Video Article

An Anesthesia, Surgery, and Harvest Method for the Evaluation of Transpedicular Screws Using an *In Vivo* Porcine Lumbar Spine Model

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Abstract

Pedicle screw fixation is the gold standard for the treatment of spinal diseases. However, many studies have reported the issue of loosening pedicle screws after spinal surgery, which is a serious concern. To address this problem, diverse types of pedicle screws have been examined to identify those with good fixation strength and osseointegration in spine bone. The porcine spine is a good alternative for the human spine in the evaluation of pedicle screws due to the anatomical size, mechanical characteristics, and cost. Although several studies have reported that pedicle screws are efficient in the porcine model, no study has described detailed protocols for the evaluation of a pedicle screw using the porcine model. Here, we describe a detailed method for evaluating transpedicular screws using an *in vivo* porcine lumbar spine model. The technical details for anesthesia, spine surgery, and harvest provided here will facilitate with the evaluation of the transpedicular screw fixation model.

Video Link

The video component of this article can be found at https://www.jove.com/video/55225/

Introduction

Transpedicular screw fixation is a gold-standard treatment for degenerative lumbar spine and bursting fracture because it involves three columns of the spine and achieves stabilization[1,2]. However, most patients who undergo such surgery also have osteoporosis[3,4]. Many studies have evaluated the fixation strength and the osseointegration status of transpedicular screws, because the loosening of pedicle screws currently in use has been reported in patients with osteoporosis[5,6].

The porcine spine is similar to the human spine in terms of size. It is less expensive compared to a primate model[7]. Furthermore, an *in vivo* mechanical study has demonstrated that the quadruped porcine spine is essentially loaded in the same way as that of the human spine[8], which is why many researchers use porcine spines for studies on the prevention of pedicle screw loosening. However, it takes several months to study pedicle screws in the porcine spine because identifying the long-term stability of pedicle screws takes times. In order to compare different types of screws in the vertebral body, it is necessary to insert the screws in similar positions. Therefore, researchers should be well-acquainted with proper anesthesia techniques, standardized surgical protocols, and harvest procedures before performing any experiments. Here, we describe a detailed method for anesthesia, surgery, and harvest for the evaluation of pedicle screw fixation using a porcine spine model, including *ex vivo* imaging, histology, and strength testing.

Protocol

The Institutional Animal Care and Use Committee of Chonbuk National University approved this study. The treatment, use, and handling of animals followed all guidelines and policies. Maintain the operating room at 24 °C.

1. Anesthesia

1. Acclimatize miniature pigs, aged 12 months, in the experimental unit for at least one week. Perform a clinical examination measuring the respiratory rate, heartrate, and body temperature. Do not feed each miniature pig for 12 h before the anesthesia procedure.
2. Inject atropine (0.05 mg/kg) and ketamin (20 mg/kg)/ xylaxine (2 mg/kg) into the lateral cervical muscle region, behind the ear, for premedication.
3. After sedation, tightly apply a rubber band around the base of the ear and clean the ear with topical alcohol.

4. Place an over-the-needle plastic catheter in the ear vein and remove the rubber band. Confirm that the catheter is correctly placed. Flush the catheter with heparinized saline and fix with tape.
5. **For endotracheal intubation, position the miniature pig in sternal recumbency. With the help of an assistant, hold the jaw of the pig with a suitable sling and open its mouth.**
    1. Pass the tip of laryngoscope into the pharyngeal cavity to displace the epiglottis from the soft palate. Use the tip of the laryngoscope blade to make the vocal cords visible and advance the endotracheal tube into the trachea during expiration.
    2. Feel for a free passage of air for proper intubation and check the chest auscultation for respiration sounds in both left and right sides of the miniature pig.
6. Fill cuff with appropriate volume of air of the endotracheal tube with air using a 10-mL syringe and fix the tube to the snout using adhesive tape.
7. Provide a 2.0% concentration of isoflurane, an inhalation anesthetic for prolonged anesthesia, through the endotracheal intubation tube. Test the corneal and palpebral reflexes to confirm the anesthetization and use ointment on the eyes to prevent dryness.
8. Monitor the cardiovascular system, the respiratory system, and the body temperature during anesthesia at least every 5 min until the miniature pig recovers.
9. 30 min before the spine surgery, apply 30 mg/kg cefazolin IV slowly, the 1st generation of cephalosporin antibiotics.
10. Administer 5 - 10 mL/kg/h of warm (37 °C) saline using an IV line to maintain homeostasis and provide 50 µg/kg/min of fentanyl to control pain.
11. After the spine surgery, perform extubation when a strong swallowing reflex is apparent.
12. Take the miniature pig into a room and monitor until it recovers from anesthesia. Provide food and water when the miniature pig is fully conscious.
13. Apply 3 mg/kg enrofloxacin antibiotic with 4.4 mg/kg carprofen daily for pain control during the first 3 days.
14. Monitor the pig daily until the removal of the sutures.

2. Spine Surgery

1. Autoclave the transpedicular screws and posterior fixator systems for sterilization, following the manufacturer's guidelines.
2. Shave the back of the miniature pig, approximately 10 cm from the center to the left or right, using a shaver while the pig is in a supine position. Clean the skin with povidone-iodine solution and 70% alcohol.
3. Make a longitudinal midline incision from the second spinous process of the lumbar spine to the first median sacral crest using a scalpel. Dissect through the subcutaneous tissue and fascia to the tip of the spinous processes.
4. Elevate the paraspinal muscles subperiosteally from the underlying laminae using a Cobb elevator. Dissect along the spinous process and lamina limited to the facet joints.
5. Open the superficial cortex of the entry point (which is just inferior to the mammillary process from L3 to L5 on both sides) with a burr or a rongeur.
6. Insert the guide pin at an open site, parallel to the superior endplate and at a 20° angle to the spinous process. Define the ideal starting point using C-arm or portable postero-anterior/lateral X-rays.
7. Insert a pedicle probe up to 25 mm according to the X-ray. Confirm the complete intraosseous trajectory by pedicle and body palpation using a pedicle sounding device.
8. Insert six pedicle screws into the prepared pedicle of L3 to L5 until the screw head is well-seated. Point the side opening of the implant head in the desired direction and align the horizontal position with the rod trajectory.
9. Insert two rods into both sides of the pedicle screw heads, respectively. Put in the sleeve and nut at the pedicle screw head using a universal handle.
10. Loosely tighten the nut using a straight socket wrench and firmly tighten the nut using a counter torque wrench.
11. Confirm the positions of the pedicle screws using portable postero-anterior/lateral X-rays.
    NOTE: Wait for the miniature pig to wake up and check the gait patterns and motor function of the hind limb to determine whether a screw has been badly implanted.
12. Irrigate the surgery site with 3 L of normal saline using a bulb irrigation syringe with suctioning.
13. Place a silicone drain in the surgery site and take out the silicone tip. Close the paraspinal muscles and subcutaneous using 1.0 metric absorbable sutures. Close the skin with 2.0 metric non-absorbable nylon sutures.
14. Disinfect the suture site with povidone-iodine and apply a dressing using sterilized gauze and tape.

3. Harvest Procedure

1. At 12 weeks postoperatively, inject xylazine (2 mg/kg) and ketamine (10 mg/kg) into the lateral cervical muscle region, behind the ear, for premedication.
2. After sedation, administer 15 mg/kg KCl directly into the ear vein catheter for euthanasia.
3. Make a longitudinal midline incision at the previous surgery scar lesion. Dissect the soft tissue and paraspinal muscles.
4. Expose the spinous process of the lumbar spines, laminas, rods, nuts, and transverse processes from L3 to L5.
5. Remove the nuts using a counter torque wrench and rods. Cut the L2-3 disc space and the L5-S1 disc space using an oscillating saw.
6. Dissect both sides of middle and anterior part of the L3-5 spine with a Cobb elevator and tower forceps. After harvest, if the spine cannot be tested immediately, wrap it in gauze soaked with saline and store at -20 °C.

Representative Results

A detailed protocol for anesthesia, surgery, and harvest for the evaluation of transpedicular screws using an *in vivo* porcine lumbar spine model is described here. This protocol is suitable for a number of downstream analyses, including mechanical testing (**Figure 1**), quantitative micro-CT evaluation (**Figure 2**), and histology (**Figure 3**). Representative mechanical testing (**Figure 1**) shows the mean extraction torsional peak torque. It represents the bonding strength between the pedicle screw and the bone using a mechanical testing gauge. Three types of pedicle screws were evaluated: uncoated, hydroxyapatite (HA)-coated, and titanium-coated. Data were collected from 14 pedicle screws from each of the three groups. The mean extraction torsional peak torque was greater in the titanium-coated pedicle screw group.

Representative micro-CT images (**Figure 2A**) demonstrate that the region of interest (the inner space of the full length of the screw) can be evaluated by a micro-CT program for the analysis of the bone volume fraction (**Figure 2B1**), bone surface density (**Figure 2B2**), and specific bone surface (**Figure 2B3**). Data were collected from 4 pedicle screws from each of the three groups.

A representative histology image (**Figure 3**) was stained with Goldner trichrome. The interface between the pedicle screw and bone was observable. The red color indicates fibrous tissue, while the blue color indicates bone. In uncoated pedicle screws, fibrous tissue was observed at the interface between the pedicle screw and bone. New bone formation was found at the interface between the threads of both HA- and titanium-coated pedicle screws and bone. In the titanium-coated pedicle screw group, the space between threads of the screw and the bone were compacted with bone (**Figure 3**).

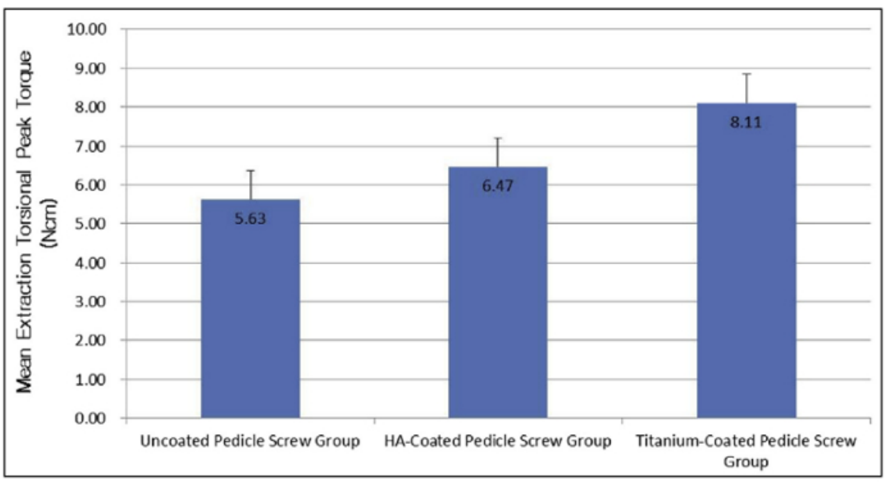

Figure 1: Mechanical Analyses of Pedicle Screws in the Porcine Lumbar Spine. The mean extraction torsional peak torque for the bonding strength between the pedicle screw and the bone was measured with a mechanical testing gauge. Modified from reference[6]. The values are presented as the mean ±SEM (*n* = 14). Please click here to view a larger version of this figure.

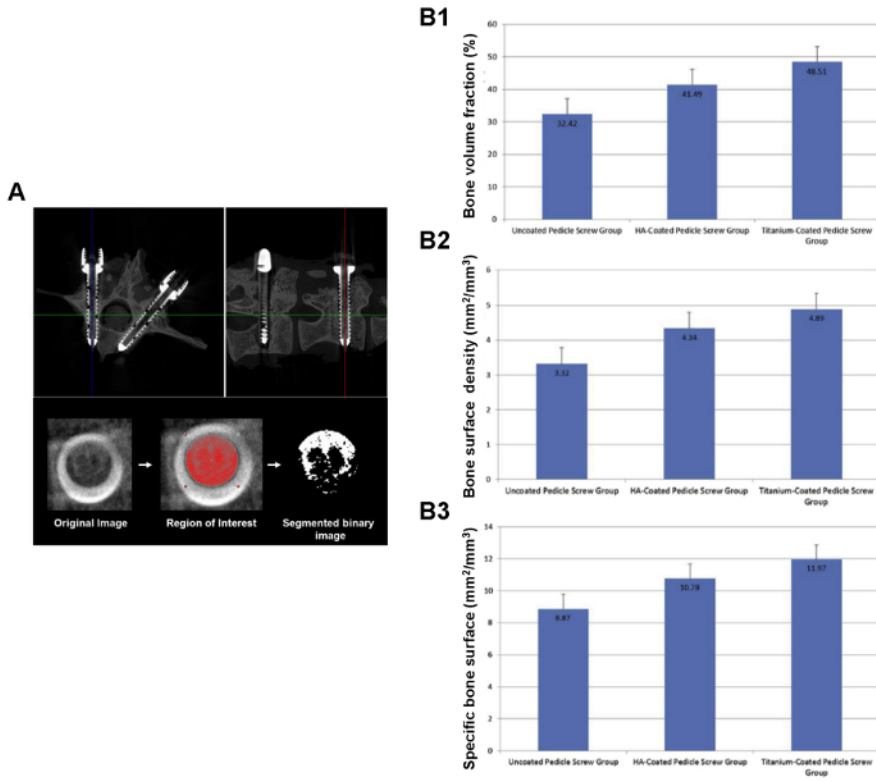

Figure 2. Histomorphological Analyses of Pedicle Screws in the Porcine Lumbar Spine. (**A**) Manual regions of interest (ROI) were set with the inner space of the full length of the screw. (**B**) The bone volume fraction, bone surface density, and specific bone surface area were measured with micro-CT. Modified from reference[6]. The values are presented as the mean ±SEM (n = 4). Please click here to view a larger version of this figure.

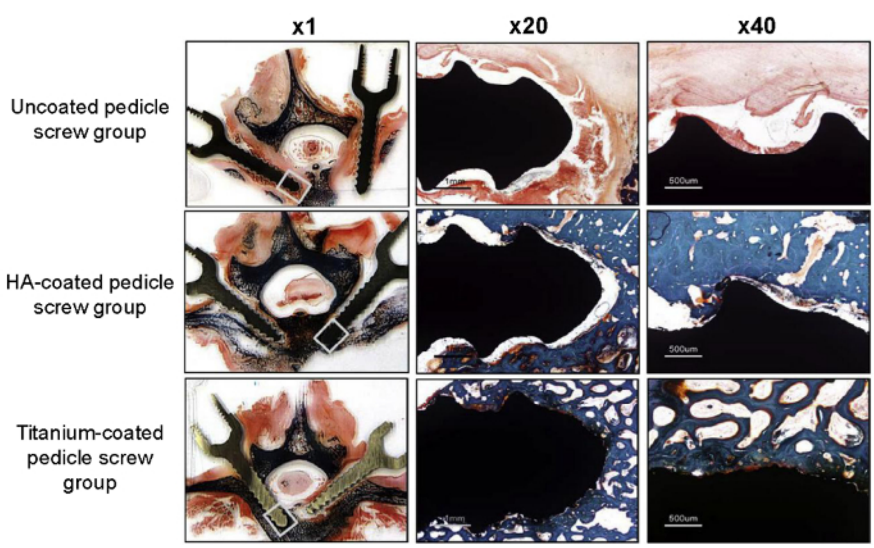

Figure 3: Histological Analyses of Pedicle Screws in the Porcine Lumbar Spine. Goldner trichrome staining (x1, x20, and x40) was performed to observe the interface between the pedicle screw surface and the bone. Modified from reference[6]. Scale bar (black) = 1 mm. Scale bar (white) = 500 µm. Please click here to view a larger version of this figure.

Discussion

The evaluation of transpedicular screws in the porcine spine requires much time and effort. First, the miniature pig is a large animal. For animal care and anesthesia, the researcher needs a specialized protocol. Second, surgery should maintain an environment similar to that of human surgery. The purpose of evaluating pedicle screws in the porcine spine is to develop an efficient screw that can be applied to humans. Third, evaluating the long-term stability of transpedicular screws requires about three months after the spine surgery. Accordingly, researchers in the field of pedicle screw fixation need to standardize the protocol by performing accurate planning. Although many studies on pedicle screws in spine models have been performed[9,10,11], no study has provided a detailed protocol for the evaluation of transpedicular screws using an *in vivo* porcine lumbar spine model.

Although an ideal model for the human spine does not exist, the porcine spine model is an alternative due to the nature of this experiment and to the anatomical size and mechanical characteristics of the spine. In addition, it is relatively inexpensive. McLain *et al.*[12] have compared the morphometry of the fourth lumbar vertebra from human, porcine, ovine, sheep, and dog specimens and concluded that porcine specimens demonstrate several advantages over other animal models as an alternate to the human spine. Furthermore, the quadruped spine of the porcine model is essentially loaded in the same way as that of the human spine[8]. Therefore, the porcine spine is used as an alternate model to the human spine for experiments involving spinal fixation and instrumentation techniques.

In this study, we described the detailed method of anesthesia, surgery, and harvest in the porcine L3-L5 lumbar spine for the evaluation of pedicle screw fixation. Many studies have evaluated pedicle screws after multiple-level spine surgeries[7,13,14]. In many cases of human degenerative disease, spine surgeries are performed through one or two spine fusions. This means that two or three spine levels of pedicle screws are used for fixation[15]. Micro-CT for the evaluation of bone formation around pedicle screws has a limited measurement range. In this case, we used two control screws for L3 pedicles, two HA-coated screws for L4 pedicles, and two titanium-coated screws for L5 pedicles. Because the anatomical dimensions of the L3-L5 lumbar spines are almost the same[16], a comparison between L3-L5 screws is more reliable than a comparison between multiple-level screws. As a result, a two- or three-level fixation of pedicle screws in the lumbar spine is more appropriate compared to multiple-level fixations.

To precisely evaluate and compare the transpedicular screws, one critical point should be kept in mind: each screw should be located at a similar position on the vertebral body. However, the majority of protocols for implanting transpedicular screws in animal models call for exposing and pre-drilling the pedicles of the spine before inserting the pedicle screws[5,13,14]. On the other hand, Upasani *et al.* proposed a surgical protocol that includes determining the position and size of the pedicle screws before surgery by using computed tomography images of the spine[2]. This protocol suggests the insertion of a guide pin at the entry point of the pedicle screw in the lumbar spine. Defining the position of the pedicle screw using C-arm or portable X-rays is also suggested in this protocol. Furthermore, by using a pedicle sounding device, the complete intraosseous trajectory can be confirmed. This protocol can be used to determine the proper positioning of the pedicle screws so that the misplacement of the pedicle can be prevented. This protocol is based on the human spine surgery technique used in our hospital.

This method has some limitations. First, the surgery was performed with a healthy porcine model. Because the purpose of evaluating pedicle screws is to reduce complications in patients with osteoporosis, this protocol should be applied to osteoporotic porcine spine models to demonstrate the effectiveness of the pedicle screws. Second, the porcine spine model requires the purchase and housing of, as well as the surgical equipment for, pigs. This can increase the cost, thus restricting the number of animals that can be used in each study group. Third, this study only included 12-month-old miniature pigs because they were easy to acquire and handle. In addition, although there are several types of devices for spine fixation, only a protocol using a rigid fixation system was used here because it is the most commonly used in spine surgery.

In conclusion, a porcine model of pedicle screw fixation provides a key clinical platform to investigate efficient fixation techniques that cause fewer complications in patients with osteoporosis. This protocol provides technical details for anesthesia, surgery, and harvest in a porcine lumbar spine model. This will facilitate the evaluation of transpedicular screw fixation using this model.

Disclosures

The authors have nothing related to this paper to disclose.

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