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Original Research

Development of a Corneal and Eye Protection Strategy in Domestic Swine

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Large animal models are essential to research in facial paralysis, face transplant, craniofacial surgery, and ophthalmology. Pigs are a well-studied species with high similarity to human anatomy and physiology for these research areas. However, in contrast to cats and dogs protecting the cornea and eye is difficult in swine due to the inability to use an Elizabethan collar (E-collar) and the complexity of placing and maintaining a temporary tarsorrhaphy for corneal protection due to the strength of the pig levator muscle. This study presents an effective method to provide corneal and eye protection in the domestic swine for at least 50 d. Furthermore, protection of the eye and face is achieved through the innovative use of a modified ophthalmologic face shield. The findings from this study will advance large animal research in these fields, enabling innovation in surgery and tissue engineering in areas of both craniofacial and ophthalmologic research.

Abbreviation: E-collar, Elizabethan collar

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Introduction

Surgical, tissue engineering, and drug development innovation require large-animal models with comparable anatomy, nerve structure, and size to humans. The pig is an ideal model for studying facial paralysis, face transplant, and retina cone cell regeneration studies.[9,12,13] However, swine studies can be limited by logistical challenges. In particular, studies that result in loss of the inherent blink reflex present particular difficulties in protecting the cornea to avoid corneal ulcers. The facial paralysis study presented here used an effective eye protection strategy to investigate the effect of neurostimulation on restoration of the blink function in a swine model of facial paralysis. This swine model required corneal protection for the time period necessary for nerve regeneration (approximately 2 to 3 mo), as the ability to close the eye (through orbicularis oculi muscle function) was lost upon transection of the facial nerve to induce paralysis.[3] However, the muscle leading to eyelid opening, the levator, remained intact. Therefore, the swine lose inherent corneal protection due to inability to close the eyelid.

In human patients, a tarsorrhaphy can be performed in which the eyelids are sutured closed to provide corneal protection if patients cannot pursue other surgical options.[2,11] A temporary tarsorrhaphy in our swine model was therefore planned and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC) to achieve eyelid closure and corneal protection. A similar protocol to that performed in human patients was completed in 2 initial swine, with sterile foam suture bolsters used as stents and a horizontal mattress suture pattern with 4-0 Prolene suture. However, the strength of the levator muscle led to ripping of the tarsorrhaphy sutures through the eyelid within 48 h and thereby loss of corneal protection. Furthermore, the swine also were discovered rubbing the tarsorrhaphy sutures against their cage walls, further leading to tarsorrhaphy failure despite efforts to reduce this behavior (including acrylic cage inserts and replacement of metal feeders with rubber bowls). Therefore, we needed to develop a novel method of corneal protection for these animals.

The corneal protection method presented in this study expands the potential to use pigs in studies that require chronic corneal and/or eye protection. The face shield could also be enlarged in order to protect facial incisions for other maxillofacial surgeries.

Materials and Methods

Animals. This study included 7 domestic female pigs, *Sus domesticus* (between 20 to 25 kg). Three pigs were obtained from a high health status farm free of Brucellosis, TGEV, Influenza A, PRRSV, PRV, PEDv, *Actinobacillus pleuropneumoniae*, *Lawsonia intracellularis*, Porcine circovirus, *Mycoplasma hyopneumoniae*, and *Leptospirosis pomona*, *ictero*, *conicola*, *hardjo*, and *grippo* (Premier BioSource, Rensselaer, IN). Due to the high-health status of these pigs, they received no vaccineations before shipping. The remaining 4 pigs were obtained from a commercial swine operation and were free of PRRSV, *Mycoplasma hyopneumoniae*, Brucellosis, and Pseudorabies (Manthei Hog Farm, Elk River, MN). These pigs were vaccinated against Porcine circovirus, *Lawsonia intracellularis*, and *Mycoplasma hyosynoviae* and *hyorhinis* prior to shipment. Animals were pair-housed upon arrival. All incoming pigs were vaccinated against *Erysipelothrix rhusiopathiae*, *Glaesserella parasuis*, and *Mycoplasma* pneumonia upon arrival and received a booster dose 2 to 3 wk after initial vaccination. After surgery,

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pigs were single housed next to each other with screens to allow socialization with the neighboring pig.

Housing. The pigs were housed and cared for in compliance with the *Guide for the Care and Use of Laboratory Animals*, eighth edition,[9] in an AAALAC-accredited facility. All procedures involving animal care and use were approved by the IACUC at the Mayo Clinic. Pigs were initially pair-housed in cages measuring 48 × 72 in. Pigs had access to 2 cage units when pair-housed prior to surgery and one cage unit when singly housed. Cages were washed daily. Relative humidity and temperature were maintained at 30 to 70% and 16 to 27 °C respectively, under a 12:12-h light:dark cycle (on 0600, off 1800). Water was provided without restriction through an automatic lick-spout system. Pigs received a pelleted diet (Mayo Pine Island Gestation Diet, Purina Animal Nutrition, Arden Hills, MN) on arrival to our facility and were maintained on this diet throughout the study. Behavioral enrichment included toys that were changed weekly. The investigators used positive reinforcement training daily, with pigs receiving treats in association with application of the face shield covering and examination of the facial region, beginning at one week before the first stage of surgery.

Research history. In the first stage of surgery, a nerve transfer was performed in these 7 pigs to develop a means of pacing peripheral nerves to restore blink function. The trigeminal motor branch to the masseter muscle was identified and coapted to the distal portion of the zygomatic branch of the facial nerve, thus innervating orbicularis oculi. The facial nerve root was then transected, leading to hemifacial paralysis. In the second stage of surgery, performed 2 to 3 mo after the first stage, a peripheral nerve pacemaker was placed on the nerve transfer to restore blink function. Therefore, corneal protection was necessary in the intervening time between first and second stages, as the facial muscles had no motor innervation and the nerve transfer had not yet regenerated to the distal muscle target to enable peripheral nerve pacing.

Tiletamine–zolazepam (5 mg/kg) and xylazine (2 mg/kg) were used to induce anesthesia, with inhaled isoflurane (1 to 3%) and fentanyl (2 mg/kg initial bolus and 2 to 5 mcg/kg/h CRI) used for maintenance in this surgery. Pigs received appropriate analgesic (carprofen 4 mg/kg for 3 d, Bup-SR, 0.12 mg/kg one dose after surgery) and antibiotic (cefazolin 22 mg/kg and ceftiofur, 5 mg/kg, one dose of each immediately before surgery) therapy.

Nictitating membrane flap. The absence or presence of a nictitating membrane in pigs had not been clearly established in the research and veterinary literature.[15] However, our preliminary dissection of the swine eye in pig cadavers documented the presence of this membrane, which was crucial to the success of the corneal protection strategy presented here. A nictitating membrane flap was placed to aid in corneal protection using 2-0 Ethilon suture (Johnson and Johnson Healthcare, New Brunswick, NJ) and an 8 FR red rubber urethral catheter (Becton, Dickinson and Company, Franklin Lakes, NJ). An 8 mm length of red rubber catheter was cut and the suture was passed through this stent through both walls. A Bishop Harmons forceps was used to grasp the upper eyelid and the needle passed into the dorsolateral fornix of the eyelid, being mindful of the eye. The nictitating membrane was then grasped and drawn anteriorly. The T-shaped cartilage of the nictitating membrane was visualized. The suture was then passed posterior to anterior above the cartilage, then anterior to posterior below the cartilage. The suture was then passed again through the fornix about 5 mm laterally to the initial suture passed through the fornix. This suture was passed through both walls of the red rubber catheter stent. A surgeon's knot was tied with enough tension to pull the nictitating membrane into the fornix.

Tarsorrhaphy. A horizontal mattress suture was used with red rubber catheter stents to perform a tarsorrhaphy to hold the eyelids closed and protect the cornea. Six 8 mm lengths of red rubber catheter were cut for stents. The 2-0 Ethilon suture was passed through both walls of the stent, then approximately 5 mm from the upper lid margin approximately 2 mm from the medial canthus. The needle was then passed through the lower lid, again approximately 5 mm from the lid margin. The same steps were then performed in reverse through the other side of the stent, thus leading to a horizontal mattress suture. A surgeon's knot was then tied on the top stent with consideration of the tension of the stents on the upper and lower eyelids. This step was repeated for 3 additional stents along the eyelid (Figure 1). Care was taken to avoid inward rolling of the eyelids, which could lead to irritation from the eyelashes and/or ulceration of the cornea.

Face shield covering. To prevent the pigs from rubbing their tarsorrhaphy sutures on the cage, the Optivizor face shield (Protective Pet Solutions, Roseville, CA) was modified to fit the pig's face and eyes. This face shield is used by veterinary ophthalmologists for dogs and cats after ophthalmologic procedures. The size "Small" was used for pigs up to 40 kg; they were switched to the "Small-Medium" above 40 kg. The straps of the original face shield were replaced with 0.5-in.-width hoop and loop straps of customizable length for the neck and chin straps of the helmet (Figure 2A). The strap ends were sutured together with 0-silk suture, which was then reinforced with industrial, water-proof tape. The plastic straps of the helmet were also reinforced with tape. Adhesive window strip padding was added to the plastic edges of the face shields to prevent abrasion of the ears and necks. The helmets were placed on the pigs while they were still under anesthesia and straps readjusted if needed when the animal awoke. Pigs were monitored daily for eyelid closure and face shield function (Figure 2B).

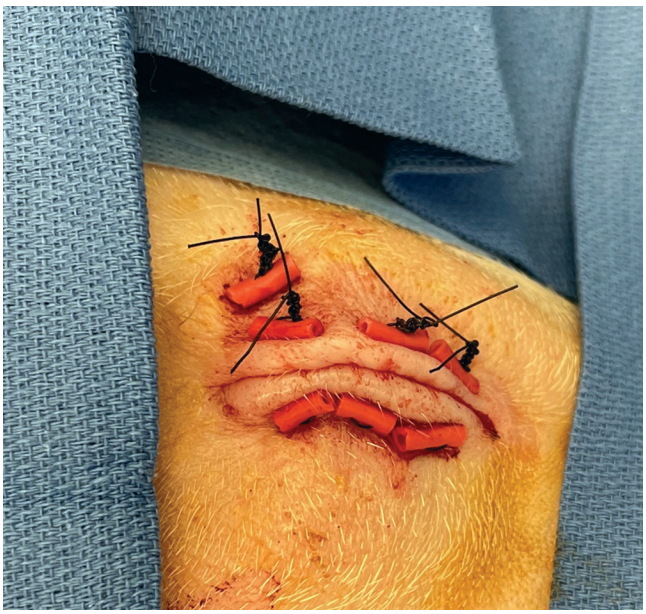

Figure 1. A nictitating membrane flap and tarsorrhaphy was performed at the end of the facial paralysis surgery to enable corneal protection with the loss of a blink reflex. 8 FR red rubber urethral catheter stents were used to distribute suture tension.

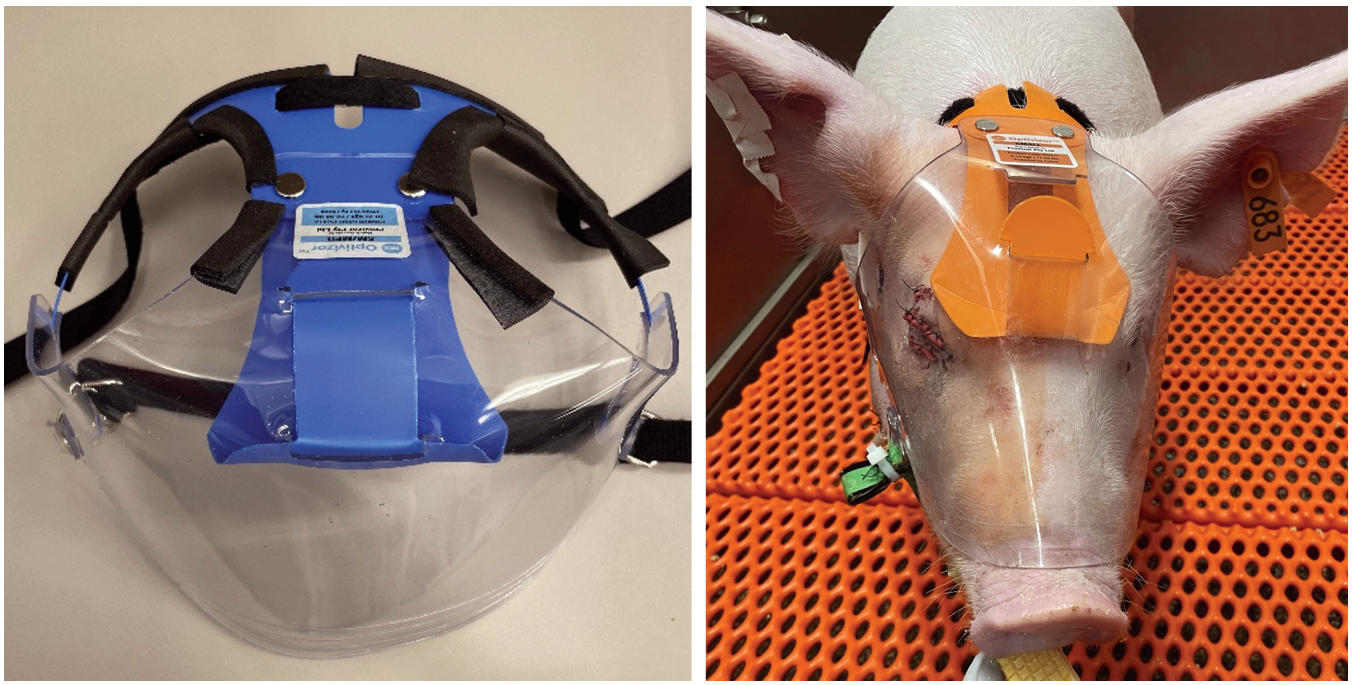

Figure 2. The Optivizor face shield was modified with window stripping padding around the ear holes to prevent abrasion to the animal (A). The animals tolerated the face shields well and both ate and slept with the shields in place with no issues (B).

Results

The eye protection protocol provided eye closure for over 50 d in 7 pigs, facilitating research on facial nerve regeneration and facial nerve neurostimulation (Figure 3). Three of the 7 pigs did not require tarsorrhaphy replacement during the 50-d postoperative period. On average, each tarsorrhaphy was intact for 30 ± 16 d. Pigs 5 and 7 each had one failure of the tarsorrhaphy and nictitating membrane flap; these occurred respectively on days 29 and 35 d after surgery. Pigs 1 and 2 each had 2 failures. Pig 1 had failures on days 18 are 43 after surgery, and pig 2 had failures on days 19 and 25 d after surgery. In cases of failure, the pig was sedated with tiletamine–zolazepam (5 mg/kg) and xylazine (2 mg/kg) and maintained under anesthesia on inhaled isoflurane (1 to 3%). Replacement of the nictitating membrane flap and tarsorrhaphy required around 10 min. Pigs received carprofen for analgesia (4 mg/kg SID for 3 d) after surgery.

During replacement of tarsorrhaphies, fluorescein dye staining was used to assess potential corneal damage. None of the pigs showed dye uptake, indicating that this approach was

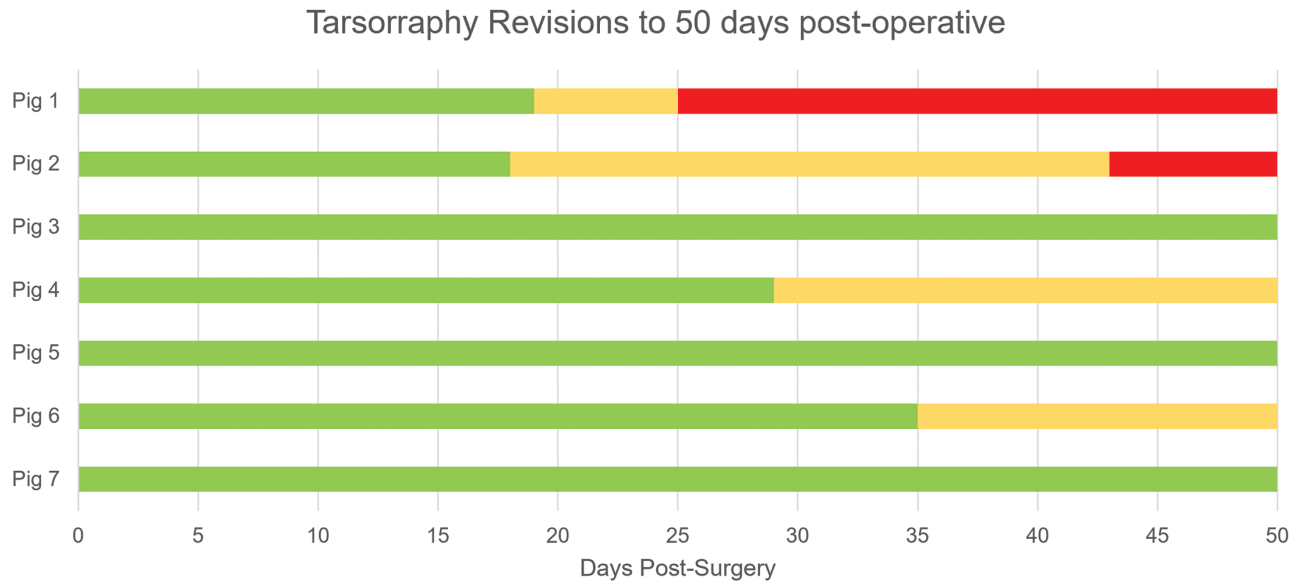

Figure 3. Graph demonstrating the time to tarsorrhaphy revision in 7 pigs for 50-d postoperative. Color changes on the bars represent a tarsorrhaphy failure and revision.

effective for corneal protection. In addition, none of the pigs developed eye infections.

Three of the tarsorrhaphy failures were due to the animals removing the face shield and rubbing the tarsorrhaphy eyelid sutures against their cages. This finding demonstrates the necessity of the Optivizor helmet in maintaining eyelid closure.

The animals tolerated the tarsorrhaphies and Optivizor eye shields extremely well. Replacing the Optivizor shield was necessary when the animals reached approximately 40 kg (from the Small to Small/Medium size). Pigs were able to eat and sleep without issues while wearing the shields.

Discussion

The current study is the first to demonstrate chronic methods to protect the swine cornea, thus supporting future use of pigs for studies in ophthalmology, plastic surgery, maxillofacial surgery, and otolaryngology. The pig is an excellent model animal for facial nerve and facial paralysis studies. Pigs, like humans, have a multifascicular nerve structure of both the facial nerve root and branches.[10] Furthermore, the facial innervations patterns of pigs are comparable to those of humans, with far less cross-innervation than occurs rats.[7] The diameter and length of the pig facial nerve are also comparable to those of humans.[1] Facial paralysis models can result in loss of blink function and corneal protection, which can be ameliorated by using the eye protection protocol described here. The focus of research in this study was neurostimulation of a facial nerve transfer. However, our protocol can also be used in research to restore facial animation. Face transplant studies in pigs can also lead to a loss of blink function. Studies of ideal immunosuppression protocols, nerve regeneration in a vascularized composite transplant model, and functional outcome measurements in face transplant must also temporarily protect the cornea until facial nerve regeneration is complete.[8]

Pigs are also ideal ophthalmology studies. The swine retina is similar to that of the human. However, pigs do not have a macula, the area of the retina that contains the foveal pit, or an area of concentrated cone photoreceptors that allow high-resolution daylight vision.[16] However, pigs instead have a concentrated region of cone photoreceptors called the "visual streak" that functions much like the macula.[4] This similarity is adequate for the study of retinal diseases in pigs.[6,14] However, manipulation of the pig retina, including the introduction of cells or drugs, can lead to retinal detachment if the pig rubs or hits the eye against the cage; this is often an end-study event for the animal. The use of an eye shield, as described here, can aid in preventing this outcome. Drug development and surgical interventions focused on corneal ulcers and exophthalmos can also use the approach presented here, as successful tarsorrhaphies have not yet been reported in swine.[5]

Protection of the pig face and eye is difficult, as traditional Elizabethan collars (E-collars) cannot be used in pigs due to their wide neck girth, which is a distinct difference from canine and feline models. The Optivizor face shield is an excellent alternative. The study team members became more experienced at securing the shield in place and adjusting the straps as the study continued. Plastic zip ties were used to tighten the straps if they were too loose after the pigs awoke from sedation. The learning curve in securing the face shields is reflected in the double tarsorrhaphy failures in pigs 1 and 2, which were due to removal of Optivizor face shield as a result of loose chin straps. This eye protection method is also extremely cost-effective to implement. Optivizor face shields cost approximately $40 each and, when reinforced as described, can be used for months. The total disposable surgical supplies for the tarsorrhaphy and nictitating membrane flap (red rubber catheter and sutures) do not exceed $20 per pig. However, the padding and strap length on the Optivizor face shields must be adjusted as described to prevent abrasion because the shields were created for use in dogs and cats, which have far less neck girth.

Daily monitoring of the pigs by individuals who are comfortable with handling the pigs and replacing the shields is essential. In several cases, the pigs had removed the face shields but, due to daily monitoring checks, the shields were replaced before the tarsorrhaphy or nictitating membrane flaps were affected. Daily monitoring also revealed that all pigs eventually developed a dry, brown, waxy debris build up around the tarsorrhaphy stents. This condition was remedied by gently cleansing the eye with gauze and sterile saline when build up occurred. Positive reinforcement training is crucial to allowing safe handling and monitoring of the tarsorrhaphies. By implementing training even before the first stage of surgery, the pigs learned to tolerate face shield adjustment and replacement without sedation.

In summary, the use of a nictitating membrane flap, tarsorrhaphy with stents, and eye protection with a face shield provided good corneal protection for at least 50 days in swine with facial paralysis. Effective protection of the cornea in pigs expands the potential to use this species in craniofacial and ophthalmologic research.

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