is working to finalize third molar tooth bud ablation technology development and enter first-inhuman clinical trials in 2022.

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This study tested a specialized refinement of microwave ablation technology, which was specifically designed to yield predictable, appropriately shaped soft-tissue microablation volumes. The microablation technology was combined with guided positioning and guided ablation volume control to selectively thermocoagulate tooth bud tissue. The specific aims of this study were to (1) develop a 3TBA procedure in pigs that will enable credible evaluation of 3TBA efficacy and safety in longer-term animal studies, (2) verify the microablation probe's center of ablation was in the center of the bony crypt of targeted 3M tooth buds, (3) confirm that preoperatively prescribed energy doses ablated the entire volume of each tooth bud, and (4) visually confirm that the zone of thermocoagulation appeared to be limited to the targeted tooth bud tissue inside the bony crypt of each tooth bud.

Adaptation of microwave tumor ablation technology, in combination with 3-dimensional (3-D) x-ray imaging and high-precision surgical guides, has the potential to provide an alternative to 3M surgery for many patients. This study seeks to provide important new information on a minimally invasive procedure that aims to leave a sutureless, 1 to 2 mm puncture at the site of each tooth bud.

The clinically driven goals of future fully guided 3TBA microablation procedures in children are 4-fold, which are as follows: (1) 100% efficacy at inducing complete 3M tooth agenesis, (2) elimination of common postoperative complications by implementing a minimally invasive technique, (3) elimination of collateral tissue damage outside the bony crypt of the tooth bud, and (4) 3TBA procedure times that are practical for outpatient pediatric treatment using microablation cycle times of 2 minutes or less. To meet these challenging clinical goals, a rapid, high-precision approach is required.

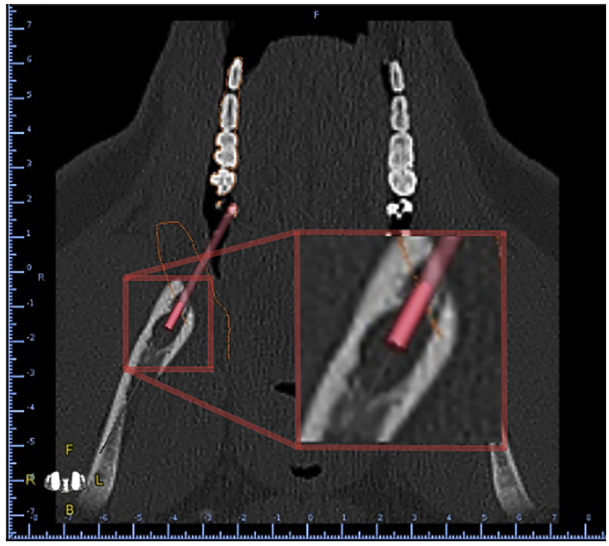

FIGURE 1. The axial view of pig #1 in surgical guide design software. The enlarged insert shows the planned position for the center of ablation of the microablation probe in this plane.

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Material and Methods

STUDY DESIGN/SAMPLE

To address the research purpose, the investigator designed and implemented a mandibular split-mouth animal study that compared right mandibular 3TBA with left-side, unablated tooth buds. The left, unablated tooth buds served as the control group.

Researchers chose pigs as test subjects because the animals' 3M tooth bud development resembles that of humans. Initiation of 3M development in both humans and pigs takes place after birth. This is significant because complete bony encapsulation does not occur until after tooth structure becomes radiographically evident. Research protocols were submitted and approved by the Institutional Animal Care and Use Committee of Oregon State University under IACUC application no. 4194.

This study employed 5 female Yorkshire-cross pigs that were 20 weeks of age at the time of ablation. The subjects' stage of 3M development at 20 weeks was nearly identical in size to that of humans before mineralized tooth structure begins to form. Body weight on arrival ranged from approximately 45 to 50 kg. The animals were acquired from an Oregon-based breeder/supplier approved by Oregon State University. In anticipation of long-term studies, researchers chose female test subjects because they are less aggressive to one another, and they are safer for staff to handle under laboratory conditions.

The group size is based on guidance from the Food and Drug Administration for other medical devices (www.fda.gov/regulatory-information/search-fda-guidance-documents/general-considerations-animal-studies-medical-devices). While the Food and Drug Administration does not specify a model or species, the recommendation for the number of animals is generally a number greater than 4 that allows sufficient data to evaluate the technology. A cohort of 5 animals was chosen, based on preliminary preclinical studies and published reports providing recommendations for a sufficient number of animals to allow evaluation by a veterinary pathologist (Schuh, Joann; Medical Device Regulations and Testing for Toxicologic Pathologists; Toxicologic Pathology, 36:63-69, 2008). In addition,

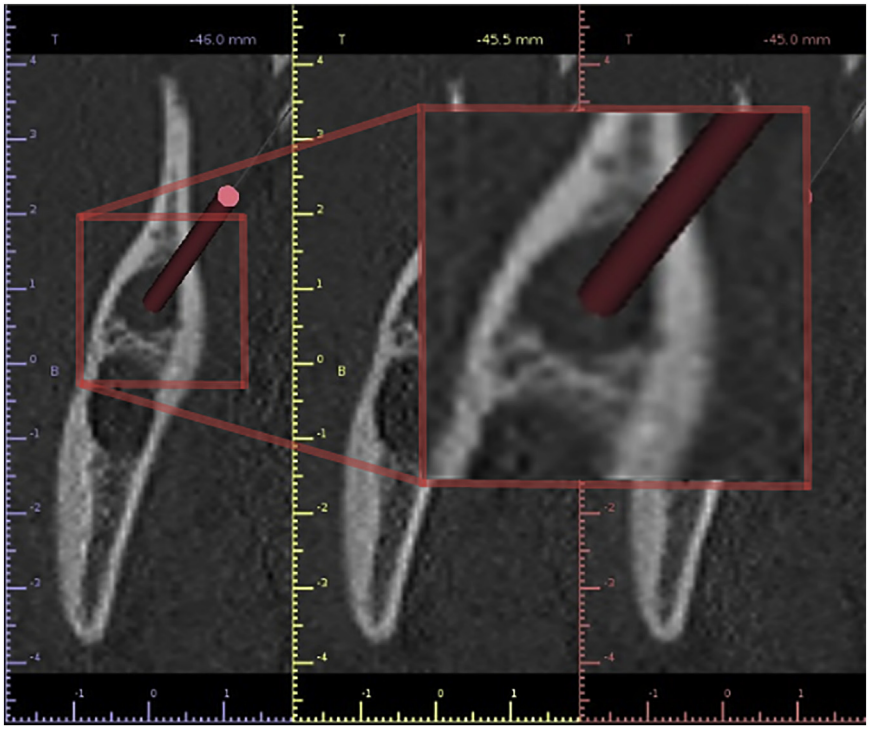

FIGURE 2. The coronal view of pig #1 in surgical guide design software. The enlarged insert shows the planned position for the center of ablation of the microablation probe in this plane.

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because the expectation is that tooth buds will be ablated, the estimated sample size (n = 5) was required to be 5 for statistically significant results with an alpha of 0.01 and beta of 0.20 (Bolton, Sanford and Bon, Charles; Pharmaceutical Statistics, Practical and Clinical Applications; Marcel Dekker, Inc, New York, NY; 2004.)

DATA COLLECTION METHODS

This study was organized around 2 "appointment" days for all 5 test subjects. The purpose of the first appointment was to obtain dental impressions and computed tomography (CT) scans to create custom 3TBA guides for each subject. The 3TBA guides would

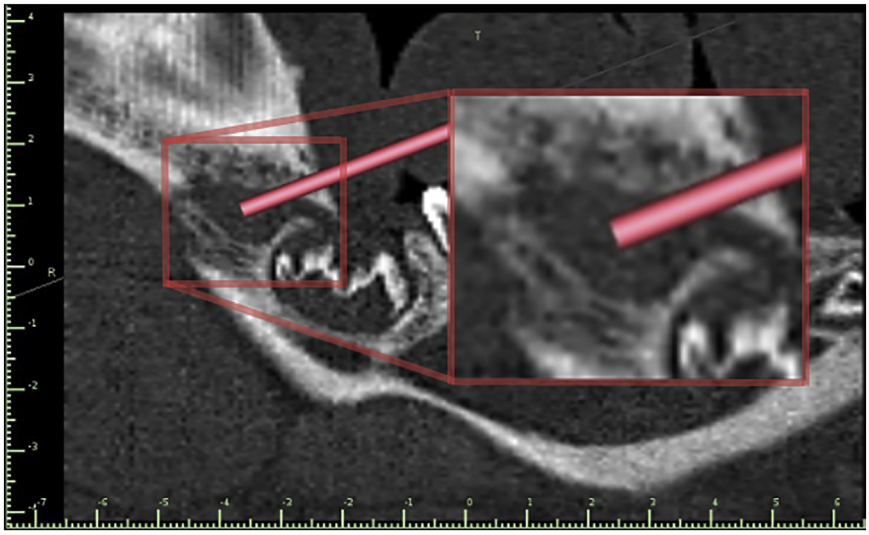

FIGURE 3. The sagittal view of pig #1 in surgical guide design software. The enlarged insert shows the planned position for the center of ablation of the microablation probe in this plane.

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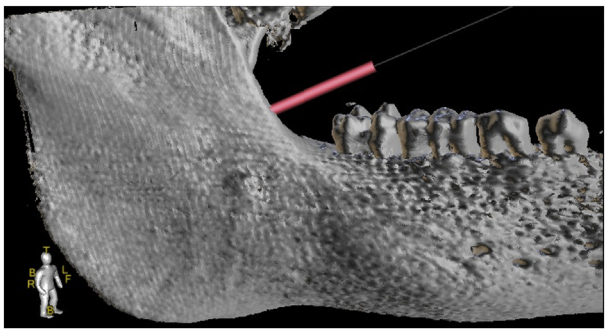

FIGURE 4. The lateral view of the planned surgical path for placement of the microablation probe into tooth bud number 32. Image is derived from the surgical guide report created in BlueSkyPlan, the dental treatment planning software used for this study. This image is representative of all subjects.

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be used to complete tooth bud ablation procedures during a second appointment day.

At the first appointment, each animal was restrained with a pig board and sedated using a combination of ketamine (15 mg/kg), xylazine (2 mg/kg), and butorphanol (0.2 mg/kg). Technicians administered the sedative via intramuscular injection into the muscle mass lateral to the T1 area on each animal's back. Investigators then affixed identification ear tags, and the animals were placed in the supine position on a CT scanning table. Care was taken to secure the pigs so that they would not be able to move their heads during the CT imaging procedure. Layers of surgical tape were used to carefully position mandibles so that the study animals' plane of occlusion was 90° relative to the CT imaging bed. The animals' heads were supported by sand bags, so that the mandibular plane of occlusion and the animals' spines were at a 90° angle to the bed of the CT scanning table. This ensured a correct volume scan orientation for later software interpretation. Each pig's head was radiographically scanned using a 64-slice Toshiba Aquilion CT scanner (Toshiba America Medical systems, Inc, Tustin, California, USA) with slices acquired every 1 mm. During CT scanning, technicians maintained constant visual contact to confirm the subjects' heads did not move.

Axial, coronal, and sagittal reconstructed CT images were evaluated for the presence and 3D location of left and right mandibular 3M buds. Tooth bud dimensions were obtained from the CT images using commercially available software (eFilm, Merge Healthcare, Heartland WI, USA). The presence of bilateral mandibular 3M tooth buds, their size, and the lack of mineralized tooth structure were then confirmed. All restraints were then removed, each pig was rolled onto its left side, and each subject's mouth was propped open for cleaning. A toothbrush was then used to remove excess feed from the mandibular dentition.

Using custom dental trays fabricated from pig cadaver studies, fast-set polyvinylsiloxane dental impressions were then obtained. The half-arch impression encompassed the right mandibular dentition and jaw extending from the midline distal to the retromolar area that specifically included the ramus of the mandible. The dental impression and CT data from the initial x-ray images were then used to construct custom 3TBA guides using commercially available

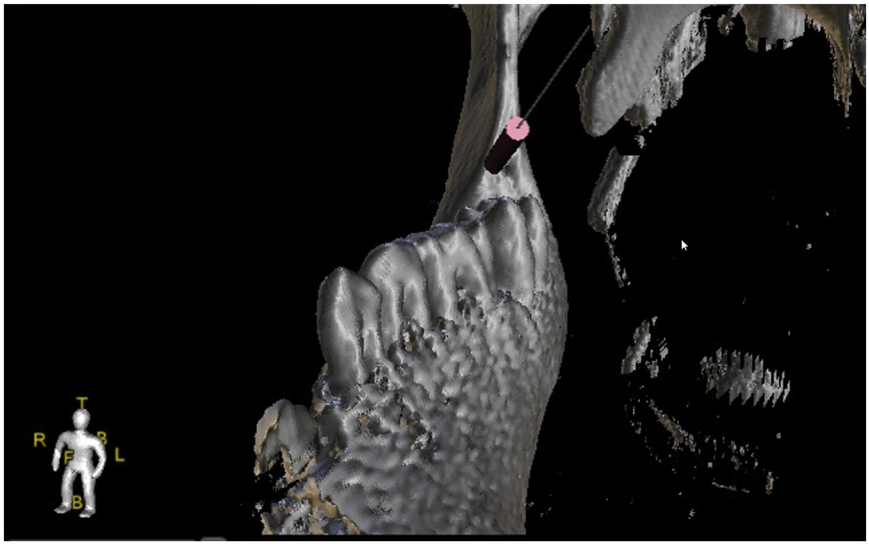

FIGURE 5. The anterior view of the planned surgical path for placement of the microablation probe into tooth bud number 32. Image is derived from the surgical guide report created in BlueSkyPlan, the dental treatment planning software used for this study. This image is representative of all subjects.

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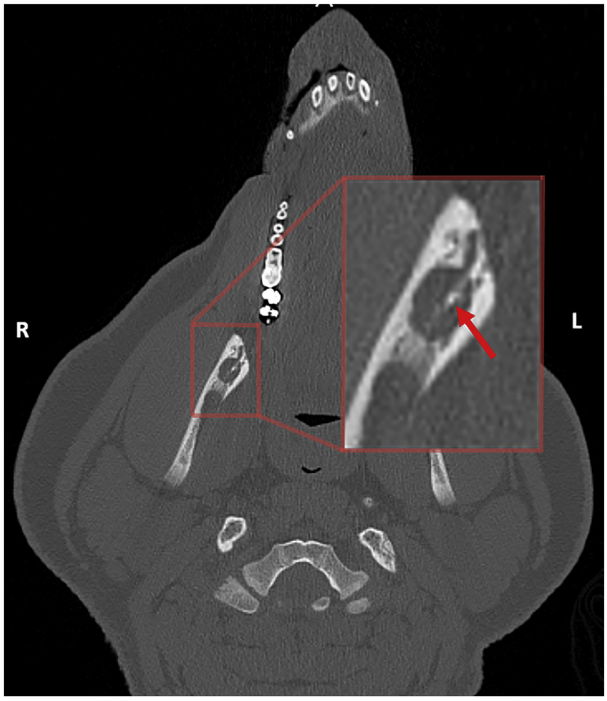

FIGURE 6. The immediate postoperative CT axial view of pig #1. The red arrow points to the distal tip of the x-ray marker, which is fully seated in the surgical guide. The marker is nearly centered anterior/posteriorly in the third molar tooth bud bony crypt and slightly medial of center by approximately 1 mm. The 1-mm slice resolution of the CT scanner limited the ability to localize the distal end of the radiographic marker. CT, computed tomography.

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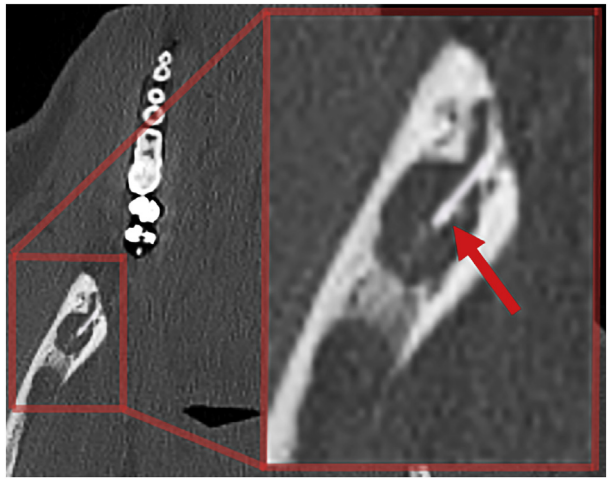

FIGURE 7. An enhanced close-up of the post-3TBA x-ray marker shown in Figure 6. 3TBA, third molar tooth bud ablation.

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software (BlueSkyPlan, Blue Sky Bio, LLC) with a design goal of placing the microablation probe with its center of microablation within the center of the targeted tooth bud.

Following the first appointment, the animals were monitored for signs of distress during recovery from the imaging procedure and remained in a recovery stall postoperatively for 4 to 6 hours before returning to their preoperative environment.

The microablation procedure was performed at a second appointment within 4 days of obtaining the impressions and initial CT scans. The short, 4-day period between the 2 appointments was necessary because the pigs were growing at a rate of 1 to 1.5 pounds per day. As with the first appointment, all test subjects received the 3TBA procedures in succession on the same day.

At the second appointment, the pigs were sedated and placed on their left side on the CT scanning table. Each animal's mandible was propped open, and a toothbrush was used to remove excess feed and other debris from the mandibular right dentition. The 3TBA guide was seated and a solid, stable fit confirmed before proceeding. Using the 3TBA guide and a 2.0-mm orthopedic bone drill, an osteotomy was created to allow access through the dense cortical bone of the anterior of the ramus. Anterior access through the ramus of the mandible is required in the pigs because medial access to the 3M tooth bud is not practical due to the pig's narrow, elongated mandible and large tongue. After the anterior osteotomy was created, a proprietary microwave ablation probe was then inserted into the 3TBA guide and pressed fully to the mechanical stop on the probe. The surgical positioning of the microablation probe was predetermined, so that the position of the probe's center of ablation was in the center of the tooth bud.

After the microwave microablation probe was positioned in each mandibular right 3M tooth bud, a microwave energy dose was delivered using microwave radiation at 8.0-GHz and 5-watts continuous power for 120 seconds using an Emblation Microwave model ISYS800 (Emblation Microwave, LTD, Scotland, UK). In ex vivo testing using surrogate tissues at body temperature, 120 seconds was estimated to deliver adequate energy to ablate 100% of the tooth bud and dental follicle tissues in the pigs with the observed tooth bud dimension. Based upon ex vivo microwave ablation testing of fresh beef liver and pork loin tissue samples at body temperature, the demonstrated soft-tissue ablation diameters were planned to be identical to the tooth bud diameters measured on the subject animals. The percent reflected microwave energy was monitored during each ablation and confirmed to be less than 10% to assure consistent energy delivery into the targeted tooth bud tissue. Upon delivery of the prescribed energy dose, the microablation probe was immediately removed and a metal x-ray marker made from 12-gauge copper wire was then

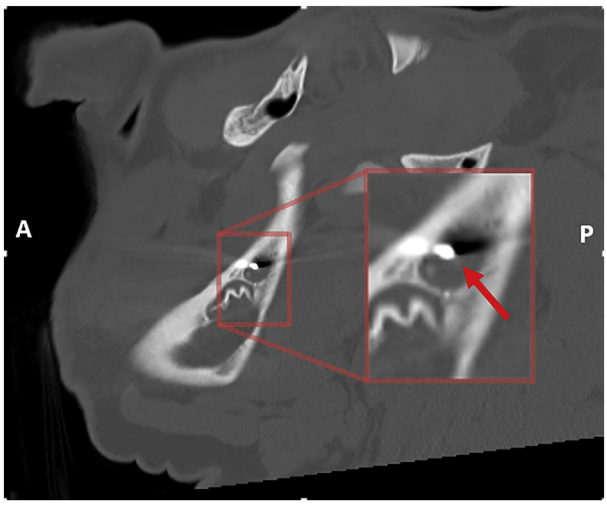

FIGURE 8. The immediate postoperative CT sagittal view of pig #1. The *red arrow* points to the distal tip of the x-ray marker, which is fully seated in the surgical guide and nearly centered within the third molar tooth bud bony crypt. The 1-mm slice resolution of the CT scanner limited the ability to localize the distal end of the radiographic marker. CT, computed tomography.

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inserted and fully seated to its mechanical stop in the 3TBA guide. The mechanical stop for each x-ray marker had a length that matched the center of ablation of the microablation probe and the center of the targeted tooth bud for each subject animal. The 3TBA guide was secured on teeth of each animal with the x-ray marker in position, and an immediate postoperative CT scan was obtained at the same resolution as the preoperative CT x-ray scan. The 3TBA guide and radiation marker were then removed. Each study animal was subsequently euthanized.

DATA ANALYSIS

During the necropsy, each mandible was retrieved intact and labeled. Each mandible was then denuded and a window cut through the dense cortical bone on the medial aspects of left and right sides to expose the 3M tooth bud tissue and cavity. The right-side tooth bud tissue was exposed and photographed to record the degree of soft-tissue thermocoagulation. The 3TBA guide was then repositioned on the right mandibular dentition, and both the microablation probe and the x-ray markers were used to determine the surgical positioning of the center of ablation of the microablation probe inside the bony crypt of the tooth bud. Once the ablated tooth bud tissue was removed for gross inspection, the x-ray markers with stops on them were then seated and used to verify ablation probe positioning within the bony bud crypt. A caliper was used to measure the final x-ray marker tip position relative to the now-empty 3M bony crypt. Unablated tooth bud tissue controls on the left side were exposed and photographed for comparison to the right-side ablated tooth buds. The distal aspect of the second molar tooth bud was used to visually compare with the ablated tissue of the targeted tooth bud to determine the extent of thermocoagulation.

Results

Five pigs (identified as #1 through #5) were treated according to the 2-appointment 3TBA protocol described previously. A sixth test subject was eliminated from the study because of health issues that developed before research began.

The investigator used 3D surgical guide planning software (BlueSkyPlan) to determine the center of ablation and surgical path for each subject. Figures 1-3 show the planned center of ablation and planned surgical path determined from the first appointment with pig #1. Figures 1-3 show axial, coronal, and sagittal planes, respectively. Figures 4 and 5 show the planned surgical path of the microablation probe (lateral and anterior views, respectively). Figures 1-5 are representative of all test subjects.

The anatomy of the test animals affected the surgical path for microablation used in this study. Because of limited medial access to 3M tooth buds in pigs, it was determined that the best path for the microablation probe was through the anterior of the ramus of the mandible. This resulted in a 48- to 52-mm working length from the center of ablation to the anterior seat of the 3TBA guide.

Following the first appointment, custom 3TBA guides for all animals were created on a 3D printer. The center of ablation for each microablation probe was preoperatively measured. Custom mechanical stops were placed on each microablation probe for

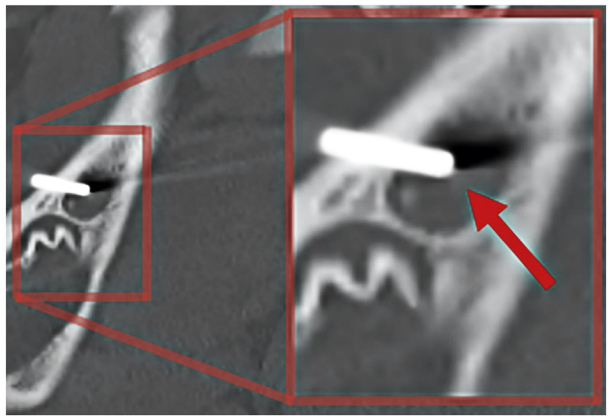

FIGURE 9. An enhanced close-up of the post-3TBA x-ray marker shown in Figure 8. 3TBA, third molar tooth bud ablation.

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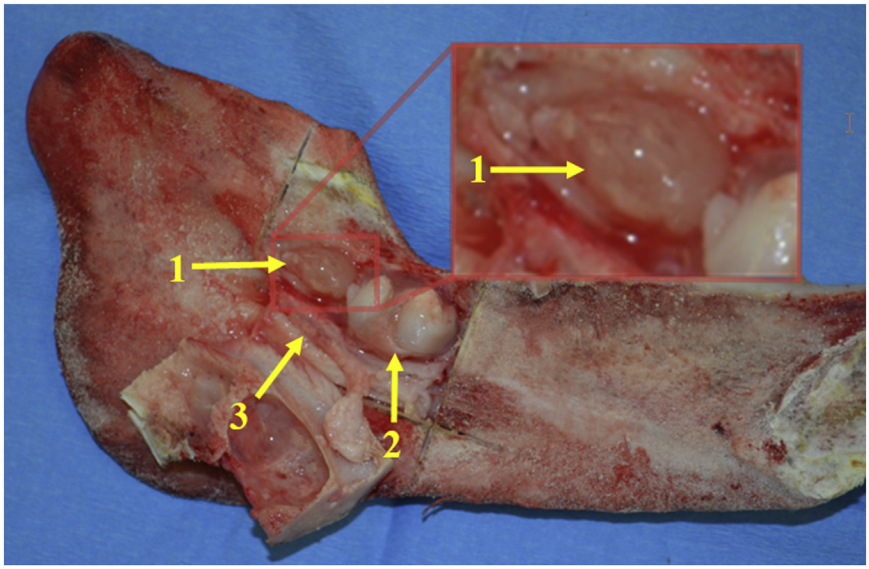

FIGURE 10. The unablated left mandible control of pig #1 is shown below with a medial window cut into the ramus to expose the third molar tooth bud (*arrow 1*) and the coronal portion of the forming second molar tooth follicle (*arrow 2*). The enlarged image shows the unablated left-side third molar tooth bud control. The distal surface of the developing second molar tooth bud follicle is nearly touching the mesial third molar tooth bud. Note the soft pink, lightly perfused appearance that matches the developing second molar anterior to the right mandibular ablated third molar tooth bud in Figure 11. The mandibular canal neurovascular bundle containing the inferior alveolar nerve, artery, and vein (*arrow 3*) is separated by a 3- to 4-mm thick osseous wall inferior to the third molar tooth bud. (Photo: Courtesy of John Mata).

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quick intraoperative positioning once each probe was fully seated to its mechanical stop.

On the second appointment day, 3TBA procedure times were less than 4 minutes for each of the 5 animals. This included the time to prop the pig's mouth open, clean the pig's teeth, firmly seat the 3TBA guide, perform the osteotomy through the ramus, insert the microablation probe to its stop, and perform the 120-second microablation.

Ablation times were determined by in vitro body-temperature ablation volume testing and the measured tooth bud volumes evident on CT scans. Because the tooth buds were nearly identical in size, the 120-second ablation at 5 watts was determined to be appropriate for all 5 animals. Intraoperative monitoring of the percent reflected energy showed that less than 10% energy was reflected (ie, not delivered), confirming delivery of the total planned microwave energy into the tooth bud tissue.

With the guide remaining in place, the researcher immediately removed the 3TBA probe and inserted an x-ray marker. The x-ray marker tip was configured to match the center of ablation of the microablation probe. Upon seating the x-ray marker, CT imaging was obtained with the guide and marker in place. Upon reviewing each set of post-3TBA CT images for the 5 subject animals, the distal tip of the x-ray marker was demonstrated to be positioned in the center of the 3M tooth bud bony crypt. This was observed with a maximum variation from center measured at 1 mm. Figure 6 shows the tip of the post-3TBA x-ray marker in subject animal #1 in the axial CT plane. Figure 7 is an enhanced close-up of the x-ray marker in Figure 6. Figure 8 shows the tip of the post-3TBA x-ray marker in subject animal #1 in the sagittal CT plane. Figure 9 is an enhanced closeup of the x-ray marker shown in Figure 8.

The CT scanner used in this study was intended for scanning large animals at the Veterinary Teaching Hospital of Oregon State University. The machine's large scan volume adversely effected the spatial resolution and image quality of the radiographic markers. The scanner's 1-mm CT plane slices limited measurement resolution and the ability to accurately determine the x-ray marker's tip position compared to higher-resolution cone beam CT imaging. The results illustrated in Figures 6-9 are representative of the results for all test subjects. Subsequent studies by the author used higher-resolution cone beam CT imaging.

Figure 10 shows the denuded and dissected left half of the mandible used as an unablated control. The medial aspect of the cortical plate over the ramus and body of the mandible has been reflected to expose the developing second molar and 3M tooth bud tissues. The enlarged insert in Figure 10 shows the immediate proximity of the 3M tooth bud to the adjacent second molar tooth bud. It also shows a 3- to 4-mm wall of osseous tissue separating the 3M tooth bud from the mandibular

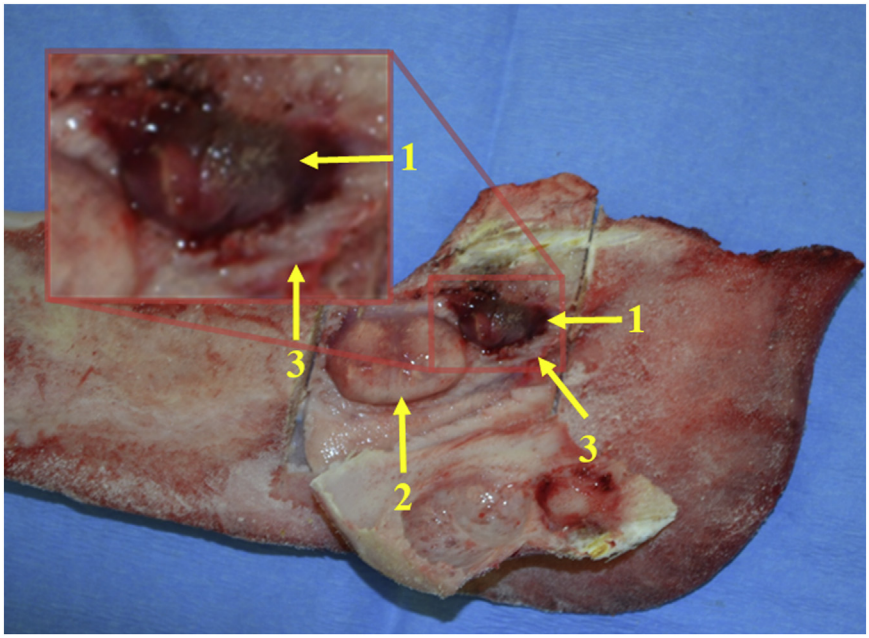

FIGURE 11. The right mandible of pig #1 with the microablated third molar tooth bud (*arrow 1*). The effects of microwave-induced thermocoagulation are immediately visible compared to the distal portion of the adjacent tooth bud follicle containing the developing second molar (*arrow 2*) and by comparison with the left-side unablated third molar tooth bud control in Figure 10. The 3- to 4-mm wall of osseous tissue (*arrow 3*) inferior to ablated third molar tooth bud that separates the mandibular canal and contains the inferior alveolar nerve, artery, and vein present appears unaffected. (Photo: Courtesy of John Mata).

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canal and the neurovascular bundle containing the inferior alveolar nerve, artery, and vein.

Figure 11 shows the denuded and dissected right half of the mandible containing the microablated 3M tooth bud. Comparison of Figures 10 and 11 show the localized effects of thermocoagulation. The thermocoagulated 3M tooth bud tissue (Fig 11) appears dark and bloody compared to light pink unablated control tissues (Fig 10). The adjacent second molar tooth bud follicle appears unaffected in all the 5 animals, suggesting that the zone of thermocoagulation was confined to the 3M tooth bud tissue within the bony crypt of the tooth bud.

Following physical exposure of the right-side microablated 3M tooth bud on each subject animal, the tooth bud tissue was then removed and confirmed to be 100% ablated based on gross appearance relative to the unablated control. Using a caliper, measurements were then obtained from the tip of the post-3TBA x-ray marker relative to the walls of the bony crypt. Figure 12 shows the custom 3TBA guide reseated on the denuded and dissected mandible of pig #1. The metal x-ray marker is fully seated into the custom 3TBA guide. The x-ray marker tip represents the center of microablation and is centered vertically and anterior/posteriorly in the 3M tooth bud bony crypt.

Figure 13 shows a closer view of the 3TBA guide and seated post-3TBA x-ray marker on pig #1. The tip of the x-ray marker, which represents the center of microablation, appears well centered in the 3M tooth bud crypt vertically and anterior/posteriorly. Figure 14 shows a close-up view of the x-ray marker tip seated in its 3TBA guide on pig #2. This image shows the 3 to 4 mm of osseous tissue separating the 3M tooth bud from the mandibular canal and the very close proximity (approximately 1 mm) of the unablated, highly vascularized second molar tooth bud follicle containing the developing second molar. All dissections and x-ray marker placements correlated well with the preoperative 3TBA guide designs and immediate postoperative CT imaging of the x-ray marker tips for each test animal. Three-dimensional positioning error for the center of ablation in relation to the center of each 3M tooth bud was confirmed to be 1 mm or less.

Discussion

The purpose of this study was to implement a fully guided surgical technique that selectively ablates 3M tooth bud tissues without damaging structures outside the boney crypt of the targeted tooth bud in a pig model. The investigator's hypothesis was that the fully guided 3TBA procedure used in this study would result in complete thermocoagulation of the entire volume of targeted 3M tooth bud tissues, with no visual

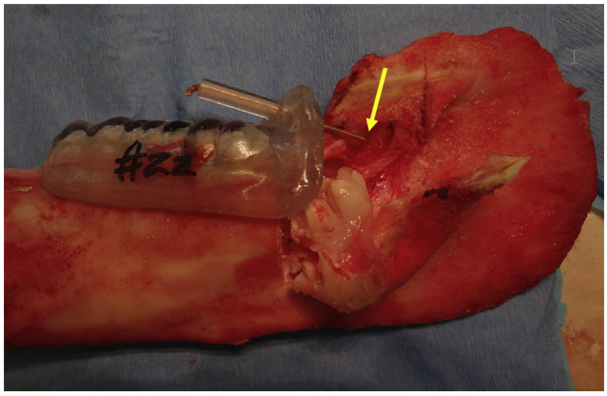

FIGURE 12. The microablation surgical guide for pig #1 is shown seated on the right mandibular posterior teeth. The x-ray marker has been seated into the microablation surgical guide. The x-ray marker tip (at *arrow*) appears to be well centered in the middle of the third molar tooth bud crypt vertically and anterior/posteriorly and represents the predetermined center of ablation. (Photo: Courtesy of John Mata).

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evidence of damage to structures beyond the bony crypt. The specific aims of this study were to (1) develop a 3TBA procedure in pigs that will enable credible evaluation of 3TBA efficacy and safety in longer-term animal studies, (2) verify the microablation probe's center of ablation was in the center of the bony crypt of targeted 3M tooth buds, (3) confirm that preoperatively prescribed energy doses ablated the entire volume of each tooth bud, and (4) visually confirm that the zone of thermocoagulation appeared to be limited to the targeted tooth bud tissue inside the bony crypt of each tooth bud.

KEY FINDINGS

The fully guided 3TBA technique employed in this live animal feasibility study represents a methodical approach predicated on precisely localizing tooth bud tissues and predetermining tooth bud microablation volumes using 3D imaging. The center of ablation – regardless of the type of ablation used – can then be accurately positioned into the predetermined center of the tooth bud. Predetermination of the ablation volume can be prescribed for each tooth bud's unique ablation volume. Once the ablation probe is accurately positioned, the ablation procedure can then be initiated and the prescribed ablation volume delivered in a predictable fashion. When employing this approach, the developing zone of ablation radiates outward from the tooth bud's prescribed center in a controlled manner. The key benefit of this approach, as demonstrated in this feasibility study, is that the ablation process can then be limited almost exclusively to the tooth bud tissue inside the bony crypt of the tooth bud. As demonstrated by the gross dissections performed in this study, the heat-affected zone was limited to the soft tissue of the bony crypt of the tooth bud.

The 3TBA procedure demonstrated in this live animal trial confirmed that the center of ablation of the microwave ablation probe was predictably positioned at the center of the 3M tooth bud before initiating the predefined ablation energy. Postoperative x-ray CT imaging and direct measurement on dissected mandibles demonstrated acceptable positioning in all animals with a maximum positioning error of 1 mm noted. This study demonstrated that it is possible to prescribe and deliver microablation energy doses resulting in completely thermocoagulated tooth bud volumes without collateral tissue damage beyond the bony crypt of the tooth bud.

PRIOR STUDIES

Despite the long-standing controversy associated with prophylactic extraction versus a monitor and treat strategy for managing 3Ms, the improved oral health profile of those who naturally do not form 3M s has never been in dispute within the dental community. As a result, prophylactic 3M tooth removal at preeruption stages as a strategy to reduce surgical risk is not a new idea.

In 1936, Henry demonstrated that prophylactic 3M tooth germectomies through lateral trephination was a superior alternative to waiting for troublesome 3Ms to form roots and then cause significant problems later in life. Henry promoted mandibular 3M germectomies with radiographic evidence of nearly complete crown formation and no root formation in children generally of age 10 to 14 years using a lateral trephination procedure. Henry actively performed 3M lateral trephination germectomy procedures for more than 35 years.[1,2] In 1969, he reported no significant long-term complications associated with doing so.[3] The *White Paper on Evidence Based Third Molar Surgery,* published in 2011 by the American Association of Oral & Maxillofacial Surgery, and germectomy trials, reported in 2017 by the International Journal of Oral & Maxillofacial Surgery, repeat Henry's findings of lower morbidity with improved outcomes.[4,5]

Surgical enucleation of the 3M tooth bud tissue at an even earlier age to prevent tooth formation was supported in the mid 1970s by Ricketts et al.[6] Surgical enucleation involves removal of the tooth bud tissues when they were detectable on extraoral radiographs before little or no tooth structure was present. Treatment is performed by creating a surgical flap over the area of the tooth bud followed by blunt dissection to mechanically enucleate the tooth bud tissues from the bony crypt on the medial aspect of the mandible.

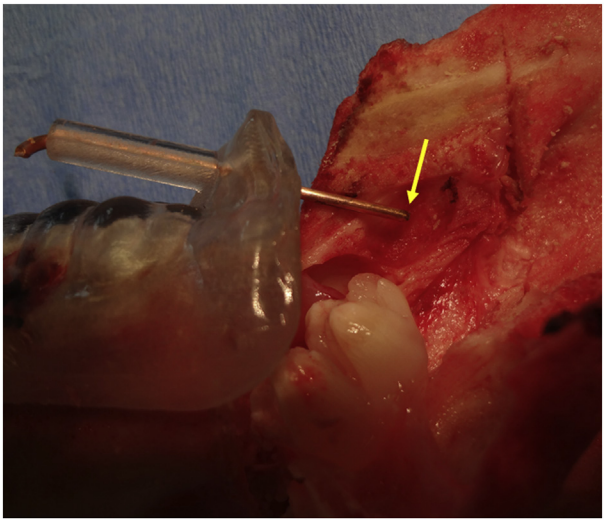

FIGURE 13. A closer view of the custom surgical guide seated on pig #1 shows the x-ray marker fully engaged. The tip of the marker (at *arrow*) matches the center of ablation of the microablation probe used to microablate pig #1. (Photo: Courtesy of John Mata).

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However, identifying the 3M tooth bud tissue with no collateral tissue damage in an open surgical field can be difficult in an 8- to 10-year-old child. As a result, the procedure was never adopted by the pediatric dental profession.

In an effort to develop a less-invasive approach to manage 3Ms, Gordon and Laskin[7] reported on the use of unguided cryoablation of live-animal premolar tooth buds in 1979. They hand placed cryoablation probes in an attempt to cryoablate premolar tooth buds in dogs without knowing the precise 3D location of each tooth bud, the tooth bud volumes, or the efficacy of cryoablation at inducing tooth bud tissue death under ex vivo or in vivo conditions.

Gordon and Laskin[7] reported mixed success. They observed complete agenesis in 62% of third premolars and 25% agenesis of fourth premolars. Partial agenesis was reported in 25% of third premolars and 56% of fourth premolars. However, the researchers report no radiographic evidence of damage to adjacent teeth and bone structure in the months following cryoablation. In addition, they noted a correlation between the amount of agenesis observed and tooth bud size. As tooth buds increased in size, clinically induced premolar agenesis rates decreased. This is significant because the researchers were unable to preoperatively adjust or size the zone of cryoablation to fit tooth bud volumes as the age of their study animals varied.

The only documented attempts to prevent 3M tooth formation at an earlier stage of tooth bud development were reported by Silvestri et al.[8-10] The researchers' unguided approach did not involve ablating detectable tooth-forming tissues. Instead, the approach by Silvestri et al was directed at blocking the microscopic tooth bud initiation process that leads to the growth of tooth-forming tissues. In the absence of a method to image or localize the microscopic tooth bud initiation process, Silvestri et al implemented a shotgun approach. That method delivered excess energy through the overlying visible oral mucosal into undefined submucosal tissue volumes.[8-10]

In 3 separate animal trials using electrosurgical energy in rat pups and long-pulse laser energy in beagle puppies, Silvestri et al attempted to destroy the lamina dura tissue that would give rise to tooth bud progenitor tissues. The lamina dura is the first tissue that forms once the genetically preprogrammed triggering event occurs. This is located on the underside of the visible oral epithelium. In each of these studies, Silvestri et al reported adverse side effects associated with the unguided application of ablation-level energy through oral mucosal tissues and into underlying soft and hard tissues.[11] The use of unfocused electrosurgical energy was too powerful and uncontrollable to reliably confine damage to tooth-forming tissues alone. Tissue destruction from long-pulse laser energy was so widespread that oral mucosal burns and bony sequestration was reported 2 weeks following treatment. In addition, adjacent developing second molars became malformed.[12-14]

Such an uncontrolled approach puts vital structures at risk, including the adjacent forming 2nd molar and the inferior alveolar neurovascular bundle inside the mandibular canal. In the decade following the research by Silvestri et al, advancements occured in understanding the mechanisms of microwave ablation technology to better control the margins of the prescribed thermocoagulation zone in targeted tissues.[15-17] Kim notes that microwave ablation therapy has become widely accepted as a means of minimally invasive tumor ablation. Compared to radiofrequency tumor ablation, for example, microwave ablation can be reliably performed on a wide range of tissue types with shorter ablation times and larger ablation volumes without tissue charring because of unique tissue penetrating mechanisms.[18] In addition, the nonionizing mechanism of action of microwave energy in body tissues is limited to a tissue heating effect with no risk of mutagenicity.[19,20]

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[21] 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.

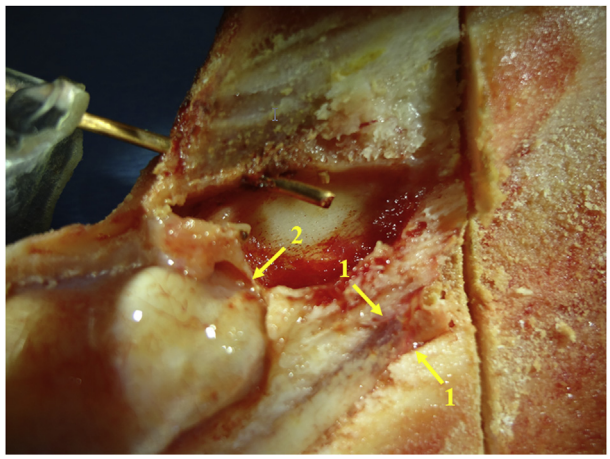

FIGURE 14. This slightly oblique close-up view of the bony crypt of the third molar tooth bud on pig #2 shows the mandibular canal (between *arrows 1*) with a 3- to 4-mm osseous separation from the third molar tooth bud crypt. It also shows the close proximity of the tooth bud follicle of the developing second molar to the anterior and inferior aspect of the third molar tooth bud crypt (*arrow 2*). There was no visible evidence of thermocoagulation of the distal aspect of the second molar tooth bud follicle or visible evidence of bone tissue damage in the 5 subject animals used in this study. (Photo: Courtesy of John Mata).

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LIMITATIONS

Extensive work went into developing a practical animal model for this study. The pig model appears feasible, but it has limitations for a number of reasons. There were no relevant data available regarding 3M tooth formation in pigs, other than tooth charts indicating that 3M tooth eruption generally occurs at 18 months. This is the age at which the animals' skeletal growth is nearing completion. Initial 3TBA attempts were designed to gain medial access to the mandibular tooth buds residing 1 to 2 mm under the oral mucosal surface separated by a thin layer of cortical bone. Once access through the anterior of the ramus of the mandible was employed, the procedure was manageable even though positioning accuracy was reduced. Accessing the tooth buds in this way required an osteotomy and a longer ablation probe with up to 20 mm of reach beyond the surgical guide. This resulted in a working length considerably longer than anticipated for use in humans, which is estimated to be 12 to 18 mm total working length in the surgical guide and less than 6 mm beyond the surgical guide.

Substantial advancements in relevant technology have occurred since this study was conducted. Higher-resolution cone beam CT imaging, digital dental impressions, higher-resolution 3D printing, and improvements to software used to integrate these technologies make it possible to produce 3TBA guides with greater precision. These advancements can also improve the accuracy of prescribed ablation volumes.

Another limitation of this study is that it did not include histologic analysis. There was no attempt to section the bone surrounding the 3M tooth bud to determine adjacent osteocyte viability or determine whether the bony crypt of the tooth bud was thermally affected by microablation. The aim of this study was to develop an appropriate animal model to facilitate future research. Regardless, the histologic evaluation of thermally affected tissue immediately postoperatively will not show the zone of ablation. Determination of thermally affected tissues can only be determined in live animals using an acute healing model with histology obtained at 7 days postablation, once an animal model has first been demonstrated to be feasible.

Additional efforts could have been made to better visualize neighboring tooth buds and surrounding tissues. Debridement of the ablated tissue could have been performed and direct visualization of the resultant bed could have been obtained. Subsequent studies by the author include these procedures.

The clinical goal of 3TBA is to minimize surgical risk and improve clinical outcomes compared to established 3M management strategies. Fully guided 3TBA as demonstrated in this study represents a potential minimally invasive means to prevent 3Ms from ever forming.

The fully guided 3TBA approach, demonstrated in 5 successive 3TBA procedures in pigs, met the surgical goals of this study. Demonstrating fully guided 3TBA first required (1) development of a practical animal model, (2) development of the associated clinical protocols to predetermine the location and microablation volume prescription for each target tooth bud, (3) the design of microwave ablation probes that were capable of delivering energy doses inside the bony crypt of the tooth bud, and (4) construction of 3TBA guides to position the center of ablation in the center of the tooth bud.

Center of ablation positioning error was observed to be no greater than 1 mm prior to delivering the predefined level of microablation energy under live conditions. Positioning accuracy was observed when extending 20 mm out of the distal end of the 3TBA guide to the center of the targeted tooth buds. All the 5 3TBA procedures resulted in completely ablated tooth bud volumes with the zone of thermocoagulation, and all 5 visually appeared to be limited to the targeted tooth bud tissue inside the bony crypt of each tooth bud. Postoperative dissections demonstrated no visual evidence of thermal damage to sensitive collateral tissues, which included the highly vascularized developing second molar tooth bud follicle less than 1 mm anterior to the ablation zone and inferior alveolar neurovascular bundle located 2 to 3 mm inferiorly.

With microablation energy originating from the center of the tooth bud and extending outward in a predefined fashion, it was possible to verify that in vivo margins of thermocoagulated tissue zones were similar to predicted heat-affected zones obtained by preliminary computer modeling and surrogate ex vivo tissue microablation models.

Subsequent to this study, additional studies were performed using this animal model to successfully demonstrate molar agenesis. Studies include short-term healing evaluation and long-term healing with histologic evaluation.

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