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Straight-segment mandibulectomy: a reproducible porcine mandibular critical-size defect model

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Abstract

Porcine mandibular defect models are commonly used for the preclinical evaluation of reconstruction techniques. Existing studies vary in technique, complexity, and postoperative outcomes. The procedures are complex and often described without sufficient detail. We describe in detail a simple and reproducible method for creating a critical-size mandibular defect in a porcine model. Seven hemimandibular critical size defects were created in five male Yorkshire-Landrace pigs, three with unilateral defects and two with bilateral defects. A transverse incision was made over the mandibular body. Periosteum was incised and elevated to expose the mandibular body and a critical-size defect of 30 × 20 mm created using an oscillating saw. The implant was inserted and fixed with a titanium reconstruction plate and bicortical locking screws, and the wound closed in layers with resorbable sutures. Intraoral contamination was avoided. Dentition was retained and the mental nerve and its branches preserved. The marginal mandibular nerve was not encountered during dissection. All pigs retained normal masticatory function, and there were no cases of infection, wound breakdown, haematoma, salivary leak, or implant-related complications. The procedure can be performed bilaterally on both hemimandibles without affecting load-bearing function. All pigs survived until the end point of three months. Postoperative computed tomographic scans and histology showed new bone formation, and a three-point bend test showed the restoration of biomechanical strength. Straight-segment mandibulectomy is a simple and reproducible method for the creation of critical-size mandibular defects in a porcine model, simulating a load-bearing situation.

Keywords: Mandible; Mandibulectomy; Mandible reconstruction; Porcine model; Critical size defect

Introduction

Porcine mandibular defect models are often used for the evaluation of reconstructive techniques, as similarities in morphology and physiology between porcine and human mandibles allow for meaningful preclinical evaluation.[1–3] Technical variations exist and descriptions of surgical steps lack detail.[4–7] Complexity varies, with some requiring dental extraction[8] and resection of mandibular neurovascular bundles,[9] which increases the risk of infection and early euthanasia of the animals.[10] We describe in detail a simple and reproducible technique for creating a straight-segment critical-size defect over the lower mandibular border in a porcine model (Fig. 1).

Method

Pre-procedure

Seven hemimandibular critical-size defects were created in five male Yorkshire-Landrace pigs (three animals with unilateral defects, two with bilateral defects). All procedures were approved by our Institution's Animal Care and Use Committee (National University of Singapore R19-1245). The animals weighed 30–40 kg and were acclimatised for

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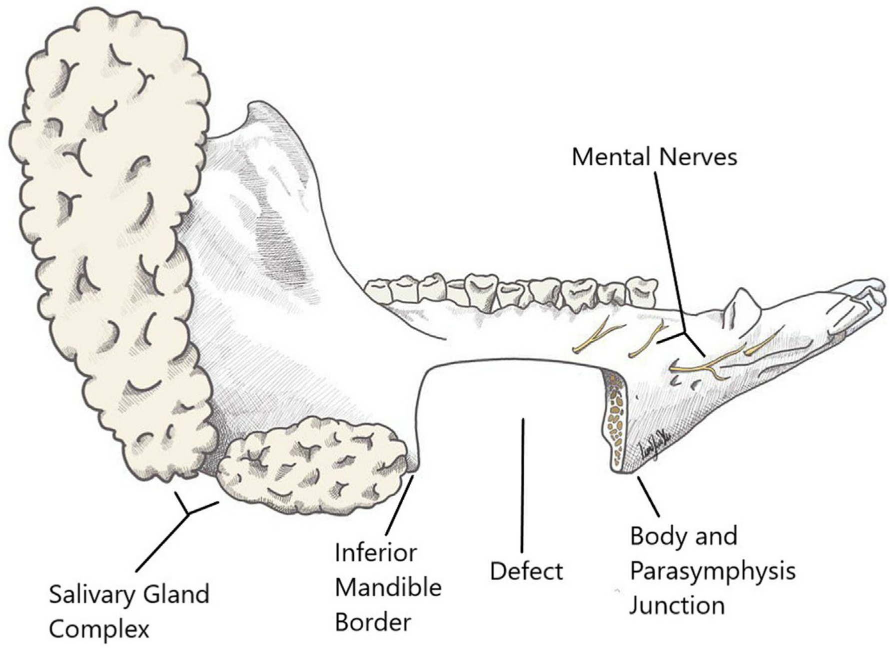

Fig. 1. Straight segment mandibulectomy created a critical-size defect over the body of the mandible for evaluation of reconstruction methods. The defect was bordered by the following structures: superiorly by mental nerves; inferiorly by the inferior mandibular border; anteriorly by the junction between the body and parasymphysis; and posteriorly by the salivary gland complex. Four primary steps were involved: approach, defect creation, defect reconstruction, and closure.

one week. They were housed individually and had access to food ad libitum.

Anaesthesia

General anaesthesia was administered. Premedication was given with atropine (0.044 mg/kg IM), midazolam (0.5mg/kg IM), and ketamine (12 mg/kg IM). Isoflurane 5% was used for induction. Animals were intubated and placed on assisted ventilation. Isoflurane 2%–3% was used for maintenance. Analgesia (carprofen 4mg/kg intramuscularly (IM) and buprenorphine 0.1mg/kg) was administered and regional nerve block (bupivacaine 1–2 mg/kg) and local anaesthetic field block (lidocaine 1–4 mg/kg) given. Maintenance 0.9% normal saline drip was administered at 5–10 ml/kg/hour. Vital signs were monitored throughout surgery.

Surgical steps

Approach

The animal was positioned laterally and preoperative markings were made (Fig. 2). These included the mandibular angle, inferior border, and parasymphysis, an outline of the salivary gland complex (parotid and submandibular glands), and the incision. The longitudinal incision measured 10 cm and was marked 1–2 cm above the inferior border of the mandible.

The surgical site was disinfected with povidone iodine and chlorhexidine solutions, and sterile drapes were placed. A full-thickness skin incision was made with a size 15 blade. Subcutaneous tissue, fascia, and muscle were incised with monopolar cautery. At the posterior end of the wound the anterior border of the salivary gland complex was encountered just beneath the muscle. Blunt dissection was performed with a haemostat, developing a plane between the salivary gland complex and muscle. Muscle was incised with electrocautery, preserving the capsule of the salivary gland complex, which was retracted posteriorly to facilitate further dissection.

Dissection was carried down to periosteum, and the periosteum incised with a 15-blade. It was scraped along its length using a periosteal elevator to expose the underlying bone then elevated as periosteal flaps using the periosteal elevator both superiorly and inferiorly. Superiorly, the periosteum was elevated until the mental nerve branches were visualised and preserved. Inferiorly, it was elevated to the inferior mandibular border. The marginal mandibular nerve was contained within the inferior flap and was not encountered. The lateral surface of the mandible was exposed, and the medial surface dissected with electrocautery and completed by finger dissection. The medial surface of the mandibular body was dissected similarly to the level of the mental nerve branches. This can be ascertained by performing a ‘pinching’ manoeuvre of the exposed mandibular body using the thumb on the lateral surface and index finger on the medial surface. The bone was dissected until the implant and reconstruction plate could be accommodated (Fig. 3),

Defect creation

A defect 30 mm long and 20 mm high was created over the inferior mandibular body. The defect was bordered by the following structures: superiorly by the mental nerves and

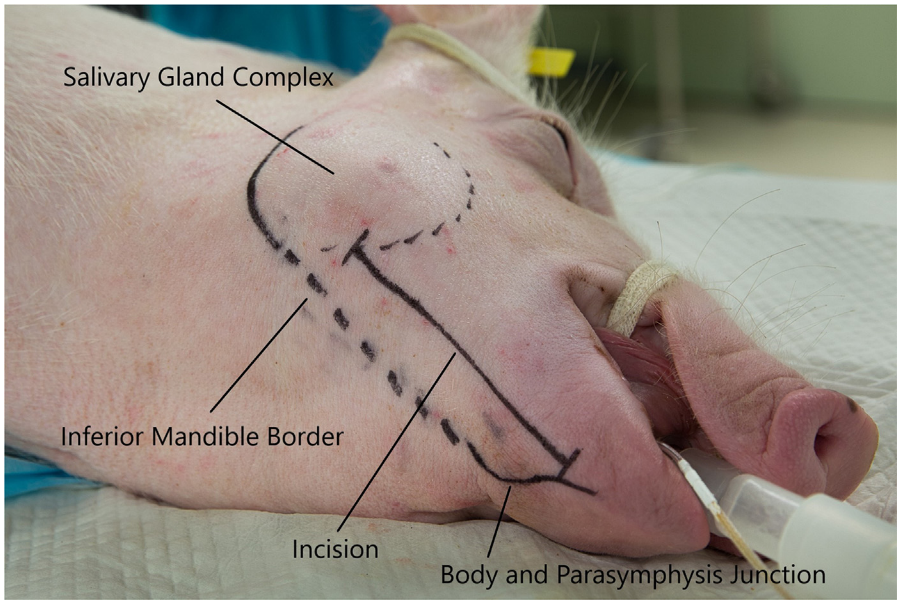

Fig. 2. Preoperative markings. A longitudinal incision was positioned 1–2 cm above the inferior mandibular border. It extended from the anterior border of the salivary gland complex posteriorly to the body and parasymphysis junction anteriorly.

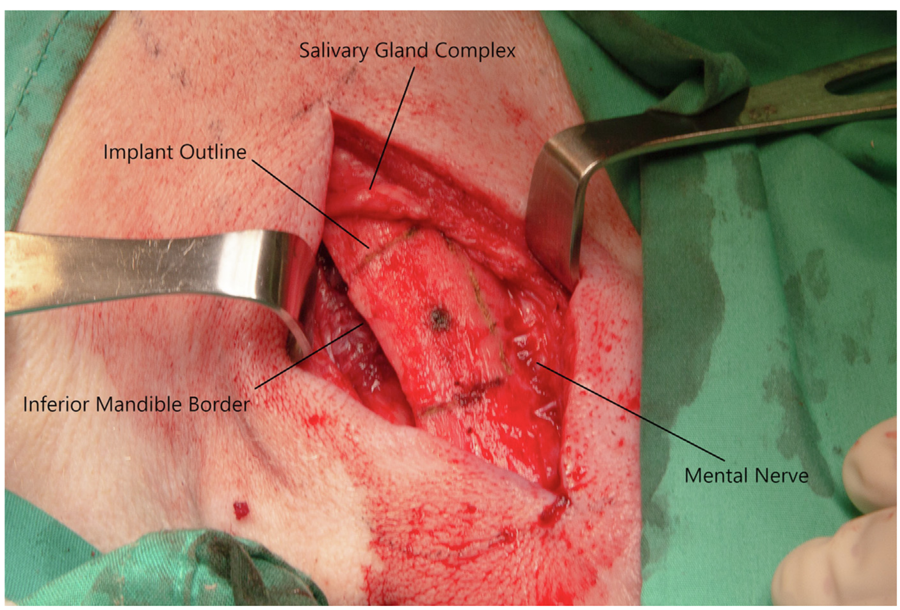

Fig. 3. Approach. Dissection was performed in layers down to the bone, preserving the mental nerves and salivary gland complex. The outline of the implant to be tested was made with cautery.

tooth roots; inferiorly by the inferior mandibular border; anteriorly by the junction between the body and parasymphysis; and posteriorly by the salivary gland complex. A defect at least 20 mm in length was required to qualify as a critical size in a porcine model.[11] The height of the defect was limited by the mental nerves and tooth roots superiorly.

A 2.0 mm thick MatrixMANDIBLE™ (DePuy Synthes) 12-hole straight titanium reconstruction plate was trimmed to the length of a 10-hole plate using shortcut plate cutters. The cut end of the plate was smoothed with the diamond file located on the handle of the plate cutters, and the plate placed over the lower border of the mandibular body, ensuring contact with bare bone throughout its length. To achieve this, a small amount (5°–10°) of out-of-plane bending over the middle third of the plate was performed using bending pliers with nose. This step was performed first to ensure that there was adequate longitudinal exposure to accommodate the reconstruction plate for the subsequent fixation of implants.

In this study a 3D-printed polycaprolactone-tricalcium phosphate (PCL-TCP) implant (Osteopore®) 30 × 20 mm was used to reconstruct the defect. It was placed in the proposed defect position on the lower border of the mandibular body. The outline of the implant was marked using monopolar cautery then it was removed and replaced with the 10-hole reconstruction plate. Predrilling of the screw holes was performed with a Colibri II® (DePuy Synthes) handpiece with drill bit (1.5 mm in diameter). Bicortical drilling was performed through the proximal three holes and distal three holes of the reconstruction plate. The middle four holes of the plate where the PCL-TCP implant would be positioned were left empty. A depth gauge was inserted to determine the length of the screws. Locking screws (2.0 mm in diameter) were screwed into all the holes and removed. The screws and reconstruction plate were then placed in a dish submerged in chlorhexidine.

An oscillating saw attachment was attached to the Colibri II® handpiece, and a saw blade (coarse teeth, cutting thickness 0.6 mm) attached to the saw attachment. The defect was created by sawing full-thickness through the marked outline (Fig. 4). The soft tissue deep to the defect was protected with a malleable retractor. Bleeding from the exposed medulla was expected, and haemostasis was achieved with minimal cautery of the bleeding points, gauze packing, and digital pressure.

Defect reconstruction

The 30 × 20 mm 3D-printed PCL-TCP implant was inserted snugly into the defect. Fixation of the construct was done using the10-hole 2.0 mm titanium bridging, load-bearing, locking reconstruction plate (Fig. 5). The plate was placed across the defect, with the PCL-TCP implant positioned in the middle. The reconstruction plate was fixed using bicortical locking screws (3 proximal and 3 distal to the implant) with two additional screws through the implant.

Closure

The wound was irrigated with chlorhexidine and saline. Haemostasis was secured. Closure was performed with synthetic absorbable monofilament sutures (Monosyn®, B Braun Medical Ltd): Monosyn® 2-0 to the muscle-periosteal layer in interrupted buried fashion, Monosyn® 3-0 to the dermis in interrupted buried fashion; and Monosyn® 4-0 in a continuous subcuticular fashion. No dressing was required.

Post-procedure

The animals were housed individually with access to food ad libitum. A soft diet was continued for two weeks before escalation to a pellet diet. Antibiotics (amoxicillin/clavulanic acid 10–20 mg/kg, orally once a day) and analgesia (carprofen 4mg/kg, orally once a day; fentanyl patch 2–3 mcg/kg/hour, transdermally one dose/72hours) were administered for one week. The animals were monitored daily for systemic and local complications.

Results

Five animals underwent surgery. Unilateral mandibular defects were created in three and bilateral mandibular defects in two. There were no systemic complications. The animals remained well with stable weight gain, and normal masticatory function was retained. Local complications included two animals with stitch granuloma that resolved after two to three weeks of topical antibiotics. No other local complications were observed. All the animals survived and were euthanised at the study's end point of three months. The mandibles were harvested. Gross examination, computed tomographic scans

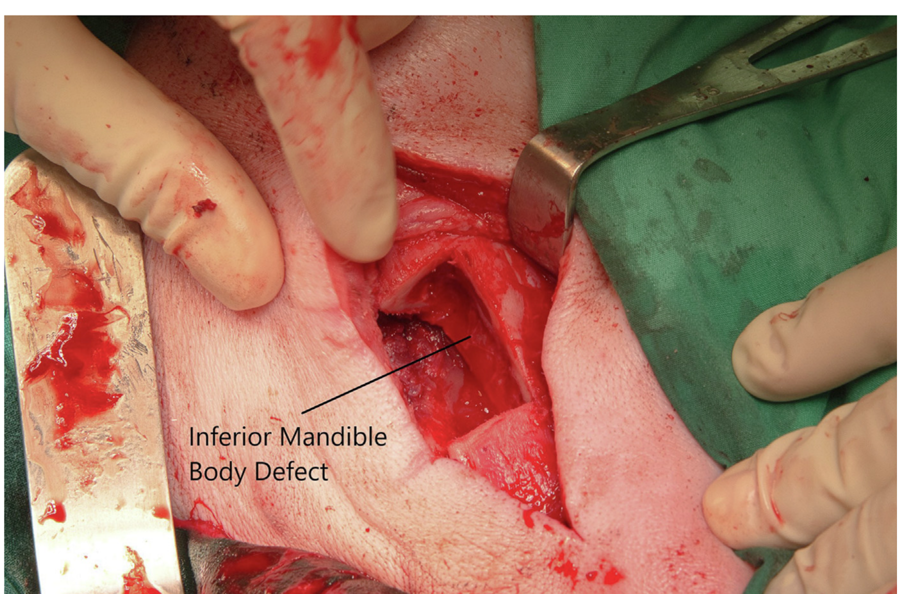

Fig. 4. Defect creation. The defect was created using an oscillating saw. A malleable retractor was positioned underneath during the process to protect underlying soft tissue structures.

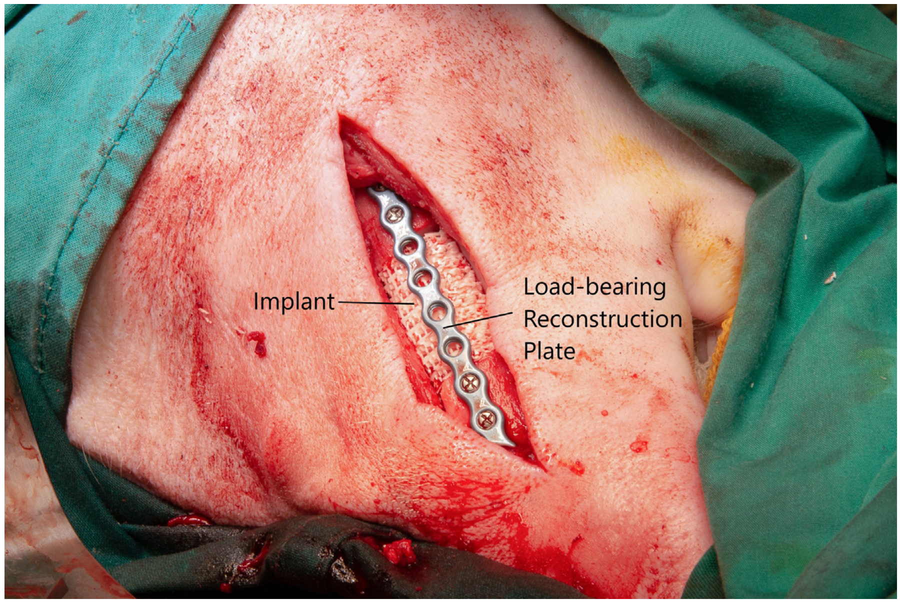

Fig. 5. Defect reconstruction. Fixation of the implant was performed using a titanium load-bearing reconstruction plate with bicortical locking screws. Three screws were positioned on each side of the implant and two were inserted through the implant (a total of eight screws).

and histology showed new bone formation. Biomechanical testing with a modified three-point bend test showed comparable maximum flexural force between the reconstructed and unoperated hemimandibles.

Discussion

Mandibular reconstruction is performed following head and neck tumour resections or major trauma with significant bone loss. Critical-size defects require the use of a vascularised bone flap, a commonly used example being a free fibular osteocutaneous flap.[12] Vascularised bone flaps are associated with donor site morbidity and perfusion compromise that might result in flap failure.[13] Advances in craniomaxillofacial reconstruction, however, have demonstrated the potential of alloplastic implants with osteogenic and osteoconductive properties.[14] These implants have the potential to reconstruct critical-size defects without the need for a vascularised bone flap.

Animal models are important in implant research. A simple and reproducible technique can obtain meaningful results in an efficient and cost-effective manner while minimising risk to the animals.[1–3] Techniques described previously have varied significantly in terms of surgical approach, laterality of defects, number of defects created in each animal, defect size and configuration, and need for tooth extraction, which can affect the outcome, complications, and survival of the animals throughout the duration of the study (Table 1).[4–10] Some techniques may involve unnecessary steps that could jeopardise the outcome and predispose the animals to unwanted risks. A safe and reliable technique minimises complications and increases survival of the animals. It also allows for convenient comparison of the results across different studies.

The technique created a critical-size defect over the inferior portion of the mandibular body. The defect would experience stresses and strain similar to those of a marginal mandibulectomy defect.[15]

There were four main components: approach, defect creation, defect reconstruction, and closure (see accompanying video). The duration of surgery ranged from 1.5–2 hours for a unilateral procedure, and from 3–4 hours for a bilateral procedure.

This simple procedure coupled with a short operating time minimised surgical and anesthetic stress for the animals, and the risk of infection was reduced with an extraoral approach. Wide exposure and a straight-segment defect allowed the surgeons to easily control and modify the size of the defect according to the needs of the experiment. The procedure was predictable without the risk of major complications or iatrogenic injury to important facial structures.

Approach

A single longitudinal incision positioned directly over the inferior mandibular body provided direct access to the mandibular body without the need for extensive elevation and dissection of the flap. This differs from a standard submandibular approach to the mandible, which requires elevation of the superior flap over a greater area and gives limited exposure so that subsequent procedures are performed within a cavity that is challenging.

Dissection proceeded in an area below the buccal nerve which can be easily preserved. A bottom-up approach allowed the mental nerves to be easily identified and preserved. The marginal mandibular nerve and its branches were contained within the inferior skin flap and were not encountered during dissection.

Table 1
Comparison of methodologies and outcomes of previous studies using porcine models for mandibular reconstruction.

First author, year, and reference	Species of animal	No. of animals	Sex	Approach	Laterality of defect	No. of defects over each hemimandible	Defect size (mm)	Defect configuration	Tooth extraction	Duration of surgery	Complications (No. of animals, specific complications)	Early euthanasia or death before study end-point (No. of animals, complication resulting in early euthanasia or death)
Schliephake 1997[4]	Göttingen minipigs	20	Female	External skin incision	Mixture of unilateral and bilateral	1	20 and 40	Mixture of marginal and segmental mandibulectomy	No	Not stated	2, pneumonia; 2, surgical site infection; 1, fistula with implant loss	2, pneumonia
Scarano 2017[5]	Minipigs	6	Unspecified	Unspecified	Bilateral	3	5 × 5	Cylindrical	No	Not stated	No	No
Yeo 2012[6]	Micropigs	10	Male	Intraoral incision	Unilateral	1	45 × 12 × 5	Marginal mandibulectomy	Yes	Not stated	8, implant exposure on histological analysis	No
Probst 2020[7]	Münchener Trollschweine minipig	16	Females and males	External skin incision	Unilateral	1	30 × 10 × 20	Marginal mandibulectomy	No	Not stated	2, local inflammation	No
Gröger 2003[8]	Göttingen minipig	6	Unspecified	External skin incision	Unilateral	1	20 × 10	Marginal mandibulectomy	No	Not stated	1 - salivary cyst	No
Wang 2004[9]	Göttingen minipig	5	Female	External skin incision	Unilateral	1	50	Segmental mandibulectomy	Yes	Not stated	No	No
Dorafshar 2014[10]	Yorkshire minipig	8	Female and males	External skin incision	Unilateral	1	60	Segmental mandibulectomy	No	250–398 minutes (average 346 minutes)	4, infection (3 mandible surgical site infection; 1 donor site infection)	4, infection

Compared with an intraoral approach, contamination and risk of infection were reduced. The operative field was not obstructed by an endotracheal tube. There was no disruption of the dentition and the animals regained normal masticatory function immediately after surgery. Compared with a preauricular or a retromandibular approach, the salivary gland complex was spared extensive dissection. This avoided injury to the parotid duct, and formation of a sialocele or salivary fistula.

Defect creation

A 30 $\times$ 20 mm defect was created over the inferior mandibular border. A defect size of at least 20 mm long has been found to be of critical size in porcine models.[11] The height of the defect was determined by the position of the mental nerves and tooth roots superiorly. The geometry of the defect was simplified and adaptable with a straight-segment defect over the mandibular body. The defect could be easily sawed off by following a rectangular-shaped marking.

Biomechanically, a marginal defect situated over the inferior border of the mandible evaluates the strength of the implant under a loaded situation during mastication. It receives loading and compressive forces, exerted by the muscles of mastication - predominantly the digastric and masseter muscles. Forces from the medial and lateral pterygoids, and the temporalis muscle would be also be received. Tension forces over the superior border will not be pronounced with the upper tooth-bearing border of the mandibular body still intact. Bilateral defects can be created in a single setting without resulting in biomechanical instability. The simplicity of a straight-segment defect allows for creation of a longer defect that can be adapted to individual experimental requirements. Tooth roots are preserved, and there is adequate mandibular height, preventing pathological fracture.[16]

Defect reconstruction

The versatility of a simple straight-segment defect allows for the testing of a variety of implants and reconstructive techniques. Fixation can be performed easily with a titanium reconstruction plate trimmed to the required length. Minimal out-of-plane plate contouring is required over the relatively flat lateral surface of the mandible.

Closure

Buried synthetic monofilament sutures reduce the risk of bacterial entrapment and infection. Minimal dead space is present with layered closure. A surgical drain is not required, negating the need for sedation and drain removal.

The described technique involves marginal resection of the mandible and does not provide an accurate representation of a segmental defect. The biomechanics of a marginal mandibulectomy differ significantly from those of a segmental mandibulectomy.[17] The residual mandible differs in terms of bone stock, structure, and geometry. Forces generated by the muscles of mastication, together with the reaction forces at the teeth and temporomandibular joints, exert varying degrees of deformation, stress, and strain to the mandible. These factors ultimately affect bone regeneration and remodelling.[18] A segmental defect is prone to infection due to its inherent instability, especially in animal models.[4,10] Developing a safe and reliable segmental defect model is challenging and warrants further study.

Conclusion

Straight-segment mandibulectomy is a simple and reproducible technique for the creation of mandibular defects in a porcine model. The technique creates a critical-size defect in a loaded situation. Predictable outcomes were obtained, improving experimental efficiency while minimising harm to the animals. A variety of reconstructive methods from implants, free vascularised bone flaps, and bone chips can be evaluated. A standardised technique allows for the comparison of results across experiments that have been independently performed.

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Conflict of interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Ethics statement/confirmation of patient permission

National University of Singapore Institutional Animal Care and Use Committee (IACUC R19-1245). Patients' permission NA.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bjoms.2022.11.003.

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