



Experimental comparison of autograft and DBM Flex (Grafton) for spinal lumbar fusion in rabbits

Tavşanlarda spinal lomber füzyon için otoplastik ve DBM Flex'in (Grafton) deneysel karşılaştırması

Cem Demirel¹, Dursun Türköz², Tuncal Yılmaz²

Abstract

Aim: The choice of graft materials used for spinal fusion possesses a great importance due to their crucial roles in bone remodelling. Although autogenous bone grafts are the “gold standard” for spinal fusion surgeries, they can cause various complications. Aim of this study was to compare the efficacy of demineralized bone matrix (DBM) and autograft in lumbar spinal fusion in a rabbit model of spinal lumbar fusion (SLF).

Methods: Twenty New Zealand rabbits were randomly divided into two groups and underwent SLF by using either iliac crest autologous bone graft (Autograft, n=10) or DBM Flex (Grafton, n=10). Eight-weeks after surgery, animals were sacrificed and spinal fusion was evaluated by computerized tomography (CT), manual palpation, macroscopic analyses, and histological assessments.

Results: CT results revealed that autograft led to significantly higher fusion scores than DBM Flex (p=0.0004). Mobility was significantly lower in autograft group (p=0.0007). Significantly lower bone formation scores were observed in DBM Flex group compared to autograft group (p<0.0001). Histology of spine in the autograft group was significantly better than DBM Flex group (p=0.0002).

Conclusion: Autograft was superior than DBM flex in SLF and these results indicate that autograft will continue to be the “gold standard” in SLF in the future.

Keywords: Autograft, DBM Flex, Demineralized bone matrix, Grafton, Spinal fusion surgery

¹ Samsun University, Faculty of Medicine, Department of Neurosurgery, Samsun, Turkey.

² University of Health Sciences, Samsun Education and Research Hospital, Department of Neurosurgery, Samsun, Turkey.



CD: 0000-0002-6084-4075

DT: 0000-0003-3599-0895

TY: 0000-0002-5055-8913

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Sorumlu yazar / Corresponding author:

Cem Demirel

Adres/Address: Samsun University, Faculty of Medicine, Department of Neurosurgery, Samsun Sağlık Uygulama ve Araştırma Merkezi, İlkadım, Samsun, Turkey.

e-mail: drcemdemirel@hotmail.com

Tel/Phone: +90 362 311 15 00

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Öz

Amaç: Spinal füzyon için kullanılan greft materyallerinin seçimi, kemiğin yeniden şekillenmesindeki önemli rolleri nedeniyle büyük önem taşımaktadır. Otojen kemik greftleri spinal füzyon ameliyatlarında “altın standart” olmasına rağmen çeşitli komplikasyonlara neden olabilir. Bu çalışmanın amacı, tavşan spinal lomber füzyon (SLF) modelinde demineralize kemik matrisi ve otoplastik etkinliğini karşılaştırmaktır.

Yöntem: Yirmi Yeni Zelanda tavşanı rastgele iki gruba ayrıldı ve iliak krest otoplastik kemik grefti (Autoplastik, n=10) veya DBM Flex (Grafton, n=10) kullanılarak SLF uygulandı. Ameliyattan sekiz hafta sonra hayvanlar kurban edildi ve spinal füzyon bilgisayarlı tomografi (BT), manuel palpasyon, makroskopik analizler ve histolojik değerlendirmelerle değerlendirildi.

Bulgular: BT sonuçları, otoplastik DBM Flex'ten anlamlı derecede daha yüksek füzyon skorları sağladığını ortaya koydu (p=0,0004). Hareketlilik otoplastik grubunda anlamlı olarak daha düşüktü (p=0,0007). DBM Flex grubunda, otoplastik grubuna kıyasla anlamlı derecede daha düşük kemik oluşum skorları gözlemlendi (p<0,0001). Otoplastik grubundaki omurga histolojisi, DBM Flex grubuna göre anlamlı olarak daha iyiydi (p=0,0002).

Sonuç: Otoplastik, SLF'de DBM flex'ten daha üstün bulunmuştur ve bu sonuçlar da otoplastik gelecekte de SLF'de “altın standart” olarak kabul edileceğini göstermektedir.

Anahtar kelimeler: Otoplastik, DBM Flex, Demineralize kemik matrisi, Grafton, Spinal füzyon cerrahisi

Introduction

Functional spinal unit (FSU) is defined as the smallest segment exhibiting biomechanical characteristics of entire spine and consists of two adjacent vertebrae, intravertebral disc, facet (zygapophyseal) joints, joint capsules and supporting ligamentous structures [1]. Spinal instability is the development of pain and neurological deficits that prevent daily activities due to the inability of the spine to maintain its integrity, to prevent abnormal displacements, to prevent damage to the spinal cord and nerve roots under physiological loads on the spine as a result of acute or chronic insufficiency in FSU [2].

Spinal fusion surgeries with or without instrumentation have been widely performed to treat the patients with spinal pathologies. There are numerous factors such as surgical methods, graft materials, postoperative rehabilitation [3] and comorbidities [4] may affect the success of the fusion surgery. Since the fusion does not occur in a short time after fusion surgery is performed and systemic complications due to long-term immobilization may develop, use of spinal instrumentation with fusion surgery has been brought to the agenda [5, 6]. On the other hand, instrumentation without fusion is not a long-term solution for fixation as in a long-term, loosening will occur, therefore, long-term stabilization will only be possible with osseous fusion [7, 8]. The choice of the graft materials used for the spinal fusion possesses a great importance as they play important roles in bone remodelling and therefore, an optimal graft should have the properties as osteoconductive, osteoinductive and osteogenic [9].

Autogenous bone grafts, the grafts that have been harvested from another anatomical location of the patient and transplanted to another location, have been considered as the "gold standard" for spinal fusion surgeries [10]. Although the success rate of them with strong fusion capacity without the risk of rejection, they have some disadvantages such as the variations in graft quality due to the metabolism and age of the patients [11] and risk of blood loss and pain at the site of harvest [12], and autograft harvest-associated morbidity was estimated to be observed in 10-39% of the patients [13].

The alternatives materials for autografts including allograft, demineralized bone matrix (DBM), various synthetic materials such as calcium sulphates and calcium phosphates growth factors and cell- or platelet-based therapies have become alternatives for the autografts [9, 14]. DBM is the bone that has been treated with acid to demineralize the bone while keeping the organic matrix and growth factors intact [15]. Its osteoconductive and osteoinductive capacities are limited [15, 16], however, it has no osteogenic capacity [15]. On the other hand, DBM does not lead to any immunological reaction in the host because of acid treatment during the preparation of the material [17]. Moreover, DBM has been shown to induce spine fusion successfully in the experimental animals [18, 19].

In this study, we aimed to compare the efficacies of DMB flex (Grafton) and autograft on a model of posterior spinal incision of New Zealand white rabbits.

Material and methods

Experimental Groups and Animal Husbandry

This study was approved by the local Animal Experiments Local Ethics Committee. Skeletally mature 20 New Zealand White Rabbits (3 - 4 kg body weight) with both sexes

were randomly divided into two groups as DMB flex (n = 10) and iliac crest autograft (n = 10) groups. All animals were housed under controlled temperatures ($21 \pm 1^\circ\text{C}$) and controlled lighting conditions (12-h light/ dark cycle) in individual cages with ad libitum access to food and water.

Surgical Procedures

One hour previous to the experiments, each animal was coded with a number and received 20 mg/kg cefazolin as antibiotic prophylaxis. For surgery, animals were anesthetized by intraperitoneal administration of 3 mg/kg xylazine (Rompun®-Bayer) and 40 mg/kg ketamine hydrochloride (Ketalar®- Parke Davis). After anaesthesia was achieved, animals were positioned in prone position on operating table, surgical site was shaved, and skin was cleaned with 10% povidone iodine solution.

In DBM flex group of animals, a five cm midline incision was carefully performed, and subcutaneous layers were dissected. Fascia was opened from sinister to the midline in line with the L4-L6 vertebrae. Lamina of L5 and facet joints at L4/5 and L5/6 were decorticated by using a high-speed burr with 3 mm ball-end. DBM flex (Grafton) with an approximate size of 20 x 15 mm was grafted between the facet joints, and closure of fascia and subcutaneous tissues was achieved by using 3/0 vicryl sutures and skin closure was achieved by using 3/0 silk suture following homeostasis. The incisions were cleaned with 10% povidone iodine solution and animals were placed in their individual cages.

In iliac crest autograft group of animals, a five cm midline incision was carefully performed. After lumbar fascia was dissected bilaterally, the soft tissues on the iliac crest was dissected carefully. The bone graft with a size of 20 x 15 x 4 mm was harvested by bone rongeur. Homeostasis of the iliac crests was achieved by bone wax application. The autograft bone material was decorticated by using a high-speed burr with 3 mm ball-end. Lamina of L5 and facet joints at L4/5 and L5/6 were bilaterally decorticated by using a high-speed burr with 3 mm ball-end. Surgical region was cleaned, homeostasis was achieved, and iliac crest graft was placed. The closure of fascia and subcutaneous tissues was achieved by using 3/0 vicryl sutures and skin closure was achieved by using 3/0 silk suture following homeostasis. The incisions were cleaned with 10% povidone iodine solution and animals were placed in their individual cages.

Postoperative Care

After the surgery, 2 mg/kg meperidine for analgesia and 20 mg/kg cefazolin sodium for antibacterial activity were administered intraperitoneally to each animal. General condition, neurological findings, mobility and infection findings in the animals were closely monitored and recorded for a week. Neurological deficits and alteration vital signs were not observed in any of the subjects in the early postoperative period. Animals were fed with standard diet and followed for eight weeks. Eight weeks later the animals were sacrificed by high dose lythethenon administration.

Computerized Tomography

After sacrifice, animals were taken to the radiology department and surgical region was scanned by thin slice computerized tomography (CT) and reconstructed into 3D images. Images were evaluated in terms of fusion and bone

formation. The fusion was scored as follows: net fusion = 2, likely fusion = 1, no fusion = 0.

Manual Palpation and Macroscopic Evaluation of Bone Formation

After CT scans, the spinal region between L7 and L2 including the objective lumbar spine (L4-L6) was dissected and surrounding soft tissue was removed. Fusion was assessed by manual palpation as previously described [20, 21]. The motion was assessed by a blinded examiner, and in case of that no motion was detected in the segments (L4/5 and L5/L6), the implanted graft was assessed as fused and scored as 2. If there was a motion in the segments assessed, the implanted graft was assessed as not fused and scored as 1.

Bone formation was also evaluated macroscopically and scored as follows: no new bone formation = 0, minimal new bone formation = 1, moderate new bone formation = 2, obvious new bone formation = 3.

Histological Assessments

The spinal segment that was dissected en bloc and fixed in 10% formalin solution for 24 h and dehydrated in ethanol solutions. After dehydration, harvested samples were decalcified in 10% nitric acid for seven days and embedded in paraffin. Longitudinal slices with five μm thickness were taken from the paraffin embedded samples and were stained with Hematoxylin and Eosin (H&E) by using the standard protocol. The success of the fusion was evaluated by an experienced pathologist who were blinded to groups as previously described by Emery et al. [22].

Statistical Analysis

Statistical analyses were conducted by using the Statistical Package for the Social Sciences (SPSS) version 15.0 (IBM). Data are expressed as mean \pm standard deviation (SD). Normal distribution of the data was evaluated by Shapiro-Wilk normality test. Data with normal distribution was evaluated by independent Student's t-test while the data without normal distribution was evaluated by Mann-Whitney U test. The associations between the variables were evaluated by Chi Square test. A p value lower than 0.05 was accepted as statistically significant.

Results

Computerized Tomography

CT results revealed that one animal had likely fusion and the rest of the animal did not have any fusion in DBM Flex group (Figure 1A). On the other hand, six animals received autograft had net fusion, three animals had likely fusion and one animal had no fusion (Figure 1B). Mean fusion scores was significantly higher in the autograft group compared to DBM Flex group (Figure 1C; $p = 0.0004$).

Manual Palpation and Macroscopic Evaluation of Bone Formation

Examination by manual palpation showed that in iliac crest autograft group, no motion was observed in any of the animals while in DBM Flex group, motion was detected in eight of the animals ($p = 0.0007$).

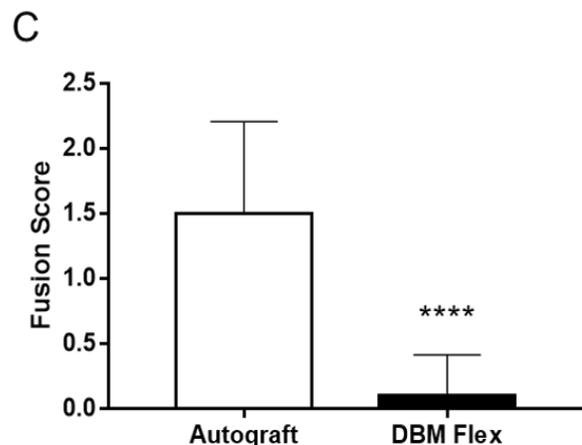
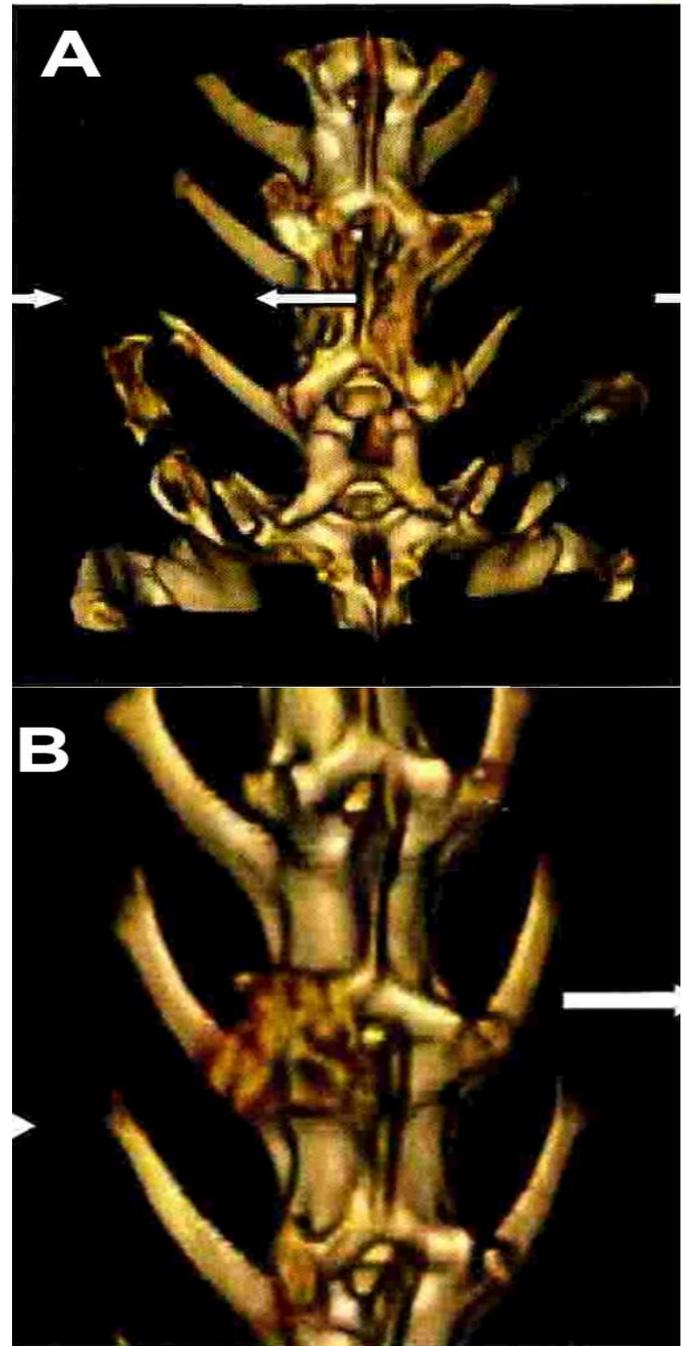


Figure 1. Representative 3D reconstruction of CT images of (A) Autograft, (B) DBM Flex groups. Arrows indicate the grafting region and bone formation four weeks after the grafting. (C) Fusion score of both groups. (Statistical analyses: Mann-Whitney U test. **** $p = 0.0004$).

DBM Flex grafting led to no new bone formation in nine of the animals and minimal new bone formation in one animal in the macroscopic evaluation of the bone formation. However, in the autograft group, an obvious new bone formation was observed in nine animals and moderate new bone formation in one animal ($p < 0.0001$).

Histological Assessments

DBM Flex group of animals exhibited significantly lower histopathological score (1.20 ± 1.40) compared to autograft group of animals (3.50 ± 2.07 ; Figure 2A - C; $p = 0.0002$).

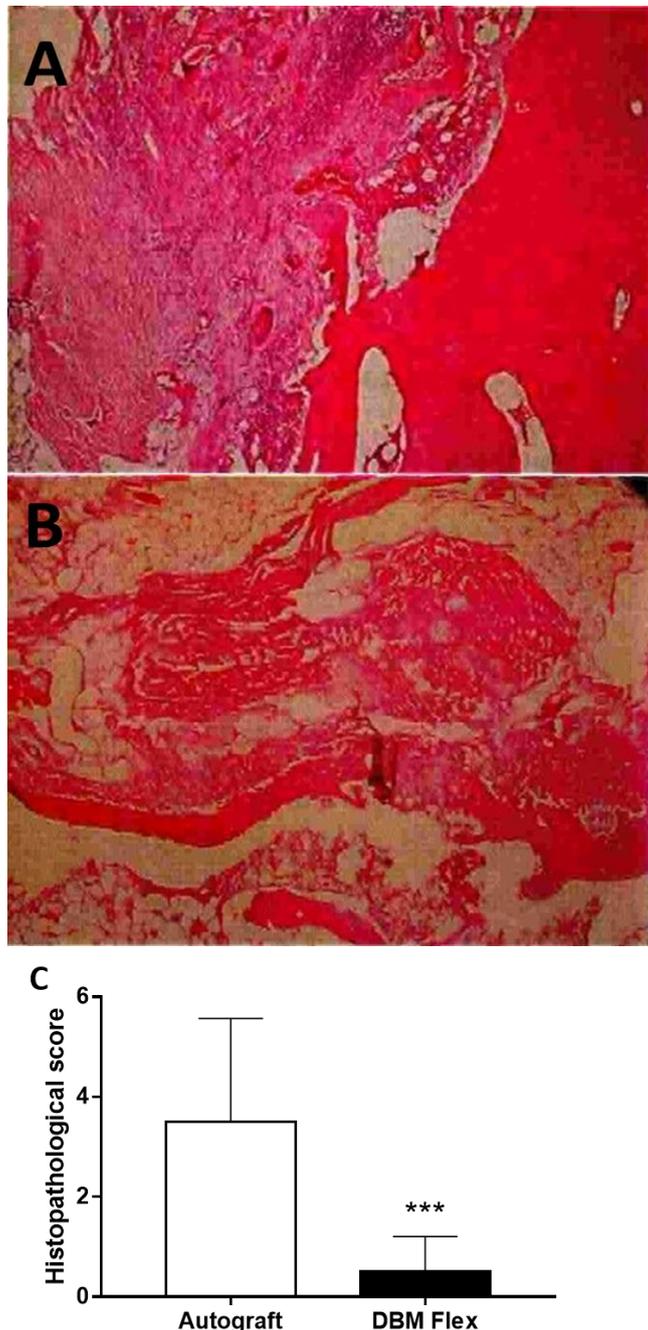


Figure 2. Representative histopathological images of (A) autograft group and (B) DBM Flex group. (C) Histopathological scores of both groups. (Statistical analyses: Mann-Whitney U test. *** $p = 0.0002$).

Discussion

Achieving satisfactory spinal fusion has been the most important issue in the surgery of spinal pathologies [14, 23, 24]. Today, although we can provide spinal stabilization in the early period by using complicated spinal instruments, the success in long term is achieved with the development of fusion. In this study, we compared the efficacy of autograft and DBM Flex (Grafton) in spinal fusion and found that autograft is superior than DBM Flex in spinal fusion.

The most commonly used substitute materials in spinal fusion are autograft and allograft bones [25] and autogenous bone graft has been implicated as the “gold standard” for spinal fusion grafting [10]. Iliac crest bone grafts are the most commonly used substitute in spinal fusion [26]. Although autografts have various advantages including promotion of extensive fusion with perfect histocompatibility, autograft harvest-associated morbidities can be observed in a significant part of the patients [13]. Therefore, use of different graft materials were required in spinal fusion surgeries.

DBM is a synthetic substitute that is particularly osteoinductive and is partially osteoconductive [23]. On the other hand, bone formation was indicated to be supported by DBM through osteoconductive mechanisms [19]. The results regarding the success achieved in spinal fusion by use of DBM are variable. A study conducted by Cook et al. on dogs revealed that either alone or together with allograft, DBM is not effective in formation of stable spinal fusion [27]. Helm et al., in their study indicated that DBM has an inhibitory role on spinal fusion in dogs [28]. In rabbits, although autograft was found to yield higher spinal fusion rates compared to allograft and allograft together with DBM, the difference between the groups were not significant [29]. However, several animal studies indicated that different forms of DBM act as a graft extender/enhancer and provide superior fusion rates than autograft only [19, 30]. Similar controversial results regarding the success after DBM grafting in spinal fusion surgery were also obtained by various clinical studies [31-37]. On the other hand, autograft alone was shown to induce bone formation to a higher extent than DBM alone in dogs [30].

Scoring was used in all three examinations, since spinal fusion has stages of inflammation, vascularization, osteoinduction, osteoconduction and remodeling. We observed that autograft yielded significantly higher lumbar spinal fusion than DBM Flex as revealed by the manual palpation and fusion score. Moreover, use of autograft for spinal fusion surgery led to a significantly better histology than DBM Flex grafting. This might be attributed to the extensive osteoinductive, osteoconductive and osteogenic properties of autografts [38-40].

In conclusion, our results suggest that autograft is superior than DBM Flex in spinal fusion. In our opinion, use of autograft in spinal fusion surgery will continue to be the “gold standard” for spinal fusion in the future. Further studies to find a suitable and efficient candidate substitute for autografts are required to avoid the complications that may arise due to autologous bone harvesting.

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