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Pseudoarthrosis repair using autologous cultured osteoblasts in complex type-1 neurofibromatosis spinal deformity. A case report and review of the literature

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Study Design: A case report and literature review

Objective: To report a late dislocation of the vertebra caused by progressive dural ectasia combined with type-1 neurofibromatosis (NF-1) and the clinical results of pseudoarthrosis repairs using autologous cultured osteoblasts.

Summary of Background Data: NF-1 is a well-known genetic disorder that is commonly characterized by spinal deformities including kyphoscoliosis. Late dislocation of the vertebra resulting from progressive dural ectasia after surgical correction of NF-1 scoliosis is a very rare occurrence, and pseudoarthrosis frequently develops after surgical intervention for this complex spinal deformity.

Methods: A 32-year-old female patient with NF-1 scoliosis underwent surgical correction with posterior instrumented fusion (PIF). Seventeen years later, dislocation of the lumbar spine with implant failure resulting from massive progressive dural ectasia was observed. She underwent anterior interbody fusion (AIF) 3 times and PIF 4 times for pseudoarthrosis followed by surgical deformity correction. For the last operation, autologous cultured osteoblasts were used as a therapeutic approach to repair the pseudoarthrosis, and a three-dimensional printing technique was used to understand the surgical anatomy of the dislocated lumbar spine in detail.

Results: After the final operation, bone union was achieved and confirmed by clinical and radiological examination.

Conclusions: Spine surgeons should be knowledgeable about the possibility of late destabilization of the spine, due to pulsatile dural ectasia, and a high rate of pseudoarthrosis in neurofibromatosis. Autologous cultured osteoblasts may prove to be a modality that can be applied pseudoarthrosis repair to treat complex spinal deformity.

Key Words: Neurofibromatosis, dural ectasia, osteoblast, three-dimensional printing

Level of Evidence: 5

Introduction

Neurofibromatosis type-1(NF-1) is a well-known genetic disorder, and is characterized by cutaneous manifestations, multiple neurogenic tumors, and skeletal problems including spinal deformities. Kyphoscoliosis is a common abnormality, and spontaneous dislocation of the vertebral body combined with dural ectasia has been reported in dystrophic spinal defomities.[1-4] Dural ectasia can also occur in NF-1 and is characterized by dilatation of the dural sac, which may result from cerebrospinal fluid pulsation, and can lead to spinal instability.[3,4] This vertebral body dislocation secondary to dural ectasia is very rare, and only a few cases have been reported.[4-9] Moreover, non-union is a common problem after surgical intervention for NF. Dystrophic bone, deformed anatomy and osteopenia in NF-1 have been considered as possible causes of this problem. However, with advances in the pedicle screw system, biologic trials that apply enhancing fusion such as bone morphogenic protein and anterior-posterior fusion have recently exhibited increased success in deformity correction and fusion.

The authors treated a NF-1 patient with progressive spinal deformity with dislocation caused by dural ectasia. Pseudoarthrosis at the dislocated level occurred and persisted, despite several surgical attempts to repair the non-union. Biologic trials using autologous cultured osteoblasts and three-dimensional (3D) printing technique have been applied for this complex case, and the authors discuss the results in this report.

Case Report

In 1992, a 32-year-old female patient with NF-1 underwent surgical correction of dystrophic NF kyphoscoliosis (Figure 1A-C). She visited the clinic in 2009 and complained of intractable back pain and progressive deformity with mild radiculopathy. No significant neurological deficit was found. Plain radiographs showed instrumentation failure with dislocation of the L3 and the L4 vertebra (Figure 2A-C). A massive dural ectasia that extended to the pedicle and paraspinal area was observed on magnetic resonance imaging. On computed tomography, bilateral pedicle fractures, widened spinal canal and dislocation of the L3 and L4 vertebra were observed (Figure 3A-F). A first revision surgery was performed, the broken instruments were removed, and extended posterior instrumented fusion (PIF) was performed.

Eleven months after the 1st revision, the patient revisited the clinic and complained of worsening low back pain. Follow-up radiographs showed breakage of the left rod and dislocation of the right rod. A 360° anterior-posterior re-revision surgery was performed. The rods were replaced with additional autogenous iliac bone graft for posterior fusion (L3-5) and a massive dural ectasia that protruded into the anterior vertebral column was resected through a right-sided anterior retroperitoneal approach. Intraoperatively the dura was found to be of normal consistency, but very thin. A cerebrospinal fluid leak after resection of dural ectasia was prevented by meticulous suturing and the use of fibrin glue. Anterior interbody fusion (AIF) using autogenous iliac bone from L3 to L5 was performed simultaneously (2^{nd} revision). Unfortunately, 11 months after the 2^{nd} revision, a rod breakage recurred (Figure 4A-D). Fracture of the anterior bone graft was also observed on CT, although a decrease in the size of dural ectasia was noted. A left-sided anterior retroperitoneal approach for AIF and PIF was performed using iliac autogenous graft bone mixed with demineralized bone matrix

(3rd revision). After surgery, absolute bed rest was recommended for 3 months to achieve union. Thirteen months later, however, pseudarthrosis was evident with breakage of the rods (Figure 5A-D). Rod breakage was noted at the same level with the radiologic evidence of non-union. Antero-posterior surgery was planned for the 4th revision. Preoperative 3D printing using a CT scan revealed definite bony defects in the posterior fusion mass at the rod fracture level (Figure 6). Before the posterior revision surgery, autologous bone marrow aspirates (10 ml) were obtained from the iliac crest for autologous cultured osteoblasts. Isolation, culture and induction of osteoblast from bone marrow mesenchymal stem cells were conducted according to the manufacturer's protocol (RMS-Ossron™; SEWONCELLONTECH, Seoul, Korea). Briefly, the bone marrow aspirates were kept with normal culture media (Gibco-BRL, Grand island, NY) that contained 10% fetal bovine serum, antibiotic and 350 units of heparin. After isolation of the cells, induction into osteoblasts was done using 50μg L-ascorbic acid (Sigma) and 10^{-7}M dexamethasone.[10] The phenotype of the cultured cells were confirmed using fluorescence-activated cell sorting (FACS) analysis of type I collagen and bone specific alkaline phosphatase before transplantation (over 96% expression of target protein in the cultured cells was achieved). Before transplantation, excision of the fibrotic tissue and decortication of the upper and lower lamina around the non-union site were conducted and the broken rod was replaced. The 3rd passage of $6x10^7$ cells with 2.0 ml of culture media was implanted with biocollagen (Surgifill™, SEWONCELLONTECH, Seoul, Korea) as a scaffold (Figure 7A-D). AIF that used an autogenous fibular graft was also performed through a staged transperitoneal approach.

Serial CT scans showed bridging bone formation at the non-union (transplanted) site, which suggested fusion progression. Eighteen months postoperative radiographs demonstrated evidence of consolidation of the anterior graft, and the sagittal and coronal

alignment was maintained without implant failure (Figure 8A-E). However, the patient experienced low back pain without a neurologic deficit. The pain was attributed to multiple spinal surgeries.

Discussion

Dural ectasia in NF-1 is usually associated with changes in the vertebral column including spinal canal and neural foramina enlargement with pseudomeningocele, cortical thinning of the pedicles and laminae, and bony erosion due to expansion of the dura mater, which may result in progressive kyphoscoliosis, and dislocation of a vertebra.[4, 11,12] The dural ectasia was of unknown origin, but the association with NF-1 suggests a role of congenital malformation with a triventricular obstructive hydrocephalus associated with Silvio's aqueduct stenosis.[13,14] However, lumbosacral spondylolisthesis combined with dural ectasia in neurofibromatosis is very rare and only a few cases have been reported.[5,7,8]

Interestingly, in our case, massive dural ectasia developed 17 years after the initial surgery, resulting in dislocation of the lower lumbar vertebra. Dislocation might have developed due to progression of pulsatile dural ectasia which caused erosion of the surrounding bone with loss of the anterior vertebral column and bilateral pedicle fractures. However, the mechanism of progression of dura ectasia after initial surgery was unclear. One possible mechanism is that the lamina hooks contributed to the development of dural ectasia. A case similar to this was reported by Ciappetta et al[15] wherein hydrosyringomyelia developed secondary to pedicular hook dislocation. In our case, however, intraspinal dislocation of the hooks on plain radiographs and CT was not found and dura ectasia developed anterolateral of the vertebral body. Thus it is unlikely that this was caused by pedicular hook dislocation.

Combined anterior and posterior fusion surgery has been reported to achieve stable fusion treating NF-1 with spinal deformity caused by dural ectasia.[3,4] However, many surgeons are reluctant to do an anterior approach because of several possible risks and disadvantages. Hue et al[16] reported that the presence of excessive plexiform venous channels around the vertebral bodies made an anterior approach impossible. In addition, it is difficult to perform anterior arthrodesis if dural ectasia is identified because the vertebral bodies are very small, and hence a posterior-only approach is recommended.[7] However, there are still cases that limit the use of the posterior only approach. Cho et al [5] reported that dural ectasia and pedicular erosion prevented the safe use of pedicle screws at the deformity apices. Additionally, patients with type-1 NF are known to be osteopenic, although some authors argue that even though dural ectasia is present, surgeons should consider performing an anterior-posterior approach because dural ectasia may lead to spinal instability and high-grade spondylolisthesis.[6,13] Furthermore, posterior vertebral column resection and recombinant human bone morphogenic protein-2 (BMP-2) have been reported to be effective treatment approach for a severe dural ectasia and spinal deformity in order to prevent recurrent pseudarthrosis.[5,18] Moreover, Parisini et al[17] reported that the overall fusion failure rate was 53% in patients who underwent PIF alone, compared to the overall fusion failure rate of 23% in patients who underwent an additional anterior fusion.

In our case, at the time of the first revision, we thought that the anterior approach would increase the incidence of morbidity because of a huge dural ectasia that surrounded the vertebral body; hence, PIF alone was performed. However, after the PIF, the implant failure developed again and after we attempted to identify the cause of implant failure, it was concluded that the PIF alone could not maintain the axial loading of the spine without anterior column support. During the AIF including excision of the huge dura combined with PIF, we had difficulty obtaining a strong iliac bone graft because the iliac bone was too

osteopenic. Additionally, vertebral bodies erosed by dura ectasia were too small and osteoporotic to make a bridge with an iliac bone graft between upper and lower vertebral bodies. Unfortunately, pseudoarthrosis and implant failure developed repeatedly despite several revision surgeries. For the last surgery, autologous cultured osteoblasts were used for pseudoarthrosis repair, and preoperative 3D printing was useful for understanding the bony architecture and non-union.

Reconstruction using biologic materials such as BMP has been highlighted as a therapeutic approach, however, because of the possibility of tumor formation, the safety of its application has been challenged. Moreover, the use of BMP for bone fusion is still not permitted in our country. Therefore, cultured osteoblasts from autogenous bone marrow aspirates were applied. Cell therapy using various sources has been reported to achieve successful bone union. Since Quarto et al[19] recently reported the use of cultured autologous bone marrow mesenchymal stem cells in large bone defect regeneration, autogenous bone marrow mesenchymal stem cells became a topic of high interest. With advances in tissue engineering and cell therapy, more specific types of cells by induction of differentiation are now available. Clinical trials that use this type of cell therapy have been successfully reported in maxillofacial reconstruction.[10,20] However, to the best of our knowledge, this is the first report of autologous cultured osteoblasts applied to repair pseudoarthrosis in spine surgery. Although there are still many limitations to overcome for safe clinical use of cell therapy, minimal cell culture manipulation and autologous cell sources may be positive strength.

In conclusion, spine deformity in NF-1 is complex and sometimes combined with late destabilization of the spine due to pulsatile dural ectasia and high rate of pseudarthrosis. Autologous cultured osteoblasts may prove to be promising modalities for pseudoarthrosis repair for treating complex spinal deformity

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Figure Legends

Figure 1. A) Preoperative plain lateral radiographs taken before the 1st operation (December 1992). **B)** MRI showing mild dura ectasia with scalloping of multiple vertebral bodies. **C,** Postoperative lateral radiographs showing posterior correction and fusion with the use of hooks and pedicle screws for scoliosis (AP radiographs are missing).

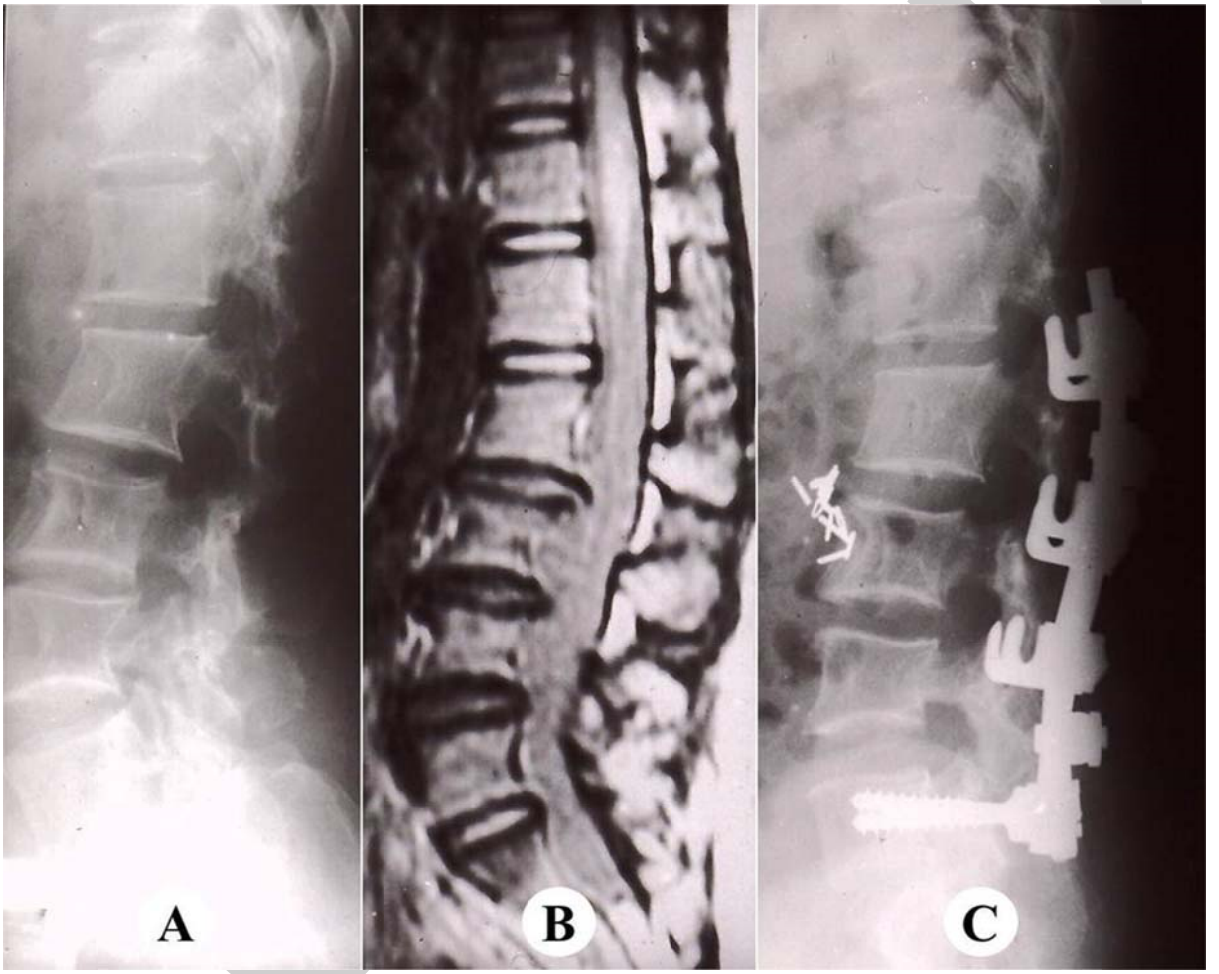

Figure 2. A) Clinical photographs. **B, C)** AP and standing lateral radiographs that demonstrate scoliosis deformity characterized by coronal decompensation and metallic failure at 17 years after the first surgery.

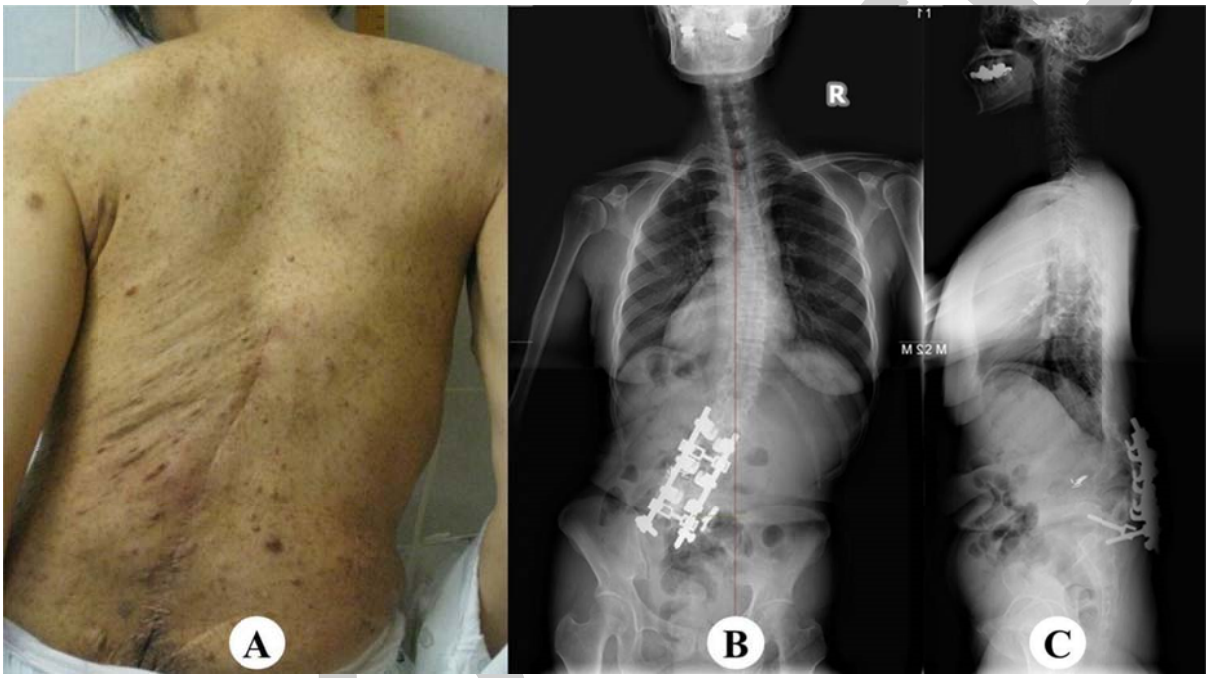

Figure 3. A- C) T2-weighted sagittal and axial MRI showing huge dural ectasia (arrow) at the lumbosacral region. **D-F**) CT scans showing anterolateral dislocation of the L4 vertebra, pedicle fractures and widened a spinal canal.

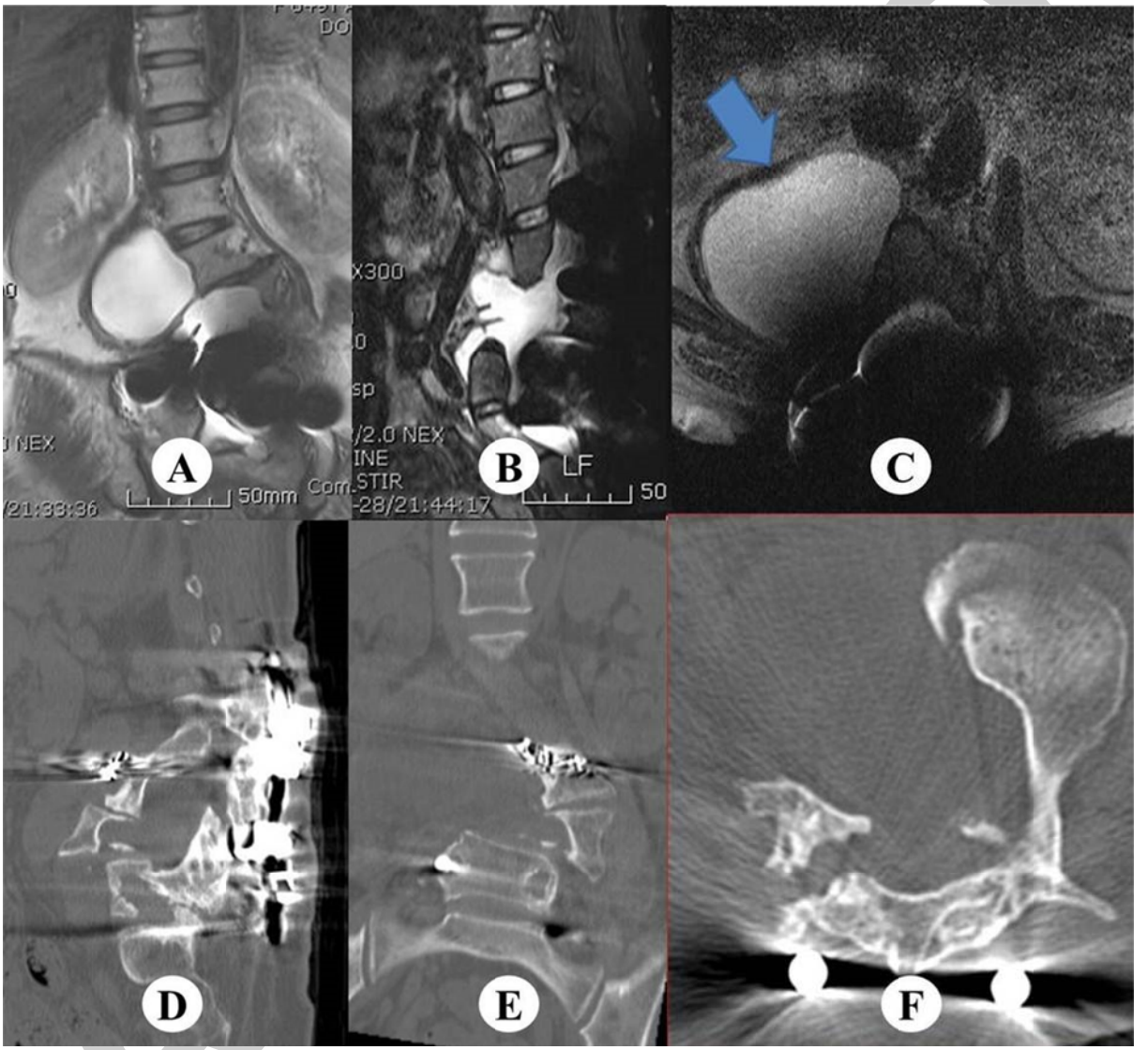

Figure 4. A, B) An anterior-posterior re-revision surgery was performed, where the rods were replaced, and a massive dural ectasia that protruded into the anterior vertebral column was resected through a right-sided anterior retroperitoneal approach. **C, D)** rod breakage re-occurred 11 months after the 2nd revision.

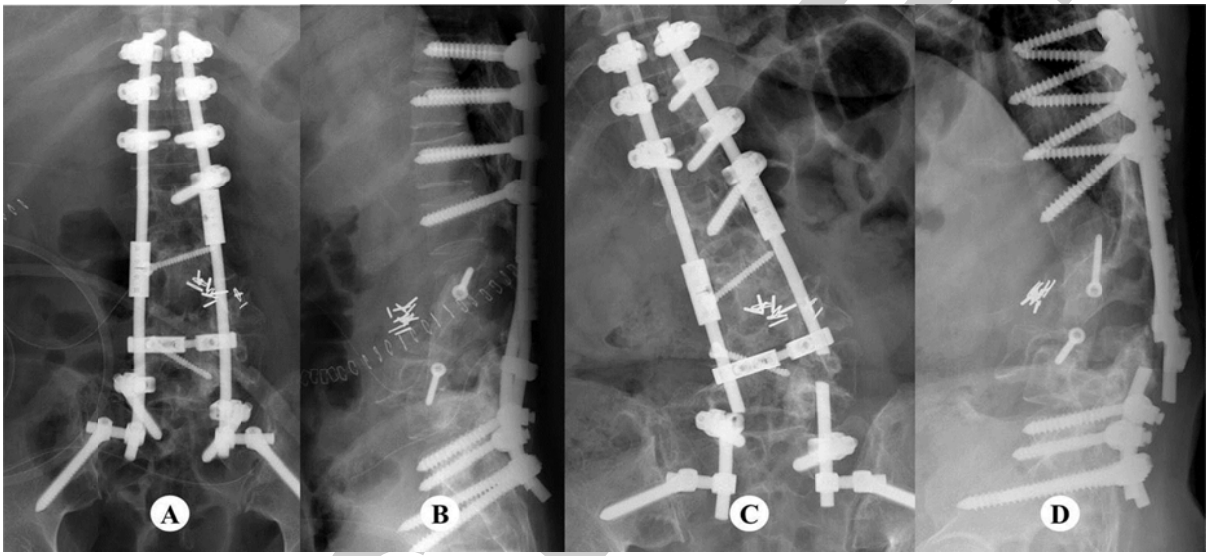

Figure 5. A, B) For the 3rd revision surgery, anterior interbody fusion by left-sided anterior retroperitoneal approach and posterior revision was performed with iliac autogenous graft bone mixed with demineralized bone matrix. After surgery, absolute bed rest was recommended for 3 months to achieve union. **C, D**) Thirteen months later, however, pseudarthrosis was evident with rod fracture on plain radiographs and CT scan.

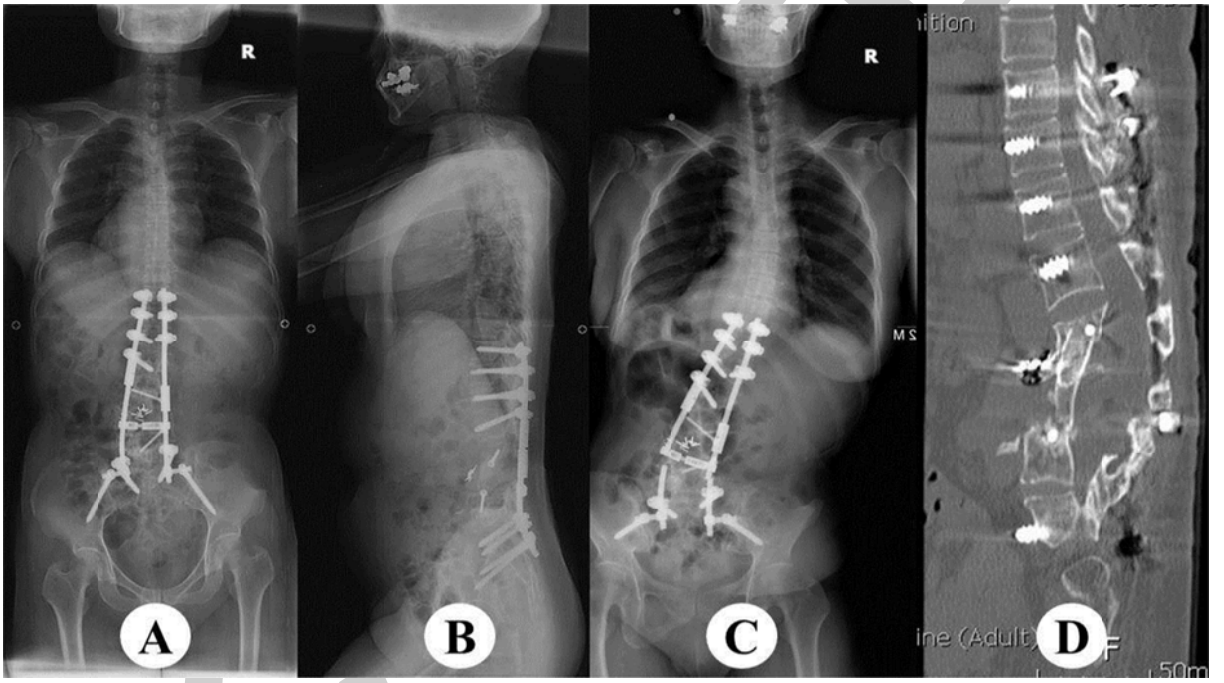

Figure 6. 3-D printing model shows a large spinal canal and the intervertebral foramen defect with dislocation of the L4. An arrow indicates a site of non-union at the posterior fusion mass.

Figure 7. **A-B)** Characteristics of cultured osteoblasts from autologous bone marrow mesenchymal stem cells are confirmed by fluorescence-activated cell sorting (FACS) analysis of type I collagen and bone specific alkaline phosphatase before transplantation (over 96% expression of target protein in the cultured cells was achieved). **C-D)** Cultured osteoblasts were mixed with fibrin glue and impregnated with biocollagen and transplanted into the nonunion site after careful decortication.

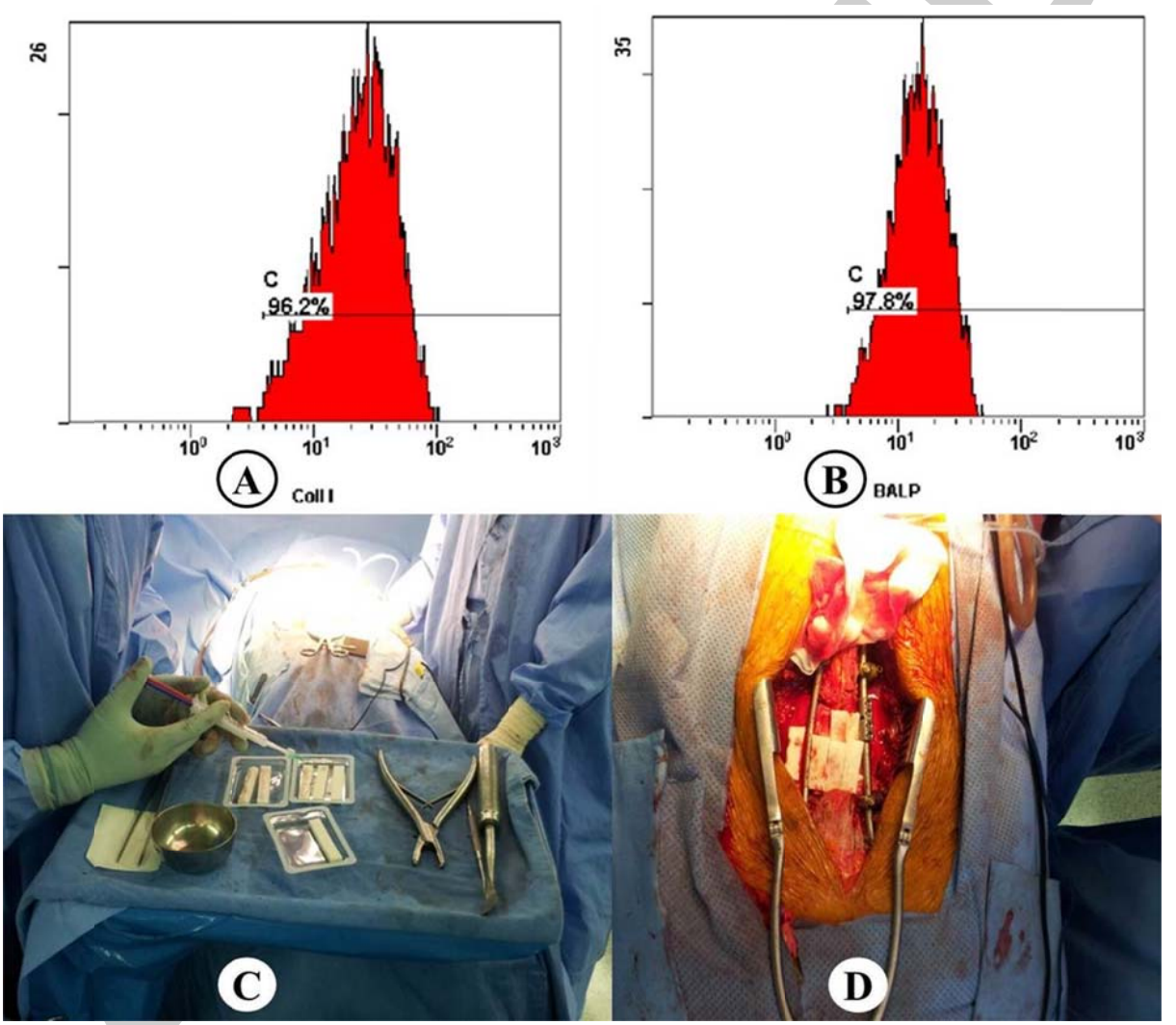

Figure 8. A-C) Serial CT scans from preoperative to 10 months postoperative show the bridging bone formation in the non-union site (arrow head). **D, E)** Eighteen months postoperative radiographs suggests union without implant failure.