Original Investigation

Comparison of Stabilization and Fusion Methods with Xenograft Plate Screws After Anterior Lumbar Corpectomy in Dogs

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ABSTRACT

AIM: Fusion development is the primary goal in spinal surgeries that are conducted for the treatment of vertebral body pathologies such as trauma, tumor and infection. Stabilization using metal plate screws together either with an autograft, allograft or xenograft is used. We evaluated fusion development in stabilizations that were carried out with xenograft (XG) with XG plate-screw (XPS) and XG with metal plate-screw (MPS) systems in dogs' lumbar vertebrae (L5-7 segment) in terms of radiological, biomechanical and histopathological aspects.

MATERIAL and METHODS: The animals were divided into 4 groups, each including 5 subjects. The experiment consisted of Control group 1 which did not go through any procedure and was stabilized, Control group 2 which underwent instability with only L6 anterior corpectomy, Experimental group 1 which was stabilized with intervertebral XG and XPS after L6 corpectomy, and Experimental group 2 which was stabilized with intervertebral XG and MPS after L6 corpectomy. Development of fusion in the Experimental groups 1 and 2 was evaluated in terms of radiological and histopathological aspects.

RESULTS: Comparison of Control and Experimental groups showed an increase in resistance in all activities on biomechanical tests (p<0.01). Fusion development was observed in the radiological and histopathological examinations of the subjects in the Experimental group. On the other hand, Experimental groups 1 and 2 did not show a significant difference in the biomechanical test comparisons (p>0.05).

CONCLUSION: Xenograft plate screws and metal plate screws provide equivalent fusion and stabilization in anterior lumbar stabilization.

KEYWORDS: Xenograft, Screw, Corpectomy, Lumbar anterolateral approach, Lumbar vertebra, Biomechanic test

■ INTRODUCTION

The number of spinal surgeries for the treatment of pathologies related to the vertebra corpus is increasing with a fast pace (34). Obtaining fusion development is usually the primary target in these surgeries. Autografts are used for this purpose. In case of insufficient autografts volumes, allografts and xenografts are used. Anterolateral approaches for lumbar vertebra corpus pathologies provide the best decompression, stabilization and fusion (11,12,25). The use of this surgical approach has increased with implant diversity and surgical techniques that improved after the first anterior lumbar fusion surgery using an internal fixative device by Humphries et al. (5,12,25,32).

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Although there is plenty of information about obtaining fusion by using autograft, allograft, and synthetic and osteoinductive factors (8-10,13,15,18,31,37), there is only one elucidative experimental study about the use of xenografts (24). Furthermore, there is currently no study on obtaining fusion through a stabilization system with plate and screws prepared from a xenograft (XG) for anterior lumbar vertebrae. There are some reasons for using a xenograft; it is cheap and easy to derive tricortical grafts with the desired size, so a xenograft is assumed to be an alternative for other grafts.

Our aim in this study on an instability model created with lumbar corpectomy was to examine the fusion that develops with intervertebral xenografts in terms of its radiological, biomechanical and histopathological aspects, in addition to the xenograft plate screw's (XPS) contribution to the stabilization and its comparison to metal plate screws (MPS).

■ **MATERIAL and METHODS**

Uludağ University's Medical Faculty Ethics Committee approved the study. Thirty-two adult, mixed breed dogs, with similar and weights varying between 20-25 kg were used. The subjects were kept under observation for 2 weeks and went through medical examinations. Study subjects were divided into 4 groups:

Control group 1 (CG-1), the L_{5-6-7} segments of the subjects were taken out without applying any previous procedures.

Figure 1: Trans-abdominal approach position.

Figure 2: L6 corpectomy.

Control group 2 (CG 2), instability was induced in the L_{5-7} segment with L_6 corpectomy.

Experimental group 1 (EG 1), L_{5-6} and L_{6-7} discectomy and L_6 corpectomy were performed with a trans-abdominal approach (Figures 1, 2). After placing XG into the corpectomy area, stabilization was obtained with XPS.

Experimental group 2 (EG 2), L_{5-6} and L_{6-7} discectomy and L_6 corpectomy were performed with a trans-abdominal approach. After placing XG into the corpectomy area, stabilization was obtained with MPS.

Fusion development and screw-plate were monitored on the 1st day, the 2nd week, the 1st month, the 2nd month and the 3rd month after the surgery with bilateral lumbar radiographies. At the end of the 3rd month, the subjects were sacrificed with high dosage Pentothal, their lumbar vertebrae were removed (Figure 3A,B) and the ligamentous, articular and intervertebral disc structures of the L_{5-7} segments' were maintained as they were dissected from the muscles. They were placed in 10% formalin.

Biomechanical tests were performed on all the vertebrae taken from all the subjects in all groups.

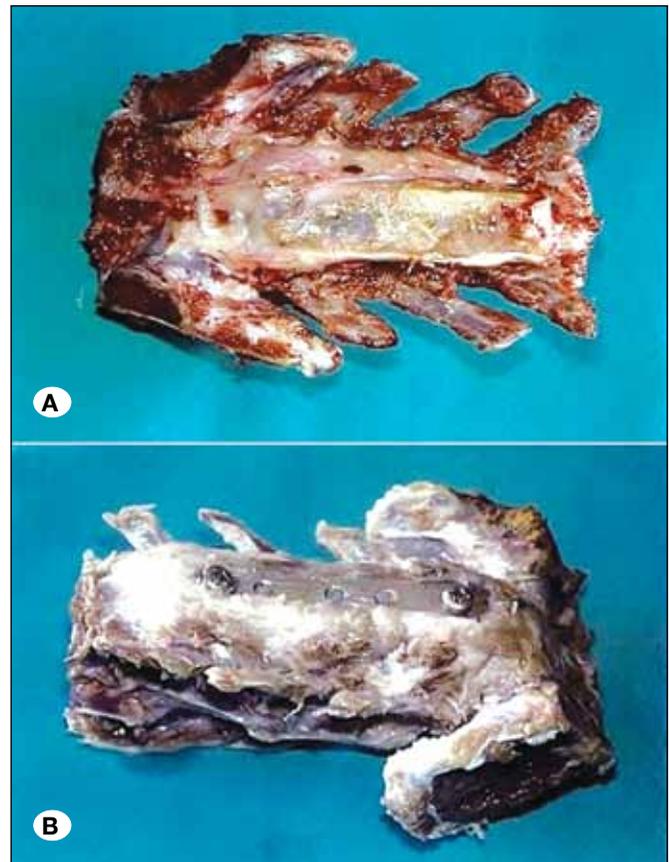After biomechanical tests, the lumbar vertebra blocks were decalcified. Axial and sagittal sections were taken for

Figure 3: Lumbar vertebrae taken out at 3rd month. **A)** xenograft + xenograft screw-plate subject, **B)** xenograft + metal screw-plate subject.

pathological examination. Their histopathological examinations were performed under a light microscope as they were stained with Hematoxylin and Eosin (H&E).

Preparation of Plate and Screw

The XGs used in the surgical operation were made of cattle tibia. The screws were prepared as 2.5 cm length and 4 mm diameter, and plates as 6, 7.5, 9, 10.5 cm length and 1 cm width, 2 mm thickness (Yunnan Machine Tool Works, China) (Figure 4A). Metal screws and plates were prepared with similar sizes (Figure 4B). The XG used for fusion was shaped during the operation according to the corpectomy area. Before the surgical operation, XG and metal implants were sterilized in an autoclave in 230°C for 30 minutes.

Surgery

The surgical procedures for the CG 2 and EG 1, 2 subjects were performed in the operating room of the Uludağ University's Veterinary School. All the subjects that were operated went through the same anesthesia and surgical procedures. The animals were left without food for 12 hours before the operation. They were sedated with 2% xylazine-HCL and 2.5 mg/kg (Rompun, Bayer) was intramuscularly injected. Extremities were determined in supine position, and heart rate and respiration were monitored. For anesthetic drugs and maintenance fluid application, a catheter (18 GA intravenous (IV) catheter angiocath, Becton Dickinson Vascular Access Sandy, Utah, USA) was placed into cephalic antebrachial vein. The anesthetic induction was with a 15 mg/kg dose of Pentothal (Thiopental Sodium, 0.5 g İE Ulugay Pharmaceutical Industry, Turkey) through the intravenous route. Following respiratory depression, the dog was intubated with the proper endotracheal intubation tube, attached to the anesthesia device (Sweden) and anesthesia was realized with 50% oxygen and 2.5% halothane. Hair in the area of the operation was shaved and antibiotic prophylaxis was administered with ceftriaxone 50 mgr/kg intravenously before the operation.

After field cleaning and covering in accordance with the surgical sterility principles, an abdominal midline incision was performed. The intraperitoneal organs were mobilized superiorly and the retroperitoneal region was reached. The inferior vena cava, iliac artery and aorta were excluded and the lumbar vertebra corpus front face was reached. After the L_{5-6}, L_{6-7} anterior discectomy, L6 corpectomy was done with the help of a mini drill (Microton GC 412; Aesculap Co., Tuttlingen, Germany) until the posterior longitudinal ligament was seen (Figure 5A). The XG was shaped according to the size of corpectomy area and placed into the intervertebral region. The stabilization between L_5 and L_7 was obtained with XGPS in experimental group 1 (Figure 5B) and with MPS in experimental group 2 (Figure 5C). The abdominal organs were replaced into their normal position and all the layers were closed according to their anatomy.

Biomechanical Tests

The biomechanical tests were conducted by Uludağ University Faculty of Engineering, Mechanical Engineering Department's Resistance Unit using the Instron 4301 (Instron, England)

universal tensile testing system and comparator device with 1/100 mm sensitivity. The lower and upper parts of the lumbar spinal column were fixed to a pallet in a standard way. The lower pallet was fixed for all the tests and the upper pallet was applied force.

Flexion, extension, right on left lateral flexion (four-way movement), axial loading and torsion tests were performed on lumbar spinal columns at 18°C room temperature. The torsion test, which is the most destructive test, was performed following other biomechanical tests.

In four-way movement measurements, tests were performed as force was applied to the moment arm, from a 15 cm distance, fixated onto the upper pallet in the center of gravity (Figure 6). The measurements were conducted by applying direct force to the center of gravity for axial loading in the vertical position. The measurements were done for torsion tests by turning the mechanism to the horizontal position (Figure 7).

15 mm/min fix loading speed was applied for the tests for four-way movements while 5 mm/min fix loading speed was applied for axial loading and torsion tests. The force was applied in a way that the total displacement of the moment arm was 3.5 millimeter (mm) for axial loading and 10 mm for torsion. For four-way loading, all subjects were imposed 200 mm bending force (Figures 9A-D, 10A-D). The measurements for resistance against the applied force were performed once every 2 mm for four-way movement and torsion movements, and once every 0.5 mm for axial loading. The resistance
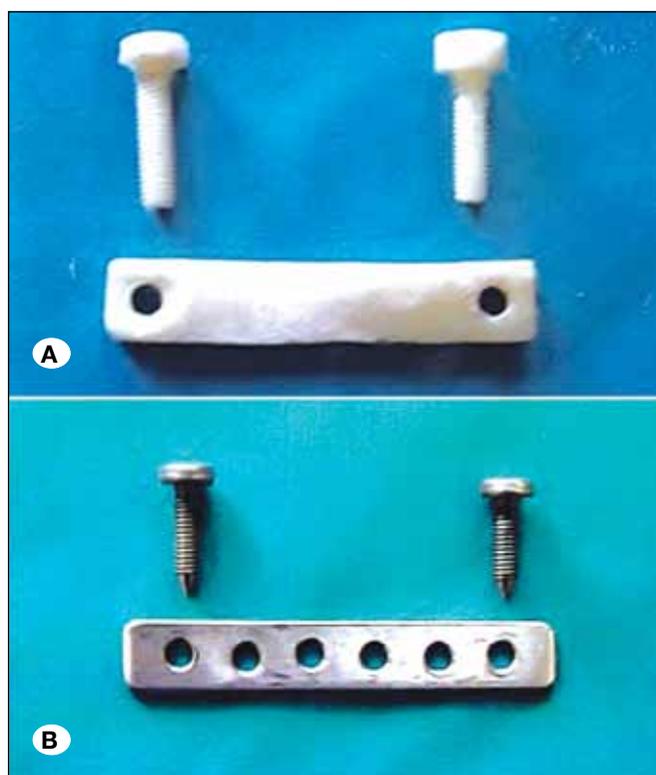
Figure 4: Xenograft and metal screw-plates. **A)** xenograft screw-plate, **B)** metal screw-plate.
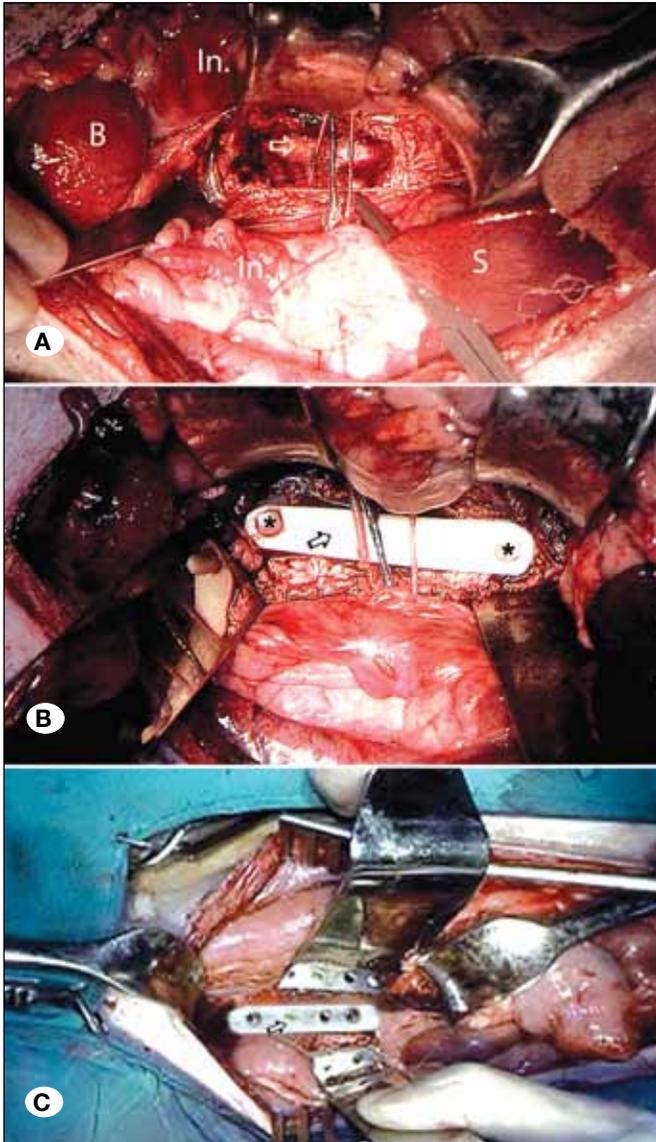
Figure 5: A) Distance after L6 anterior corpectomy (B; bladder, In; intestine, S; spleen, White arrow; posterior longitudinal ligament). **B)** Xenograft screw-plate after fixation (White arrow; xenograft screw-plate). **C)** Metal screw-plate after fixation (White arrow; metal screw-plate).

against force in the lumbar vertebra blocks was measured and recorded in Newton measurement units.

Statistics

The Kruskal-Wallis Non-parametric ANOVA test was used for comparing the biomechanical test results of the groups. When discrepancy was observed, a comparison was made between the groups with the Mann-Whitney U test, and a p value <0.05 was regarded as statistically meaningful.

■ RESULTS

A total of 10 experiments with EG 1 and EG 2 were performed on 22 operated dogs. Twelve dogs were lost due to malnutrition
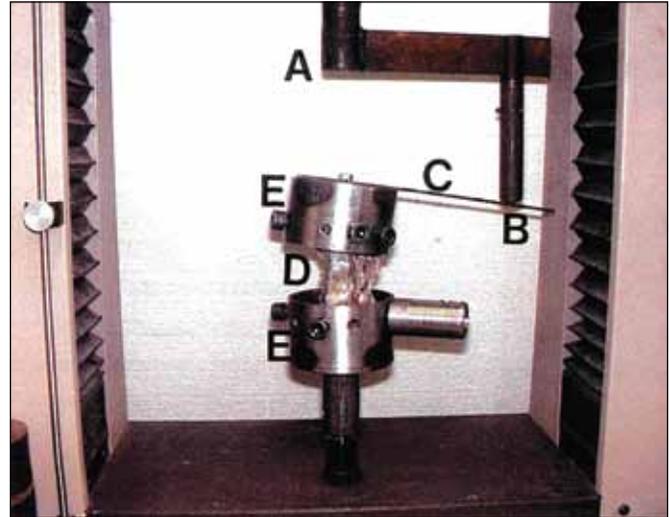
Figure 6: Mechanism for Flexion tests (A; level arm, B; application point, C: moment arm, D; lumbar spinal column, E: pallets).
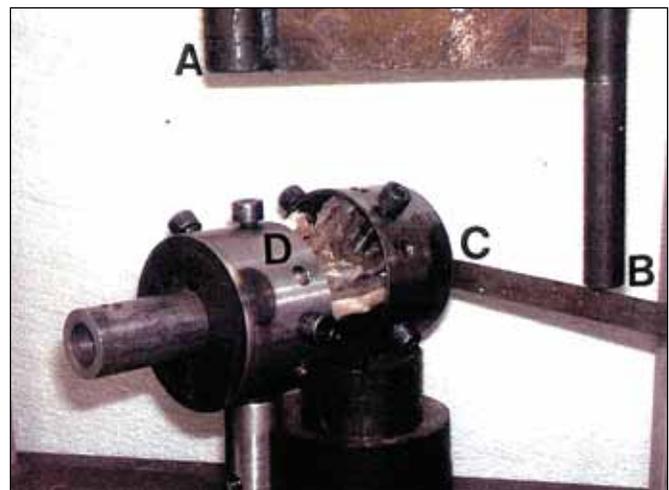
Figure 7: Mechanism for Torsion tests (A; level arm, B; application point, C; moment arm, D; pallets and lumbar spinal column).

(n=5), sepsis (n=2), bronchopneumonia (n=1), anesthesia complications (n=1) and idiopathic (n=3) reasons.

Radiologic Findings

The direct x-ray findings of the subjects are given in Table I. It was observed in the 2nd week follow-up radiography that one of the bone screws of a subject in the EG 1 group had been broken (Figure 8). It was observed that the XG plate and the XG within the space of corpectomy were in place and fusion developed without issue in the follow-up. It was also observed in the 1st day post-operative control lumbar radiography that the upper metal screws had been displaced and the plate was not in place in one of the subjects in the EG 2. The subject was operated again and metal screw-plate was revised. No additional issue was observed during the follow-up.

For all of the EG 1 subjects, directs radiographs were taken after the operation (Figure 9A).

In the EG 1 subjects, it was observed during the 1st month follow-up that there were irregularities on the XG plate contours and resorption had begun on the parts of the XG screws within vertebra corpus. A decrease was observed in the XG matrix in the corpectomy area (Figure 9B).

In the second month control radiographies, it was observed that the XG plate had got even thinner, the sharpness of the edges had diminished, and the parts of the XG screws within the corpus were totally resorbed. The density of the intervertebral XG in the corpectomy area had decreased even more, the irregularities of its contours had become more visible and partial fusion with adjacent corpuses had developed (Figure 9C).

In the third month control radiographs, it was observed that the thickness of the XG plate had decreased and it had gained a heterogeneous appearance due to resorption, the borders of the corpectomy graft had fully disappeared and full fusion had developed with the vertebrae that it was is in touch with (Table I, Figure 9D).

In the follow-up radiographs of the EG 2 subjects, it was observed that resorption and fusion development phases observed in the XG in the corpectomy area were similar to those in EG 1 (Figure 10A-D).

In the 3D tomography of lumbar vertebra, it was observed that a full fusion had developed in the group where stabilization was obtained with bone, plate and screw, and the graft had provided fusion in such a way to form a block between the upper and lower vertebras. When the bone plate was examined, it was observed that it was partially resorbed and its edge sharpness had disappeared (Figure 11A,B). Fusion formation and plate-screw location in the group where stabilization was obtained through metal plate-screws could not be evaluated in an optimal manner due to artifacts.

Results of the Biomechanical Studies

The biomechanical test results are shown in Tables II, III and Figures 12, 13.

The lumbar vertebra biomechanical test results of the CG 1 subjects were considered as physiological stabilization values and compared with the biomechanical test results of the vertebra blocks of the subjects of the other 3 groups. The lumbar vertebra biomechanical tests results of the CG 2 group were considered as unstable vertebra values.

The resistance results obtained with the forces applied in the biomechanical tests were compared between the groups. A meaningful resistance was observed in the EG 1 and 2 subjects in biomechanical tests in terms of stability obtained in all movements as compared to CG 1 and CG 2. No statistical difference was observed in the comparison of the biomechanical results of EG 1 and 2 (Table III).

When a maximum gradient of 20 mm was reached in the movements of flexion, extension, flexion towards right and flexion towards left, the average force that was required per mm was respectively 0.46, 0.3, 0.42, 0.51 Newton-meter/degree for the CG 1, 0.26, 0.08, 0.23, 0.31 Newton-meter/

Figure 8: Broken bone screw of subject no:3 in bone screw-plate stabilization group.
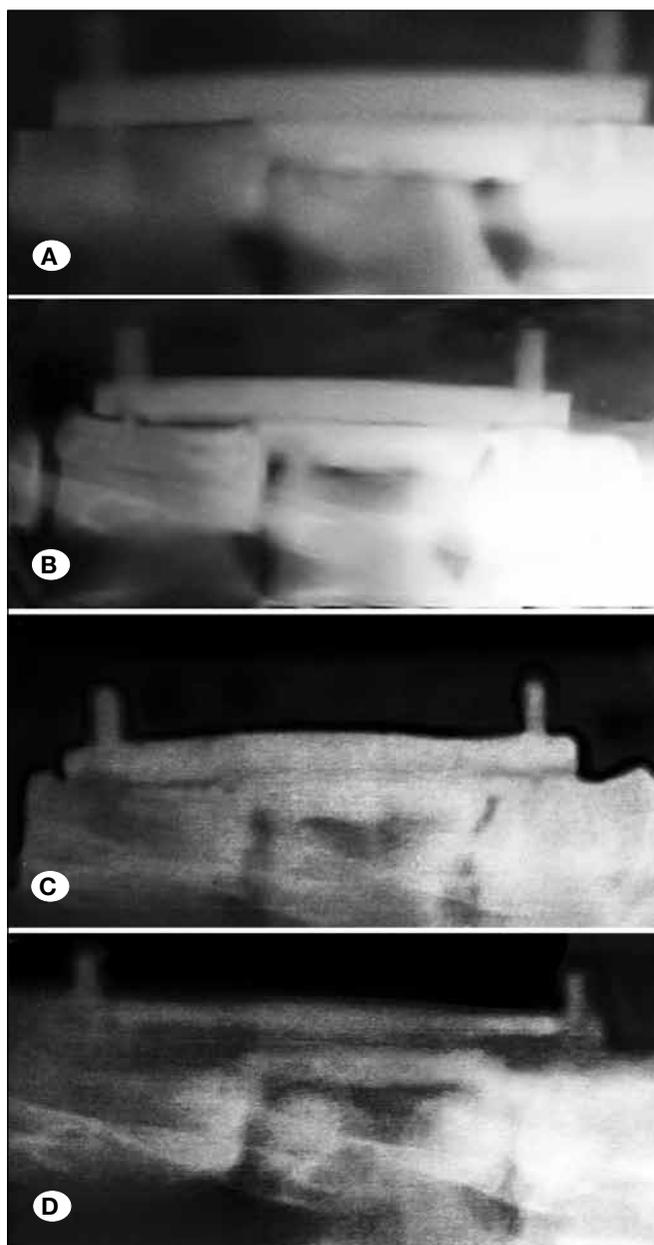
Figure 9: Radiological follow-ups of EG-1. **A)** 1st week, **B)** 1st month, **C)** 2nd month, **D)** 3rd month.
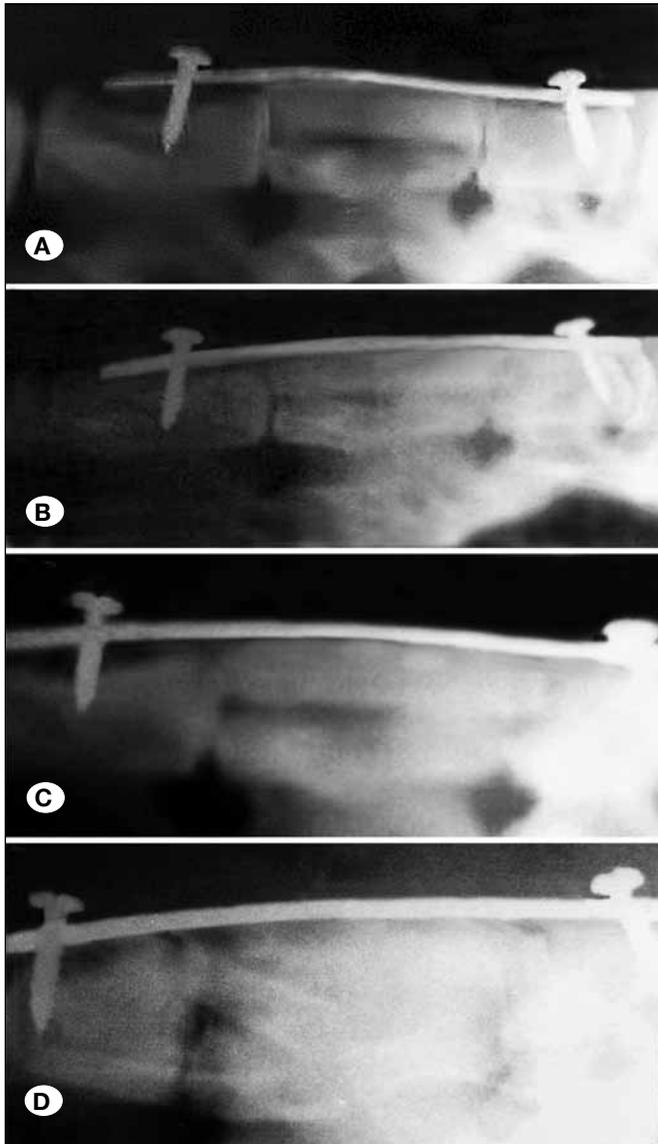
Figure 10: Radiological follow-ups of EG-1. **A)** 1st week, **B)** 1st month, **C)** 2nd month, **D)** 3rd month.

degree for the CG 2, 1.24, 1.2, 1.38, 1.79 Newton-meter/degree for the EG 1, and 1.24, 1.27, 1.39, 1.28 Newton-meter/degree for the EG 2 group (Figure 12, Tables II-1 and II-2). While a meaningful decrease in resistance ($p<0.05$) in favor of instability was observed in the CG2 as compared to the CG 1, no difference was observed between EG 1 and 2 ($p>0.05$). When both CG and EGs were compared, it was observed that the EG showed a meaningful resistance ($p<0.01$).

When a maximum compression of 3 mm was reached in the axial loading test, application of 380.8, 132.8, 881.2, 722.4 Newton force was needed for the CG 1, CG 2, EG 1 and EG2 group respectively (Figure 13).

When a maximum bending of 10 mm was reached in the torsion test, the forces needed to be applied per mm for the CG 1, CG 2, EG 1 and EG 2 group were 1.98, 0.3, 2.26, 2.54 Newton-meter/degree respectively (Tables II-1 and II-2).
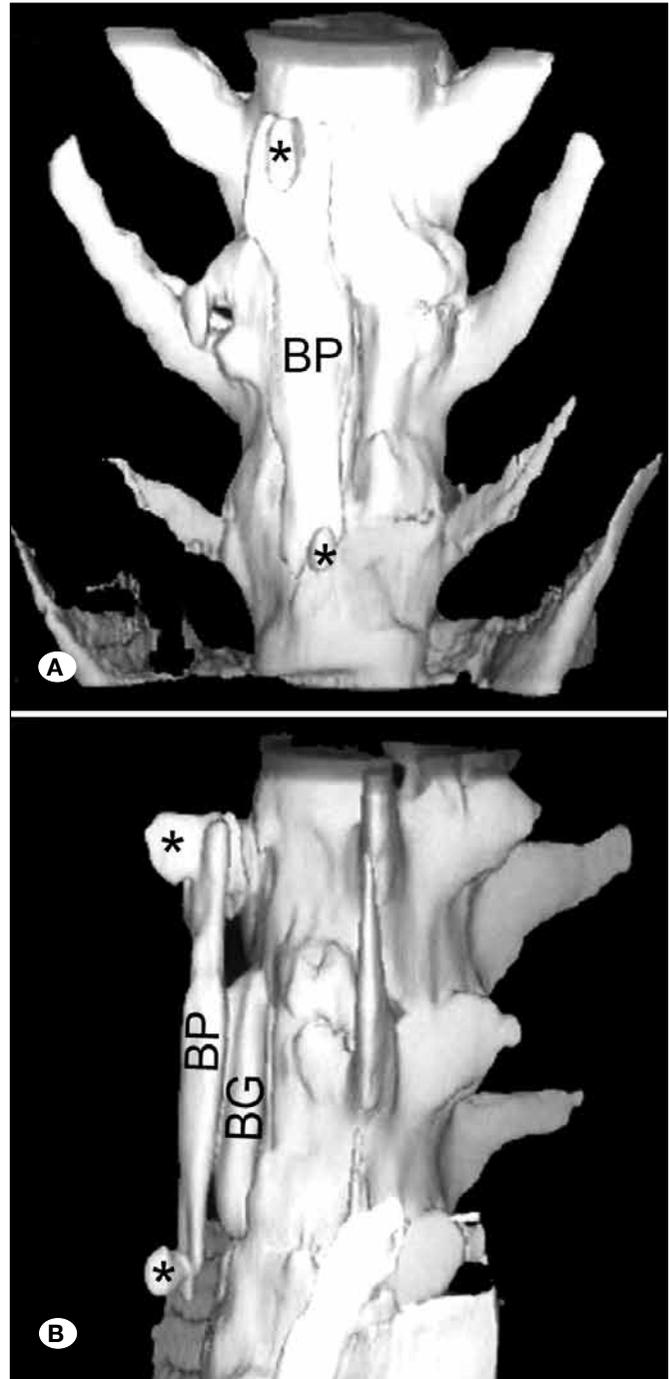
Figure 11: 3D computed tomography. **A)** Front view of fixation area of EG-1 (BP; bone plate, Black asterisk; bone screw). **B)** Side view of fixation area of EG-1, (BP; bone plate, BG; bone graft).

Histopathological Findings

Dense gray-white fibrotic tissue was observed around both the metal and XG screw and plates in the microscopic examinations of the vertebrae of the EG 1 and EG 2 subjects. The granulation tissue contained the intervertebral graft and the vertebrae had become a firm block (Figure 3A, B).

Table I: The Direct X-ray Findings of the Subjects in the Experimental Groups

	Experimental Group – 1					Experimental Group – 2				
Subject no	1	2	3	4	5	1	2	3	4	5
Level	L5-7	L5-7	L5-7	L5-7	L5-7	L5-7	L5-7	L5-7	L5-7	L5-7
Angulation	-	+	-	-	-	-	-	-	-	-
Fusion	+	+	+	+	+	+	+	+	+	+
Plate Separated	-	-	-	-	-	-	-	-	-	-
Screw Loosened	-	-	-	-	-	+	-	-	-	-
Plate Broken	-	-	-	-	-	-	-	-	-	-
Plate Absorption	+	+	+	+	+	-	-	-	-	-
Screw Absorption	+	+	+	+	+	-	-	-	-	-
Screw Broken	-	-	+	-	-	-	-	-	-	-
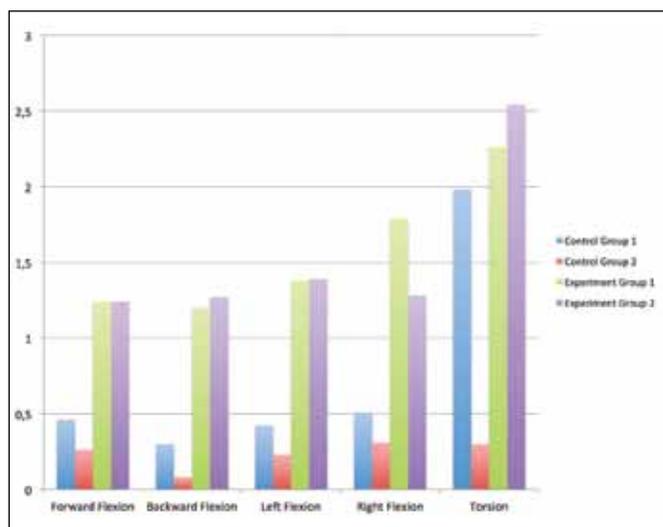
Figure 12: Biomechanical test results. Forward, backward, left, right flexions.
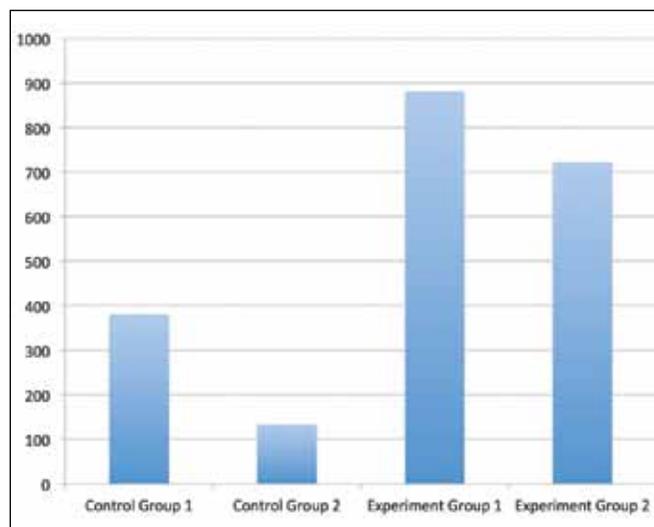
Figure 13: Biomechanical test results for loading. Result units are Newton.

On histopathological examination, it was observed that the parts of the screws within the corpus were resorbed in the EG 1 subjects.The XG plate and screws were surrounded by dense granulation tissue, and widespread vascular fields existed in the granulation tissue and the fibrotic area. The intervertebral XG in this group was examined in terms of resorption and it was found that the borders had become irregular, with granulation, vascularization, cartilaginous fields, ossification islets and fusion development (Figure 14A, B). No infection was observed in any preparation.

The metal screws and plates of the EG 2 subjects were examined after they were unfastened. Granulation, vascularization and dense fibro-cartilaginous fields and ossification fields were observed in the intervertebral XG area. Inflammation findings connected with reaction to possible foreign bodies were observed in 2 subjects in this group.

■ **DISCUSSION**

Quality of fusion is the most important factor that affects the clinical result. The most important factor that affects fusion development is the type of graft material and the surgical technique. Synthetic grafts that contain autograft, xenograft, bone morphogenetic protein (BMP) and demineralized bone matrix (DBM) are used for fusion formation (10,18,33,35). The ideal bone graft should be biomechanically stabile, osteoinductive, osteoconductive, should not carry any disease, and should have minimal antigenic features.

Today many surgeons think that iliac crest and bone autografts are the gold standard for lumbar spinal fusion. Following iliac crest and bone grafts, many studies have shown successful fusion in the lumbar spine (10,18,28,33). However, morbidity rates related to iliac crest and bone grafting are high. Some studies have shown up to 50% permanent pain at the donor

Table II.1: The Biomechanical Test Results of the Control Groups

	Control Group 1			Control Group 2		
	Average	Median	Standard Deviation	Average	Median	Standard Deviation
Forward Flexion						
2	1.60	7.0	1.8708	1.20	1.0	0.4477
4	2.40	2.0	1.5166	1.80	2.0	0.4477
6	3.40	3.0	2.0736	2.40	3.0	0.8944
8	3.80	3.0	2.4900	2.00	2.0	0.7071
10	4.60	3.0	2.6071	3.00	3.0	1.2247
12	5.40	4.0	3.2091	3.60	3.0	1.3416
14	7.00	5.0	4.7610	3.40	3.0	1.6733
16	7.25	5.0	5.2520	4.00	3.0	2.3452
18	8.25	6.5	5.3151	4.60	4.0	2.1909
20	9.25	7.0	6.7020	5.20	5.0	1.9235
Backward Flexion						
2	0.80	1.0	0.8367	0.60	1.0	0.5477
4	1.80	2.0	0.8367	1.20	1.0	0.4477
6	2.40	2.0	1.1402	1.00	1.0	0.7071
8	2.60	2.0	0.8944	1.00	1.0	0.7071
10	3.20	3.0	1.0954	1.00	1.0	1.0000
12	4.00	3.0	1.4142	1.80	2.0	1.0954
14	4.00	3.0	1.4142	1.60	2.0	0.8944
16	4.40	4.0	1.5166	1.80	2.0	1.0954
18	5.00	5.0	1.5811	2.00	2.0	1.2247
20	6.00	7.0	1.8708	1.60	2.0	1.1402
Left Flexion						
2	1.40	1.0	0.5477	0.40	0.0	0.5477
4	1.80	1.0	1.3038	1.00	1.0	0.7071
6	1.80	1.0	1.3038	1.40	1.0	0.5477
8	3.00	2.0	1.4142	2.00	2.0	1.0000
10	3.80	4.0	1.3038	1.80	1.0	1.0954
12	4.40	4.0	1.6733	4.40	3.0	1.3416
14	5.00	5.0	2.1213	3.00	3.0	1.5811
16	6.40	5.0	3.0496	3.60	3.0	1.8166
18	7.40	6.0	3.6469	4.40	3.0	1.9494
20	8.40	7.0	3.6469	4.60	4.0	1.9494

Table II.1: Cont.

	Control Group 1			Control Group 2		
	Average	Median	Standard Deviation	Average	Median	Standard Deviation
Right Flexion						
2	1.40	1.0	0.8944	1.00	1.0	0.000
4	2.00	1.0	1.7321	1.40	1.0	1.1402
6	2.60	1.0	2.6077	1.60	1.0	08944
8	3.00	1.0	4.0620	2.20	2.0	1.3038
10	4.00	2.0	4.5277	2.60	3.0	1.6733
12	4.80	3.0	5.1672	3.40	3.0	2.3022
14	6.20	4.0	5.5408	3.60	3.0	2.7019
16	7.60	6.0	6.0249	4.80	4.0	3.1145
18	9.50	7.0	7.1880	6.00	5.0	3.5355
20	10.25	8.0	7.4554	6.20	5.0	3.8987
Torsion						
2	4.40	3.0	2.8810	1.00	1.0	1.0000
4	9.00	7.0	6.2849	1.40	1.0	1.1402
6	12.20	9.0	8.9275	1.80	2.0	0.8367
8	16.00	12.0	10.0750	2.80	2.0	1.6432
10	19.80	14.0	11.4320	3.20	3.0	1.9235
12	23.60	17.0	12.8570	4.20	5.0	2.1679
14	25.80	19.0	13.8090	4.20	5.0	2.1679
16	29.40	22.0	15.3880	4.80	6.0	2.7749
18	32.33	23.0	24.3790	5.60	6.0	2.1909
20	14.00	14.0		6.00	6.0	2.5495
Loading						
0.5	122.20	97.0	66.1490	25.00	16.0	19.519
1.0	209.20	166.0	96.9730	47.20	40.0	35.710
1.5	284.80	210.0	140.9400	68.80	42.0	5353.640
2.0	330.40	262.0	157.7000	90.20	62.0	73.087
2.5	353.40	312.0	119.0000	112.20	83.0	86.860
3.0	380.80	347.0	108.1500	132.80	104.0	97.779
3.5				156.00	130.0	106.47
4.0				131.20	135.5	50.740

Figure 14: Histopathological cross-section taken from fixation area (H&E, x200). **A)** Histopathological cross-section of heterograft plate (Black arrow: fibrous tissue formation). **B)** Fibro-cartilaginous and ossification fields around heterograft bone plate (Black Asterisk: tissue invasion).

site, in addition to hematoma, paresthesia and infection (3). This has increased the tendency to develop and use substitutes of bone grafts in spinal surgery.

Historically, the most commonly used grafts are autografts are allografts. The biggest concern of clinicians regarding allografts is that they can carry infectious diseases in spite of detailed scan tests and serologic tests conducted on donors (1,2). Processing and preservation methods for allografts affect their immunogenicity, and osteoinductive and osteoconductive capacities (13,15,20,31). Preservation of allografts is done through freezing and drying by freezing. These two methods provide longer storage, but diminish immunogenicity of the graft. This might decrease the mechanical resistance of the graft and increase the risk of viral disease (2,13).

Animal models have been used to compare allograft and autografts in anterior and posterior spinal fusions (17). A slower fusion rate, more resorption and increased infection rate were observed with allografts There has therefore been limited clinical interest in using solely allografts as a substitute for autografts (17).

In lumbar vertebrae, cortical allografts are usually used for structural support in combination with autogenous bone grafts and pseudoarthrosis is rarely seen (4).It was observed in many studies that allografts are used solely or in combination with autografts for posterior spinal fusion. However, when compared to autografts, allografts showed lower fusion rate and higher resorption (8,9).

Kiel bone and Surgibone are similar products that are made of calf or cow bones, processed to become non-immunogenic and sterile (21,22). Many products are produced for load-bearing and non-load-bearing usage (16). It has been reported that better fusion and clinical results are achieved by removing organic characteristics of graft material as it also removes other disadvantages in addition to obtaining a better osteoconductive affect (7,21-23,26,29,30,35).

Some authors claim that bovine xenografts have been improved by fibrous fusion as opposed to solid bone fusion obtained with autogenous bone grafts (14,27). Both studies indicated high resorption rate, distinct inflammatory reaction around graft and very little finding for osteointegration or fusion (14,19). However, usage of bovine xenografts was encouraged in four studies (7,21,22,26). In the Cloward operation conducted by Löfgren et al. for cervical disc disease, they obtained similar rigid fusion with autografts, allografts and xenografts (16). One of the advantages of xenografts is that hematoma and pain that is observed in the donor region in the 15% to 50% of the patients who were treated with an autograft is not observed, and moreover usage of xenografts decreases operation time (3). However, immunological response, transmission of diseases, poor osteointegration or fusion development and repeated surgical procedures are regarded as disadvantages of xenografts (14,18,19,35).

In our previous radiological and histopathological studies, we showed that biomechanically meaningful fusion could be obtained with xenografts. In radiological follow-ups of the experimental group, it was observed that resorption developed from the 1st month on the intervertebral XG and XGVPs and fusion development had radiologically occurred at the end of the 3rd month. Vascularization increase, fibrosis, ossification and development of cartilaginous islets were observed in the fusion area in the subjects of the experimental group (24).

In moving vertebrae, the axial compressive loading force is transmitted from one vertebra to another through the intervertebral disc. In case of corpectomy or discectomy surgery, the transmission of the load is provided by the graft placed into the distance (9). The graft should therefore have the characteristic of carrying load (36). In order to prevent collapse of the graft material and enable it to carry load, it is suggested to have at least 30-40% contact between the vertebra surface and the graft (6,19). The cages that are used with autogenous grafts and allografts can meet this biomechanical requirement, but other synthetic grafts do not have the capacity to carry load (18,33,35). There are therefore many studies suggesting usage of a xenograft with the purpose of obtaining fusion as it has osteoconductive features (7,21-23,26,29,30,35).

Table II.2: The Biomechanical Test Results of the Experimental Groups

	Experimental Group 1			Experimental Group 2		
	Average	**Median**	**Standard Deviation**	**Average**	**Median**	**Standard Deviation**
Forward Flexion						
2	4.40	4.0	2.3020	4.40	4.0	3.5071
4	7.20	7.0	3.4928	6.80	5.0	4.7117
6	9.60	10.0	4.5056	9.60	8.0	5.4589
8	12.20	12.0	5.2631	12.00	10.0	6.4420
10	14.60	14.0	6.3875	14.80	13.0	6.6106
12	16.60	15.0	6.7676	17.40	16.0	7.0214
14	18.80	16.0	8.2280	19.60	18.0	7.5366
16	20.80	18.0	9.1488	21.60	19.0	8.1731
18	22.60	20.0	9.7622	23.60	21.0	8.4735
20	24.80	21.0	8.4735	24.80	21.0	10.257
Backward Flexion						
2	4.40	4.0	1.5166	3.60	3.0	1.9494
4	6.80	6.0	1.9235	6.80	6.0	2.5884
6	9.40	9.0	2.6077	8.60	7.0	3.2094
8	11.60	10.0	3.2094	11.40	10.0	3.9115
10	13.20	12.0	3.6332	13.20	12.0	4.0866
12	15.40	13.0	4.8270	15.20	14.0	4.0866
14	17.40	15.0	5.9414	17.40	16.0	4.0988
16	19.40	16.0	6.7676	19.60	19.0	4.4497
18	22.20	18.0	8.3785	22.40	21.0	5.4589
20	24.00	19.0	9.6695	25.40	25.0	6.5803
Left Flexion						
2	4.40	5.0	1.9494	4.60	5.0	2.0736
4	7.20	8.0	3.1937	7.60	8.0	2.3022
6	9.80	10.0	4.2071	10.00	11.0	26.58
8	12.40	11.0	5.1769	13.00	14.0	3.5355
10	15.00	14.0	6.5192	15.80	17.0	3.8987
12	17.60	17.0	7.9875	18.20	21.0	5.3572
14	20.00	19.0	9.1104	20.80	22.0	6.2209
16	22.40	21.0	10.237	22.80	24.0	7.1204
18	25.00	23.0	11.489	24.80	26.0	7.6942
20	27.60	26.0	12.857	27.80	29.0	8.1670
Right Flexion						
2	5.60	6.0	3.6469	3.80	4.0	1.3038

Table II.2: Cont.

	Experimental Group 1			Experimental Group 2		
	Average	**Median**	**Standard Deviation**	**Average**	**Median**	**Standard Deviation**
4	9.00	9.0	5.6125	6.80	7.0	1.4832
6	12.60	12.0	7.7974	9.80	10.0	1.4832
8	15.60	15.0	9.8641	12.20	12.0	2.2804
10	20.00	20.0	12.021	14.20	14.0	3.6332
12	23.00	23.0	14.018	16.80	16.0	5.1186
14	25.80	26.0	15.304	19.00	18.0	6.2048
16	29.00	30.0	16.808	21.20	20.0	7.5961
18	32.40	33.0	18.298	23.40	22.0	8.7920
20	35.80	38.0	20.327	25.60	24.0	9.6850
Torsion						
2	6.80	5.0	4.5497	6.80	7.0	1.3038
4	10.60	10.0	6.3875	11.40	12.0	1.5166
6	14.00	12.0	8.3666	16.40	17.0	2.3022
8	18.00	15.0	9.6437	21.20	23.0	4.0249
10	22.60	19.0	11.3490	25.40	27.0	5.2726
Loading						
0.5	154.00	121.0	85.7230	180.60	172.0	20.707
1.0	294.40	261.0	138.1500	311.60	295.0	28.312
1.5	456.00	312.0	244.5000	416.20	390.0	51.359
2.0	593.00	373.0	340.3900	520.80	467.0	92.963
2.5	749.60	492.0	479.3300	626.20	559.0	153.46
3.0	881.20	588.0	567.3600	722.40	634.0	207.16
3.5	767.75	604.5	394.4300	724.50	754.5	91.966
4.0	643.67	699.0	115.4200	814.00	854.0	141.56

The Kruskal-Wallis test was used to compare all groups generally and the differences are statistically significant for all parameters ($p<0.05$).
The Mann-Whitney U test was used to compare groups with one another.

Easy installation of the graft into the bed decreases the incidence of the graft breaking or being dislocated. Moreover, avoiding space between graft vertebra walls helps vascularization and bone growth from the sides, and therefore increases the fusion rate (14). It is possible to achieve this coherence between the graft and the bearing it will be placed with xenograft.

Rigidity of the spinal implant and its capacity for stabilization are the most important factors for powerful and successful fusion development (5,11,12,14,32).The spinal implant must put up resistance to prevent collapse of the graft due to axial loading, especially during the resorption phase (35). Nevertheless, if the implant carries the load excessively in an unbalanced way, the possibility of achieving a solid arthrodesis decreases. This effect is called "stress shielding". Xenograft plates that are made properly might eliminate this effect. In our study, stress-shielding effect was not observed in the groups with metal implants and xenograft plates. For this reason, the dog vertebra might be in horizontal position. However, fusion generation in all subjects can also be evaluated such that sufficient load is imposed onto the grafts as good fusion generation required placing load on the grafts (14,27).

Although a good stabilization can currently be obtained with many kinds of implants, these metallic implants cause artifacts during radiological evaluations and impede optimal evaluation of the neural canal. This is the biggest disadvantage of these

Table III: Comparison of the Groups Based on Biomechanical Test Results

	p Values					
	CG – 1 CG – 2	CG – 1 EG – 1	CG – 1 EG – 2	CG – 2 EG – 1	CG – 2 EG – 2	EG – 1 EG – 2
Forward Flexion						
2	0.157	0.016*	0.151	0.008**	0.056	0.841
4	0.317	0.016*	0.032*	0.008**	0.008**	0.690
6	0.257	0.032*	0.016*	0.016*	0.008**	0.690
8	0.066	0.016*	0.016*	0.008**	0.008**	0.841
10	0.066	0.016*	0.008**	0.008**	0.008**	1.000
12	0.066	0.016*	0.008**	0.008**	0.008**	1.000
14	0.109	0.032*	0.032*	0.008**	0.008**	0.690
16	0.109	0.032*	0.032*	0.008**	0.008**	0.841
18	0.144	0.032*	0.032*	0.008**	0.008**	0.841
20	0.285	0.032*	0.032*	0.008**	0.008**	0.690
Backward Flexion						
2	0.317	0.008**	0.032*	0.008**	0.016*	0.548
4	0.830	0.008**	0.008**	0.008**	0.008**	0.841
6	0.660	0.008**	0.008**	0.008**	0.008**	0.690
8	0.660	0.008**	0.008**	0.008**	0.008**	1.000
10	0.590	0.008**	0.008**	0.008**	0.008**	1.000
12	0.420	0.008**	0.008**	0.008**	0.008**	1.000
14	0.410	0.008**	0.008**	0.008**	0.008**	0.841
16	0.590	0.008**	0.008**	0.008**	0.008**	0.841
18	0.660	0.008**	0.008**	0.008**	0.008**	0.690
20	0.420	0.008**	0.008**	0.008**	0.008**	0.548
Left Flexion						
2	0.059	0.016*	0.056	0.008**	0.016*	0.841
4	0.257	0.016*	0.008**	0.008**	0.008**	1.000
6	0.655	0.008*	0.008**	0.008**	0.008**	0.841
8	0.157	0.008*	0.008**	0.008**	0.008**	0.841
10	0.041*	0.008*	0.008**	0.008**	0.008**	0.690
12	0.039*	0.008*	0.008**	0.008**	0.008**	0.690
14	0.039*	0.008*	0.008**	0.008**	0.008**	0.841
16	0.039*	0.008*	0.008**	0.008**	0.008**	0.841
18	0.042*	0.008*	0.008**	0.008**	0.008**	1.000
20	0.042*	0.008*	0.008**	0.008**	0.008**	1.000

Table III: Cont.

	CG – 1 CG – 2	CG – 1 EG – 1	CG – 1 EG – 2	CG – 2 EG – 1	CG – 2 EG – 2	EG – 1 EG – 2
Right Flexion						
2	0.317	0.056	0.016*	0.032*	0.008**	0.421
4	0.414	0.032*	0.008**	0.016*	0.008**	0.690
6	0.414	0.032*	0.008**	0.008**	0.008**	0.690
8	0.892	0.032*	0.008**	0.008**	0.008**	0.841
10	0.715	0.032*	0.016*	0.008**	0.008**	0.690
12	0.892	0.032*	0.016*	0.008**	0.008**	0.841
14	0.343	0.032*	0.016*	0.008**	0.008**	0.690
16	0.461	0.032*	0.016*	0.008**	0.008**	0.690
18	0.109	0.063*	0.032*	0.008**	0.008**	0.548
20	0.109	0.063*	0.032*	0.008**	0.008**	0.690
Torsion						
2	0.680	0.016*	0.008**	0.016**	0.008**	0.690
4	0.080	0.016**	0.008**	0.016**	0.008**	0.841
6	0.080	0.008**	0.008**	0.008**	0.008**	0.548
8	0.043*	0.008**	0.008**	0.008**	0.008**	0.548
10	0.043*	0.008**	0.008**	0.008**	0.008**	0.548
12	0.043*					
14	0.043*					
16	0.043*					
18	0.109					
Loading						
0.5	0.043*	0.008**	0.008**	0.008**	0.008**	0.548
1.0	0.043*	0.008**	0.008**	0.008**	0.008**	0.548
1.5	0.043*	0.008**	0.008**	0.008**	0.008**	0.690
2.0	0.043*	0.008**	0.008**	0.008**	0.008**	0.690
2.5	0.043*	0.032**	0.008**	0.008**	0.008**	0.690
3.0	0.043*	0.008**	0.008**	0.008**	0.008**	0.841
3.5		0.016*	0.016*		0.016**	0.486

*CG: Control Group, **EG:** Experimental Group, Kruskal-Wallis test is used to compare all groups generally and differences are statistically significant for all parameters (p<0.05). Mann-Whitney U test is used to compare groups with one another.*
p<0.05 values are represented with*, p<0.01 values are represented with**.

implant materials in spite of their good stabilization capacity. In our study, fusion follow-up could be performed with radiological evaluations of the subjects in whom xenograft plates were used, without an artifact issue.

In recent years, pegs made of cortical allograft bone or allograft interbody cages made of midshaft and/or diaphysial bone have become very popular in anterior lumbar spinal arthrodesis. In our study, we determined that screws and plates that we made with xenograft provided sufficient stabilization in horizontal vertebra and provided powerful fusion by participating in the fusion itself. However, lack of axial loading onto the xenograft screw-plate system prevented us from making a proper evaluation regarding resistance of the implant. The result of the lack of axial loading in the horizontal vertebrae is fusion in a short period of time, so support of the implant was not needed during fusion. Biomechanically, it is not possible to eliminate this problem, but as anti-inflammatory drugs may extend the fusion development time, it can provide a prevention against the advantage provided by unstable horizontal vertebra. Thus the consequences of longer time loading on metal and xenograft plates used for stabilization can be examined. This is the main handicap of our study.

In our study, we determined in the biomechanical tests performed after fusion was obtained by using intervertebral XG with both XG plate-screws and MPS that there was meaningful and sufficient resistance in all movements including bending backwards. We interpreted this result as a bone plate-screw system and metal plate-screw system providing similar biomechanical stability after fusion.

■ CONCLUSION

Intervertebral fusion could be formed by providing stabilization via xenograft and xenograft made screw-plate or metal screw-plate systems in an in-vivo environment in this study. Biomechanical and histopathological characteristics of different plates did not show a significant difference. This material can be a good alternative to other systems for stabilization because of easy and inexpensive acquirement of the xenograft, non-formation of radiological artifact that is caused by materials with metallic properties, being easy to shape, and also being part of the fusion while being resorbed, in addition to the low-cost.

■ ACKNOWLEDGEMENT

■ REFERENCES

1. Brantigan JW: Pseudarthrosis rate after allograft posterior lumbar interbody fusion with pedicle screw and plate fixation. Spine 19:1271-1279; discussion 1280, 1994

2. Brantigan JW, McAfee PC, Cunningham BW, Wang H, Orbegoso CM: Interbody lumbar fusion using a carbon fiber cage implant versus allograft bone: An investigational study in the Spanish Goat. Spine 19:1436-1443, 1994

3. Buck B, Malinin TI, Brown MD: Bone transplantation and human immunodeficiency virus: An estimate of risk of acquired immunodeficiency syndrome (AIDS). Clin Orthop Relat Res 240:129-136, 1989

4. Chang-Jung C, Yi-Jie K, Yueh-Feng C, Rau G, Yang-Hwei T: Anterior cervical fusion using a polyetheretherketone cage containing a bovine xenograft: Three to five-year follow-up. Spine 33:2524-2428, 2008

5. Chou D, Larios AE, Chamberlain RH, Fifield MS, Hartl R, Dickman CA, Sonntag VK, Crawford NR: A biomechanical comparison of three anterior thoracolumbar implants after corpectomy: Are two screws better than one? J Neurosurg Spine 4:213-218, 2006

6. Closkey RF, Parsons JR, Lee CK, Blacksin MF, Zimmermant MC: Mechanics of interbody spinal fusion: Analysis of critical bone graft area. Spine 18:1011-1015, 1993

7. Espersen J, Buhl M, Eriksen E, Fode K, Klaerke A, Krøyer L, Lindeberg H, Madsen C, Strange P, Wohlert L: Treatment of cervical disc disease using Cloward's technique. Acta Neurochir (Wien) 70:97-114, 1984

8. Frank EH, Gong X, Sipe RV, Buck DC, Hollinger JO: Transverse process fusion with bovine anorganic bone. J Biomed Mater Res 60:118-125, 2002

9. Goran A, Murthy KK: Fracture dislocation of the cervical spine: Value of anterior approach with bovine bone interbody fusion. Spine 3:95-102, 1978

10. Helm GA, Dayoub H, Jane Jr JA: Bone graft substitutes for the promotion of spinal arthrodesis. Neurosurg Focus 10:1-5, 2001

11. Hitchon PW, Goel VK, Rogge T, Grosland NM, Sairyo K, Torner J: Biomechanical studies of a dynamized anterior thoracolumbar implant. Spine 25:306-309, 2000

12. Humphries A, Hawk W: Anterior fusion of the lumbar spine using an internal fixative device. Surg Forum 9: 770-773, 1958

13. Jorgenson SS, Lowe TG, France J, Sabin J: A prospective analysis of autograft versus allograft in posterolateral lumbar fusion in the same patient: A minimum of 1-year follow-up in 144 patients. Spine 19:2048-2052, 1994

14. Kowalski RJ, Ferrara LA, Benzel EC: Biomechanics of bone fusion. Neurosurg Focus 10:1-7, 2001

15. Kozak JA, Heilman AE, O'Brien JP: Anterior lumbar fusion options technique and graft materials. Clin Orthop Relat Res 300:45-51, 1994

16. Löfgren H, Johannsson V, Olsson T, Ryd L, Levander B: Rigid fusion after cloward operation for cervical disc disease using autograft, allograft, or xenograft: A randomized study with radiostereometric and clinical follow-up assessment. Spine 25:1908-1916, 2000

17. Malinin TI, Brown MD: Bone allografts in spinal surgery. Clin Orthop Relat Res 154:68-73, 1981

18. Marchesi DG: Spinal fusions: Bone and bone substitutes. Eur Spine J 9:372-378, 2000

19. Pearcy M, Evans J, O'Brien J: The load bearing capacity of vertebral cancellous bone in interbody fusion of the lumbar spine. Eng Med 12:183-184, 1983

20. Pelker RR, Friedlaender GE: Biomechanical aspects of bone autografts and allografts. Orthop Clin North Am 18:235-239, 1987

21. Ramani P, Kalbag R, Sengupta R: Cervical spinal interbody fusion with Kiel bone. Br J Surg 62:147-150, 1975

22. Rawlinson J: Morbidity after anterior cervical decompression and fusion. The influence of the donor site on recovery, and the results of a trial of surgibone compared to autologous bone. Acta Neurochir (Wien) 131:106-118, 1994

23. Salama R: Xenogeneic bone grafting in humans. Clin Orthop Relat Res 174:113-121, 1983

24. Salci H, Sarigul S, Dogan S, Lekesiz H, Ozcan R, Gorgul OS, Aksoy K: Contribution of the xenograft bone plate-screw system in lumbar transpedicular stabilization of dogs: An in-vitro study. J Vet Sci 9:193-196, 2008

25. Sansalone C, Soldano S, Poli C, Tripepi M, D'aliberti G, Rossetti O: Anterior approach to the spine. Role of the general surgeon, techniques and surgical complications. The 10-year experience of the Niguarda Hospitals. J Neurosurg Sci 55:357-363, 2011

26. Siqueira EB, Kranzler LI: Cervical interbody fusion using calf bone. Surg Neurol 18:37-39, 1982

27. Steffen T, Tsantrizos A, Aebi M: Effect of implant design and endplate preparation on the compressive strength of interbody fusion constructs. Spine 25:1077-1084, 2000

28. Summers B, Eisenstein S: Donor site pain from the ilium. A complication of lumbar spine fusion. J Bone Joint Surg Br 71:677-680, 1989

29. Sutter B, Friehs G, Pendl G, Tölly E: Bovine dowels for anterior cervical fusion: Experience in 66 patients with a note on postoperative CT and MRI appearance. Acta Neurochir (Wien) 137:192-198, 1995

30. Taheri ZE, Gueramy M: Experience with calf bone in cervical interbody spinal fusion. J Neurosurg 36:67-71, 1972

31. Tsuang Y, Yang R, Chen P, Liu T: Experimental allograft in spinal fusion in dogs. Taiwan Yi Xue Hui Za Zhi 88:989-994, 1989

32. Tzermiadianos MN, Mekhail A, Voronov LI, Zook J, Havey RM, Renner SM, Carandang G, Abjornson C, Patwardhan AG: Enhancing the stability of anterior lumbar interbody fusion: A biomechanical comparison of anterior plate versus posterior transpedicular instrumentation. Spine 33:E38-E43, 2008

33. Vaccaro AR, Chiba K, Heller JG, Patel TC, Thalgott JS, Truumees E, Fischgrund JS, Craig MR, Berta SC, Wang JC: Bone grafting alternatives in spinal surgery. Spine J 2:206-215, 2002

34. Weinstein JN, Lurie JD, Olson P, Bronner KK, Fisher ES, Morgan MTS: United States trends and regional variations in lumbar spine surgery: 1992–2003. Spine 31:2707-2714, 2006

35. Wigfield CC, Nelson RJ: Nonautologous interbody fusion materials in cervical spine surgery: How strong is the evidence to justify their use? Spine 26:687-694, 2001

36. Zdeblick TA: A Prospective, randomized study of lumbar fusion: Preliminary results. Spine 18:983-991, 1993

37. Zipfel GJ, Guiot BH, Fessler RG: Bone grafting. Neurosurg Focus 14:1-8, 2003