

In vitro evaluation of allogeneic bone screws for use in internal fixation of transverse fractures created in proximal sesamoid bones obtained from equine cadavers

Naoki Sasaki, DVM, PhD; Jun Takakuwa, DVM; Haruo Yamada, DVM, PhD; Ryuji Mori, MD, PhD

Objective—To evaluate effectiveness of allogeneic bone screws and pins for internal fixation of midbody transverse fractures of equine proximal sesamoid bones (PSBs) in vitro.

Sample Population—14 forelimbs from cadavers of 3-year-old Thoroughbreds.

Procedures—Allogeneic cortical bone fragments were collected from the limbs of a male Thoroughbred, and cortical bone screws were prepared from the tissue by use of a precision desktop microlathe programmed with the dimensions of a metal cortical bone screw. A midbody transverse osteotomy of each PSB was performed by use of a bone-shaping oscillating saw and repaired via 1 of 3 internal fixation techniques: 1 allogeneic bone screw with 1 allogeneic bone pin (type I; n = 6 PSBs), 2 allogeneic bone screws (type II; 8), or 1 stainless steel cortical bone screw (control repair; 6). Mechanical tension measurements were obtained by use of a commercially available materials testing system.

Results—Mean \pm SD tensile strength (TS) was 668.3 \pm 216.6 N for type I repairs, 854.4 \pm 253.2 N for type II repairs, and 1,150.0 \pm 451.7 N for control repairs.

Conclusions and Clinical Relevance—Internal fixation of PSB fractures by the use of allogeneic bone screws and bone pins was successful. Although mean TS of control repairs with stainless steel cortical bone screws was greater than the mean TS of type I and type II repairs, the difference between type II and control repairs was not significant. Allogeneic screws may advance healing and result in fewer complications in a clinical setting. (*Am J Vet Res* 2010;71:483–486)

Fractures of PSBs in racehorses generally occur during racing or training.^{1–4} Small fragments of bone are usually removed via arthroscopic surgery, and larger fragments often require internal fixation with metal screws or stainless steel wires.⁴ Healing of fractures in PSBs requires more time than healing of fractures in long bones because PSBs are constantly tensioned by the surrounding ligaments (eg, suspensory and straight sesamoidean ligaments) and because vascular distribution to the sesamoids is poor.^{1,3} Thus, it is difficult to provide immobilization, and metal implants (ie, metal screws and stainless steel wires) used for fracture repairs can loosen and shift after placement. The amount of time needed for complete healing can be unexpectedly prolonged in these instances, and poor prognosis may result.^{1,2} For these reasons, development of a fixation material that leads to prompt bone fusion and resists tension from surrounding ligament tissues is needed to treat fractures of PSBs in

ABBREVIATIONS	
AO	Arbeitsgemeinschaft für Osteosynthesefragen
PSB	Proximal sesamoid bone
TS	Tensile strength

racehorses. In human orthopedic practice, autologous and allogeneic bone screws prepared from the cortices of long bones are used for internal fixation of bone fractures.^{5,6} Autologous and allogeneic bone screws have high biocompatibility, and growth factors (including bone morphogenic proteins) contained in these bone screws promote osteoblastic proliferation and facilitate bone fusion and regeneration.⁷ Additionally, autologous and allogeneic bone screws have the potential to function as a biomaterial platform for induction of cells that can fuse with autologous bone cells. However, biomechanical evaluation of such screws has not been performed in horses.

The objective of the study reported here was to evaluate the effectiveness and potential clinical application of allogeneic bone screws and bone pins for the repair of fractures in equine PSBs and to compare the tensile strength of such repairs with the strength of surgical repairs accomplished via an established method (ie, internal fixation with an AO stainless steel cortical bone screw) in an in vitro experiment.

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From the Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-town, Obihiro-city, Hokkaido 080-8555, Japan (Sasaki, Takakuwa, Yamada); and the Orthopaedic Department, Shimane University, 89-1 Enyacho, Izumo, Shimane 693-8501, Japan (Mori).

Address correspondence to Dr. Sasaki (naoki@obihiro.ac.jp).

Materials and Methods

Animals—Fourteen forelimbs were collected from 7 racing Thoroughbreds (1 stallion, 3 mares, and 3 geldings; age, 3 years; mean \pm SD body weight, 430.0 ± 67.6 kg). The horses had no history of signs consistent with forelimb injury and had been euthanized with sodium pentobarbital IV for reasons related to hind limb lameness. Forelimbs were wrapped and stored frozen at -80°C and thawed at 20°C immediately before use. The types of repair were randomly assigned to each limb.

Preparation of bone screws and pins—Fragments (4.5×60.0 mm) of allogeneic cortical bone were collected from the dorsal midshaft of the third metacarpal bone of a male Thoroughbred. Bone screws were prepared by use of a commercially available desktop computer numerical controlled precision microlathe^a programmed with the dimensions of a stainless steel cortical bone screw.^b Completed allogeneic bone screws had an effective diameter of 4.5 mm and were 40.0 mm in length (Figure 1). The microlathe was reprogrammed with the dimensions of a 3.5-mm-diameter drill bit, and bone pins (3.5×36.0 mm) were created. The bone screws were stored frozen at -80°C and soaked in distilled warm (ie, 38°C) water for 30 minutes prior to use. Bone pins were created immediately prior to use or were stored frozen at -80°C .

Fracture and repair of PSBs—Frozen equine forelimbs were thawed at approximately 20°C and soaked in warm water for 30 minutes prior to use. A longitudinal incision (approx 4 cm) was made between the suspensory ligament and the metacarpal bone at the proximal palmar aspect of the metacarpophalangeal joint. The palmar pouch of the metacarpophalangeal joint was dissected to expose the medial and lateral articular surfaces of each PSB. A bone-shaping oscillating saw^c was used to divide each exposed PSB into apical and basal fragments via transverse osteotomy through the middle of the long axis of the bone.

Internal fixation with allogeneic bone screws was performed via 1 of 2 methods. For type I repairs (1 allogeneic bone screw and 1 allogeneic bone pin; $n = 6$ PSBs), bone-holding forceps^d were applied on the palmar aspect of the apical and basal fragments, and a 2.0-mm drill bit^e was used to create a longitudinal hole through the dorsal-axial portion of both fragments of the PSB. A second 2.0-mm hole was created 1 cm palmar to the first hole, and a stainless steel screw^b was temporarily inserted in the second hole to hold the fragments in place. The initial 2.0-mm hole was then enlarged by use of a 4.5-mm drill bit, and screw threads were created with a custom tap^f designed for this purpose. The allogeneic bone screw was then inserted and tightened by hand. After removal of the metal screw used for temporary fixation, the second drill hole was then enlarged to a uniform diameter of 3.5 mm, and a 3.5×36.0 -mm bone pin was inserted.

For type II repairs (2 allogeneic bone screws; $n = 8$ PSBs), the fragments were apposed, and the first allogeneic bone screw was placed as for the type I repairs. A second 2.0-mm drill hole was created palmar and parallel to the first hole, temporarily fixed with a stainless

steel screw,^b and subsequently enlarged to 4.5 mm as described for type I. Screw threads were created with the tap described for type I fixation, and the second allogeneic screw was inserted and tightened (Figure 2).

The control repairs for internal fixation (1 AO stainless steel cortical bone screw^g; $n = 6$ PSBs) were created in accordance with a standard method.⁸ A longitudinal hole was drilled in the dorsal axial aspect of the 2 PSB fragments by use of a 3.2-mm drill bit. A second longitudinal hole was created 1 cm palmar to the first hole by use of a 2.0-mm drill bit, and the drill bits were left embedded for temporary fixation. The 3.2-mm drill bit in the dorsal aspect of the PSB fragments was removed, and the hole was enlarged by use of a 4.5-mm drill bit. Screw threads were created with a 4.5-mm tap, the AO stainless steel cortical bone screw was inserted, and the second drill bit was removed.

Tension testing—After the repairs were completed, the skin, superficial digital flexor tendon, and deep digital flexor tendon were excised from each limb. The straight sesamoidean ligament attachment sites of the proximal and middle phalanges and the suspensory ligament of the carpal and the third metacarpal bones were attached to the tension equipment with a retractor. The limb was fixed in place with clamps. The suspensory and sesamoidian ligaments were bisected longitudinally so that the tensions on the medial and lateral sesamoid bones were each independent of the other. Tension testing was performed by use of a tabletop model materials testing system^h with a crosshead speed of 30 mm/min (Figure 3). The TS of each sample (defined as the maximum load value recorded by the system software before the screws were pulled entirely free of the bone) was measured.



Figure 1—Photograph of an allogeneic bone screw (dimensions, 4.5×40.0 mm) prepared by use of a commercially available precision desktop microlathe programmed with the dimensions of a stainless steel surgical screw. The source of bone material was the dorsal midshaft cortex of the third metacarpal bone from the cadaver of a 3-year-old male Thoroughbred.

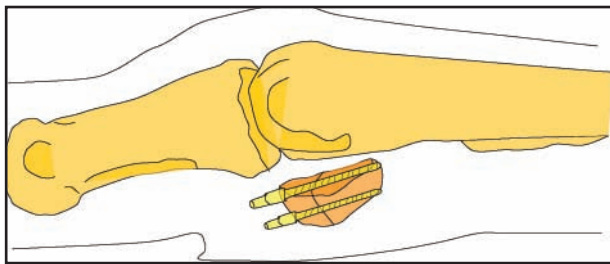


Figure 2—Diagram indicating placement of allogeneic bone screws in type II repairs for midbody fractures of PSBs in vitro. The 2 bone screws were inserted into holes drilled through the distal end of each PSB fragment. For type I repairs, a single allogeneic bone screw was placed in a similar manner through the dorsal aspect of the PSB fragments, and instead of a second bone screw, an allogeneic bone pin was placed through the palmar aspect.

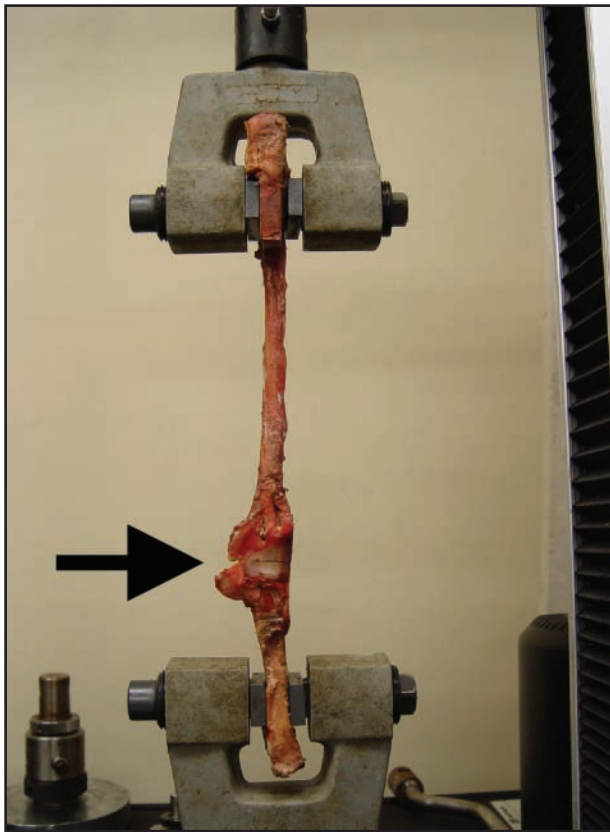


Figure 3—Photograph of a tabletop materials testing system used to perform tension tests of experimentally induced fracture repairs (black arrow) in vitro. The carpal bone and the proximal phalanx were held vertically with clamps, and tension was applied until the screws were pulled free of the bone.

Statistical analysis—A Wilcoxon signed rank test was used for statistical analysis. Values of $P < 0.05$ were accepted as significant.

Results

Equine allogeneic bone screws were successfully prepared by use of a precision microlathe system. Mean \pm SD TS of type I repairs with allogeneic bone screws (668.3 ± 216.6 N) was significantly ($P = 0.013$) less than the mean TS of control repairs made with AO stainless steel cortical bone screws ($1,150.0 \pm 451.7$ N; Figure 4). For type II repairs with allogeneic bone screws, mean TS (854.4 ± 253.2 N) was significantly ($P = 0.035$) greater than that of type I repairs. The mean TS of type II repairs was less than that of control repairs, but this difference was not significant.

Discussion

Transverse fractures of PSBs are categorized as apical, midbody, or basal, depending on location,^{1,2} with apical fractures being the most prevalent. Treatment of apical and basal fractures of PSBs usually involves removal of small fragments of bone via arthroscopy, and the prognosis is generally favorable.¹⁻⁵ In contrast, midbody fractures of PSBs are usually treated via internal fixation with metal screws or wires. The prognosis for recovery from midbody fractures, which can involve

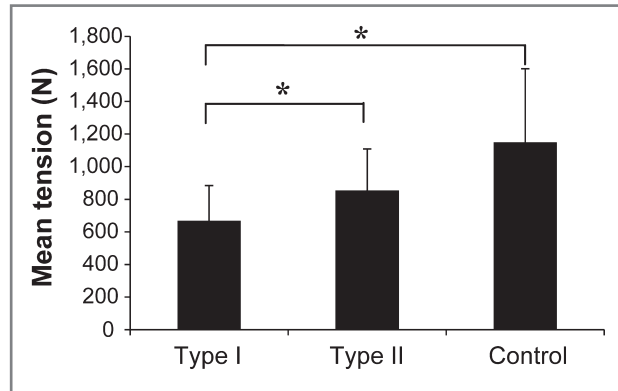


Figure 4—Mean \pm SD TS of repairs for type I (1 allogeneic bone screw and 1 allogeneic bone pin; $n = 6$ PSBs), type II (2 allogeneic bone screws; 8), and control (1 AO stainless steel cortical bone screws; 6) internal fixation techniques for repair of midbody fractures of PSBs in vitro. *Values differed significantly ($P < 0.05$) between groups.

substantial injury to the suspensory ligament, is poor, compared with the prognosis for apical and basal fractures.³ In the present study, midbody transverse fractures of PSBs were created in vitro to evaluate the potential application of allogeneic bone screws as internal fixation devices to repair this type of injury. Forelimbs were selected because this is the most common site for sesamoid fracture in racing Thoroughbreds.

In the experiments described here, allogeneic bone screws from equine cadaver limbs were used instead of screws created from autologous bone for internal fixation of experimentally created fractures of PSBs. In autologous bone grafting, surgery is required at 2 sites (ie, tissue collection and reduction sites), thereby extending the duration of the procedure. Additionally, the risk of infection and other complications associated with surgical interventions is decreased when only 1 surgical procedure is performed on a horse. Investigators in other studies^{6,7,9} determined that storage at -80°C resulted in significantly reduced immunogenicity; thus, bone screws stored at -80°C are not expected to cause a substantial immunologic reaction in a recipient of the same species. The bone screws made in the study reported here were stored at -80°C until use. Because bone strength is maintained when the tissue is moistened,⁹ the bone screws and the sesamoid bones were soaked in water for 30 minutes before fixation.

Cancellous bone from the ilia and sternbrae is often used for bone grafting in horses,^{10,11} but the TS of cancellous bone is inadequate when used as bone screw material.⁶ In the authors' experience, it is difficult to shape cancellous bone in the form of a screw. Therefore, in the present study, cortical tissue of the third metacarpal bone of adult horses was used as bone screw material. The mineral content and breaking strength of bone in 3-year-old horses is greater than that of younger horses; bone strength peaks at approximately 6 years of age and then begins to decline.¹² For this reason, bone from a 3-year-old horse was selected as the source of screws for the present study. The microlathe system was developed from a supersmall high-precision lathe for medical use, and its processing program allows precise screw-thread cutting. In the study reported here, bone

screws and bone pins were successfully prepared by use of the microlathe system.

Usually, realignment of fractured PSBs initially requires temporary insertion of a Kirschner wire or a 2.0-mm drill bit to prevent bone rotation associated with the insertion of metal screws.⁸ For type I repairs, a stainless steel screw was temporarily placed in the second drill hole, and then a 3.5-mm allogeneic bone pin was inserted, with the intention of preventing or reducing the loss of strength that could have resulted from PSB rotation. For type II repairs, a second bone screw was inserted instead of the bone pin. Because the bone screws had threads, repairs that included a second screw were expected to provide stronger apposition of PSB fragments, compared with repairs that included use of a bone pin. Thus, the overall strength of the repair would be increased. The mean TS for type II repairs was significantly higher than the mean TS for type I repairs, although it remained less than the mean TS for control repairs. Although the difference between type II repairs and control repairs was not significant, it may have been attributable to the possibility that bone screws had relatively less resistance to the shear forces created with bone rotation during tension on the suspensory and straight sesamoidean ligaments. Thus, the TS of repairs made with allogeneic bone screws may be enhanced by modifying the design to yield greater resistance to shear forces.

In the study described here, we determined that equine allogeneic bone screws can be created by use of a desktop microlathe system. Fractures of PSBs were created in vitro and repaired by use of these bone screws via 2 internal fixation techniques. Mean TS of the repairs was compared with the mean TS of repairs made by use of control AO stainless steel cortical bone screws. Although the mean TS of allogeneic bone screw repairs for type I was less than that of control screw repairs, greater TS was achieved by increasing the number of allogeneic bone screws used for internal fixation. To the authors' knowledge, the design of allogeneic bone screws with enhanced strength and their potential biological adaptability and clinical application have not been investigated. The treatment of fractures in other bones may also be enhanced by concomitant use of allogeneic bone screws with external fixation techniques, such as casts. Further assessment of potential biological reactions that may be associated with the use

of allogeneic bone screws in vivo is needed before eventual clinical applications can be determined.

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- a. MTS4 computer numerical controlled precision lathe, NanoWave, Shimane, Japan.
 - b. Ace screw, 4.5 × 40 mm, MDM, Tokyo, Japan.
 - c. Oscillating saw, Synthes, Tokyo, Japan.
 - d. Reposition forceps, 208 mm, Aesculap, Tokyo, Japan.
 - e. Drill bit, 2.0 mm, Synthes, Tokyo, Japan.
 - f. Tap, 4.5 × 100 mm, Shimane University, Shimane, Japan.
 - g. Cortical bone screw, 4.5 × 38 mm, Synthes, Tokyo, Japan.
 - h. Instron 5565, Instron, Canton, Mass.
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