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# *Ex Vivo* Gene Therapy for Spinal Fusion

Takashi Kaito, Stephanie S. Ngo and Jeffrey C. Wang
*University of California, Los Angeles*
*USA*

## 1. Introduction

When instability of the lumbar spine causes persistent pain or neurological impairment, it is usually treated by spinal fusion. Unfortunately, despite the development of spinal instrumentation such as pedicle screws, rods and intervertebral cages, nonunion rates are still close to 15%. Autogenous bone grafting has been the gold standard as the bone graft material of choice, however, its use has several disadvantages, such as a limited supply and a reasonable percentage of donor site problems. For these reasons, alternative sources of bone grafting materials or replacements have been explored and developed. One possibility is the use of bone morphogenetic proteins (BMPs), which belong to the transforming growth factor (TGF) superfamily and are known to be capable to eliciting new bone formation can provide an alternative to bone grafting (Reddi et al., 1998, Khan et al., 2002, Vaccaro et al., 2002, Veillette et al., 2007). Three types of BMP- based bone tissue engineering have been tried to date: cell therapy involving the transplantation of autogenous mesenchymal cells differentiated by BMP, gene therapy involving the transduction of genes encoding BMPs into cells at the repair site (Kaito et al, 2005), and cytokine therapy involving recombinant BMP and carriers that retain and release BMP as needed. Of the three types of BMP-based bone tissue engineering techniques, cytokine therapy has received significant attention. Several kinds of combination with cytokine and carriers were reported (Khan et al., 2004). However, uncontrolled release of high dose BMP has been known to cause inflammation, soft tissue edema, and unintended bone formation (Carlisle et al., 2005, Mroz et al., 2010). An alternative to recombinant proteins, is the use of novel methods and gene transfer techniques to achieve bone formation in the spine. Gene therapy is ideal for orthopaedic use because, in most cases, local and transient expression of osteogenic factors is necessary for bone formation (Helm et al., 2000). Regional gene therapy for spinal fusion is an attractive option to treat multi-operated unstable spines where osteogenic cells, blood supply and bone stock are limited (Wang et al., 2003).

The recent progress in molecular biology, genetics, and stem cell biology demonstrate that gene therapy for spinal fusion could result in effective bone formation by sustained and controlled release of osteogenic factors (Yoon et al., 2004). *Ex vivo* gene therapy, which is the combination of gene therapy and cell therapy, is currently the most fascinating areas of orthopedic research and surgery. In this chapter, the authors summarize the latest research in *ex vivo* gene therapy for spinal fusion and discuss the clinical implications of these treatments.

## 2. *Ex vivo* gene therapy

Regional gene therapy is ideal for spinal fusion because local and transient expression of osteogenic factors is necessary for bone formation, compared to systemic genetic disorders in which consistent and systemic expression of specific genes are required (Baltzer et al., 2004). Therefore, there is more flexibility with respect to the use of vectors for treatment of these problems and it may stave off the side effects associated with the current use of high dose and uncontrolled release of recombinant BMPs (Mroz et al., 2010). Regional gene therapy can be employed via either an *in vivo* or an *ex vivo* approach. In an *in vivo* technique, the vector is delivered directly to the target site. The advantage of the *in vivo* approach is that it involves only one step. The disadvantages of this technique are that it is more difficult to transduce cells *in vivo*, and one cannot select the target cells that will be transduced. Reports of successful fusion using the *in vivo* approach have been mostly reported in athymic rats (Alden TD et al., 1999). On the other hand, in an *ex vivo* approach, the vector is delivered into harvested cells. The transduced cells are then implanted into a specific regeneration site. The advantage of the *ex vivo* technique is that there is an increased transduction efficiency and safety as compared to when performed *in vitro*. In addition, the transduced mesenchymal stem cells (MSCs) could act not only as a production plant for osteogenic factors, but also as a source of osteogenic cells. The *ex vivo* technique also allows for attachment of a desirable carrier material which can act as an osteoconductive scaffold (Oakes DA et al., 2000). The disadvantage of this strategy is that this approach is more labor intensive, and therefore may not be cost-effective (Phillips et al., 2005).

## 3. Vectors for gene delivery

### 3.1 Non-viral vectors

Vectors may be classified broadly as either viral (Kootstra et al., 2003) or non-viral (Li S et al., 2001). Non-viral vectors are easy to handle, chemically more stable than viruses and have no limit as to the size of the genetic materials that can be introduced into the cell. However their use is limited because the transfection efficiency is low and the duration of expression of the protein product tends to be short due partly to the episomal nature of the transgene. Non-viral gene delivery includes direct injection of naked DNA, direct injection or liposomal transfection of plasmids and particle-mediated gene transfer (i.e.; gene gun). Plasmids are circular DNA initially found in bacteria and work to acquire resistance to antibiotics. They are usually used in combination with cationic liposomes or scaffolds to form what has been termed the "gene activated matrix" (GAM) (Fang et al., 1996). A particle-mediated transfer (gene gun) is a projection of a metallic particle coated with DNA by electrical charge or gas pressure into the target cells. The disadvantage of this technique is transient and limited gene expression and toxicity caused by metal particles (Benn et al., 1996). Despite many advantages such as the ability to transfer a large amount of DNA, a decreased risk of insertion into the host DNA and no incitement of immune response (repeat administration is possible), the low gene transduction rate and short-time gene expression are limiting the use of these non-viral vectors. Current investigations are attempting to overcome these limitations.

### 3.2 Viral vectors

Currently, the most efficient vectors are utilize viruses. Viral vectors are usually designed so that genes required for viral replication are replaced by a specific therapeutic gene of interest. The resulting recombinant vector is replication incompetent. However, they are still

able to deliver genetic material directly to a cell's nucleus efficiently, and then the cellular machinery can produce the therapeutic protein coded by the introduced genetic material. Vectors vary in the efficiency of gene transfer, the genetic carrying capacity, ease of production, and reactions with host immunity (Khan et al., 2000, Alden et al., 2002, Evans et al., 2004, Nishida et al., 2008).

### 3.2.1 Retrovirus

Retroviral vectors have single-stranded RNA and have been derived from oncoretroviruses such as the murine leukemia virus (MLV). The advantage of using a retroviral vector is that it transduces the genes into the host chromosomes very efficiently and reliably during cell division (Miller DG et al., 1990). Therefore the integrated genes are passed on to subsequent daughter cells, giving the gene the potential for lifelong expression. However, this integration may occur only during cell division, thus, only highly proliferative cells can be transduced with retroviral vectors. Therefore, it may not be suitable for *in vivo* direct injection. Some of the major disadvantages of retroviral vectors are the low infectivity, instability of their virions, a limited capacity of genetic materials (~8kb) and risk of oncogenesis by the insertion of genetic materials (Li et al., 2002). There have also been concerns about the potential ability of the recombinant retroviruses to convert into replication competent viruses.

### 3.2.2 Adenovirus

The most commonly tested viral delivery vehicle for gene therapy for bone healing has been the adenovirus. Adenoviruses are double-stranded DNA viruses with a genome size of approximately 35kilobases. The genetic material is inserted into the nucleus in an episomal state, and not integrated within the host chromosome. The viral genome is divided into immediate early genes, early genes, and late genes according to the time during which the genes are expressed. First-generation adenoviral vectors often have deleted the E1and E3 region which necessary for virus replication and can accommodate up to 8kb of foreign DNA (Lai et al., 2002). Recombinant adenoviral vectors can be produced at high levels, transfer genes to both dividing cells and non-dividing cells efficiently, and maintain more genetic material than other viruses (Gugala et al., 2003). The transgene remains episomal so that the therapeutic gene expression is stopped when the genetically modified cell divides. This episomal nature reduces the risk of insertional mutagenesis. The main disadvantage of the adenovirus is the high immunogenic potential. The humoral and cellular immune responses can actually remove the transduced cells, resulting in decreased gene expression (McCoy et al., 1995). Also, the immunologic memory established against the viral vector by the initial exposure limits the ability to give a second administration of gene therapy. Newer formulations of adenovirus vectors have undergone removal of many viral antigens to minimize this challenge and the so-called gutless vectors have virtually no adenovirus genes to minimize these responses (Hammerschmidt et al., 1999). As another approach for this immunological response, transient immunosuppression using cyclophosphamide has been shown to improve BMP expression after BMP adenoviral vector gene therapy (Okubo et al., 2000, Kim et al., 2003). Lieberman et al used *ex vivo* adenoviral gene transfer to create BMP-2 producing bone marrow cells for the delivery of BMP-2 to heal a critical-sized femoral segmental defect in a rat model (Lieberman et al., 1998). The genetically modified cells were implanted with inactivated demineralized bone matrix as a substrate. At two months, 92% of the defects healed radigraphically, and there was no significant difference in

biomechanical testing between the group that had been treated with genetically modified cells and those that had been treated with rhBMP-2 alone. Their results showed that BMP-2 producing bone marrow cells created by means of adenoviral gene transfer produced sufficient proteins to heal a segmental femoral defect in a rat model.

#### 3.2.3 Adeno associated virus

Adeno-associated viruses (AAVs) are a defective single stranded parvovirus (requires a helper virus such as adenovirus or herpes simplex virus for replication) that integrates its genetic material into a specific site located on chromosome 19 which can minimize the risk of insertional mutagenesis (Monahan et al., 2000). The virus infects a wide variety of dividing and non-dividing cells with high efficiency and little inflammatory response. Also, long-term gene expression has been reported in several tissues types. The disadvantages of AAVs are the difficulty in the virus production and limited titers of virus which can be generated (Kotin et al., 1994).

#### 3.2.4 Herpes virus

Herpes viruses are double-stranded DNA viruses that can cause significant human pathology, including cold sores and encephalitis. Gene therapy studies using herpes viral vectors typically use genetically modified herpes simplex Type 1 (HSV-1). Herpes viral vectors have the advantage of being able to accommodate up to 40 kb of foreign DNA, and can be used to a variety of cell populations with limited toxicity (Evans et al., 1997). However, the disadvantage is that HSVs are highly immunogenic.

#### 3.2.5 Lentivirus

Whereas retroviruses can only infect dividing cells, lentiviruses such as the human immunodeficiency virus type 1 (HIV-1) can also infect non-proliferating cells. What could be termed the third-generation lentivirus conserves only three (gag, pol, and rev) of the nine genes present in the genome of the original HIV-1. This eliminates the possibility that a wild -type virus will be reconstituted through recombination and create room for a relatively large gene delivery. Lentiviral-based gene transfer provides improved delivery, integration, and expression of target genes *in vitro* as well as *in vivo*. Furthermore, lentiviruses are capable of inserting into the host genome ensuring prolonged gene expression with a limited host immune response (Feeley et al., 2006). However, safety issues of HIV-based vectors are a major concern when adapting these vectors for clinical use (Bai et al., 2003, Dull et al., 1998, Zufferey et al., 1997, Zufferey et al., 1998).

## 4. Osteoinductive factors for *ex vivo* gene therapy

### 4.1 BMPs

BMPs comprise a large and diverse family, and many may have a distinct role not only in the cascade of bone morphogenesis but also in orchestrating tissue architecture throughout the body. More than 20 BMPs have been identified in humans and rodents (Bragdon B et al., 2011). Signal transduction of BMPs consists of interaction with both type-1(BMPR-1) and type-2(BMPR-2) BMP receptors, phospholylation of the GS domain of BMPR-1 by BMPR-2, phosphorylation of Smad1/5/8, and the translocation of the Smad1/5/8 partnering with the Smsd4 to the nucleus to activate the transcription for BMP-response genes (Reddi et al., 2001, Bragdon B et al., 2011). To date, most attempts at gene therapy to achieve spinal fusion

have involved transfection with various BMPs (Li et al., 2003). Among BMPs, BMP-2 and BMP-7 have been the most extensively studied, both in animal studies and in clinical trials of spinal fusion. Recently, some combinations of BMPs have been demonstrated to have a synergic effect on bone formation (Israel et al., 1996, Zhu et al., 2004, Zhao et al., 2005).

### 4.2 LIM

LIM mineralization protein-1 (LMP-1) is an intracellular LIM-domain protein that is directly involved in osteoblast differentiation. LMP-1 also appears to elicit the increased synthesis of BMPs. Unlike a BMP, which is a secreted protein that binds to cell-surface receptors to initiate a response, LMP-1 is an intracellular signaling molecule and must be synthesized inside cells to exert its osteoinductive effects. Thus, the use of LMP-1 to form bone must involve the techniques of gene therapy to deliver the cDNA inside the cells and result in the synthesis of LMP-1 protein in situ (Cha et al., 2003, Pola et al., 2004).

### 4.3 NELL-1

NELL-1 (NEL-like molecule-1; NEL[a protein strongly expressed in neural tissue encoding epidermal growth factor like domain]) is a novel growth factor believed to specifically target cells committed to the osteochondral lineage. NELL-1 was isolated and characterized in craniosynostosisi patients as specifically upregulated within prematurely fusing sutures (Zhang et al., 2002). The phenotype of the *Nell-1* transgenic overexpression mouse revealed cranial suture overgrowth similar to human craniosynostosis, suggesting a distinct role for Nrll-1 in bone formation. Conversely, a mouse model with mutated RNU-induced alleles, including Nell-1, resulted in cranial and other vertebral skeletal defects (Desai et al., 2006). In committed osteoblasts, Nell-1 upregulation accelerates osteogenic differentiation and bone formation. Interestingly, human NELL-1 was reported to be directly regulated by Cbfa1/Runx2, confirming its osteochondral specificity (Truong S et al., 2007). Additionally, co-administration to C2C12 myoblasts with AdBMP-2 and AdNell-1 showed a synergistic effect on osteogenic differentiation as detected by alkaline phosphatase activity and osteopontin production. Importantly, Nell-1 alone did not induce osteogenic differentiation of myoblasts.

## 5. Cells combined with *ex vivo* gene therapy

MSCs from adult tissues present fewer ethical and tumorigenicity problems in their use than embryonic stem (ES) cells. These MSCs have demonstrated not only self-renewal ability but also "plasticity" meaning they have the ability to differentiate into phenotypes not restricted to the tissue from which they are derived (Augello et al., 2010). Another key feature of these MSCs is their rapid expansion *in vitro* without loss of progenitor characteristics. Various sources of MSCs that could be used for *ex vivo* gene therapy include bone marrow, muscle and adipose tissue (Lee et al., 2008, Gottfried et al., 2008). In clinical practice, the most widely used source of MSCs is bone marrow. The advantages of using bone marrow are the technique to collect them from the iliac crest is simple and is associated with the least morbidity. However, the problem of low MSC concentration upon harvest (1 in 10,000 cells) have led to an interest in adipose derived stem cells as they have been shown to have a higher prevalence (1 in 4,000 cells). The multipotentiality of adipose-derived stem cells (ADSCs) has been demonstrated *in vivo* and they have been shown to differentiate to bone cells in reaction to BMP signaling (Zuk et al., 2001, Zuk et al., 2010, Zuk et al., 2011). Hsu et

al. reported that stem cells from human adipose cell transfected with AdBMP-2 can work as cellular delivery vehicles in an athymic rat posterolateral spine fusion model (Hsu et al., 2008). Miyazaki et al. have compared the osteogenic and osteoinductive ability between human bone marrow stem cells and human adipose derived stem cells for adenovirus mediated *ex vivo* gene therapy in an athymic rat spinal fusion model and demonstrated that both cells have similar ability for inducing new bone and spinal fusion (Miyazaki et al., 2008)

## 6. Spinal fusion by *ex vivo* gene therapy

### 6.1 BMP-2 *ex vivo* gene therapy

Riew et al attempted to prolong the bone-inducing effect of BMP-2 using an adenoviral vector carrying the human BMP-2 gene to transduce marrow-derived mesenchymal stem cells in New Zealand White rabbits. In their model, they isolated and expanded bone marrow mesenchymal cells from the resected ribs. Immunocytochemistry was used to show that approximately 80% of mesenchymal cells could be modified genetically to overexpress BMP-2 protein by treatment with an adenoviral vector encoding human BMP-2 (AdBMP-2). A similar efficiency of gen e transfer was shown with the control vector encoding a marker gene β-galactosidase (Adβ-gal). Four weeks after rib harvest, the rabbits underwent spinal fusion at L5-6. Genetically modified mesenchymal stem cells loaded onto collagen sponges were placed between the transverse processes of L5 and L6 with AdBMP-2 transduced mesenchymal stem cells placed on the left side and the Adβ-gal cells on the right. After the second postoperative week, all rabbits were examined weekly using a radiograph. Of the five study rabbits, one showed radiographic evidence of new bone formation on the side implanted with AdBMP-2 at 5 weeks after surgery. No new bone was noted on the control Adβ-gal side. No new bone formation was observed on either side in the other four study rabbits. Rabbits were sacrificed 7 weeks after the operation, and histologic examination of the rabbit with new bone revealed mature bone with a trabecular structure. The authors concluded that it was possible to transduce mesenchymal stem cells with human BMP-2 gene so that the transformed cells could produce BMP-2 *in vivo* that would exert an osteoinductive effect (Riew et al., 1998). Riew et al also reported using *ex vivo* AdBMP-2 transduction to perform anterior spinal fusion on pigs. Bone marrow cells were harvested from a resected rib and were expanded. Cells were then incubated overnight with AdBMP-2. Anterior arthrodesis was performed by a thoracoscopic technique and the researchers reported a 100% fusion rate by histologic and radiologic evaluation for six of six disc spaces treated with AdBMP-2, whereas none of the controls fused (Riew et al., 2003).

Wang et al. compared single-level posterolateral spine fusion rates between rhBMP-2 with various carriers, bone marrow cells transduced for 48 hours with AdBMP-2 and iliac crest bone graft (ICBG) in rats. They reported higher fusion rates with *ex vivo* BMP-2 transduction as compared with autogenous ICBG. All of the animals treated with AdBMP-2 and rhBMP-2 achieved solid fusion masses at four weeks, whereas none of the control groups fused. Qualitatively, the *ex vivo* AdBMP-2-treated rats produced abundant trabecular fusion masses, whereas the rhBMP-2-treated rats exhibited thinner, lacelike trabecular fusion masses. The researchers concluded that *ex vivo* AdBMP-2 transduction produced solid posterolateral spinal fusion in rats and was superior to ICBG alone (Wang et al., 2003). The same group also reported that human bone marrow cells can be infected by AdBMP-2 and produce sufficient bone *in vivo* to fuse the lumbar spine in athymic rats (Peterson et al. 2005). Miyazaki et al. have compared lentiviral and adenoviral gene therapy. They had implanted

a collagen sponge containing rat bone marrow cells transfected with either Lenti-BMP-2 or Adeno-BMP-2 and achieved solid fusion masses at eight weeks, whereas the new bone volume and levels fused are greater in the Lenti-BMP-2 treated group. They concluded that lentiviral gene therapy with BMP-2 shows great potential for application in various pathologies of the spine despite the fact that the safety of using lentiviral vectors and the side effects of prolonged BMP-2 production are required to be elucidated before the application of this technology in humans (Miyazaki et al., 2008).

### 6.2 BMP-7 *ex vivo* gene therapy

Hidaka et al. recently reported successful posterolateral spinal fusion in immune-competent rats in *ex vivo* study using rat bone marrow cells that were expanded for four weeks, then treated with AdBMP-7 or Adβ-gal for two hours. Three million cells were implanted with allograft bone at the L4-5 level using a paraspinal muscle-splitting approach. A β-galastosidase assay was performed in homogenetes of fusion masses harvested three, seven, and 14 days after surgery to evaluate the extent and duration of adenovirus mediated gene transfer in this model. The gene expression was maximal on day three, waning to background levels by 14 days. Additionally, with AdBMP-7 treatment, radiographic fusion rates were 70% and mechanical fusion rates were 80% versus 0% by either parameter in control groups at eight weeks after the index procedure. Fusion masses of AdBMP-7 treated spines had the microscopic appearance of normal trabecular bone and showed a 23-fold higher uptake of fluorochrome indicating increased bone formation. (Hidaka et al., 2003).

### 6.3 BMP-9 *ex vivo* gene therapy

Dumont et al. reported that human bone marrow mesenchymal stem cells were transduced with AdBMP-9 or Adβ-gal for 12hours and then, the cells (1 million cells/ml) were injected into the lumbar region with the use of fluoroscopic guidance in an athymic rat model. At eight weeks, the sites treated with the cells transduced AdBMP-9 showed copious bone formation, whereas the control injections showed no osteogenic activity. They did not mention about the fusion rate or bone volume of the newly induced bone (Dumont et al., 2002).

### 6.4 Combination of different BMPs

Although a variety of BMPs still are being investigated for their therapeutic potential, a combination of different BMPs and other growth factors may prove to be the most effective initiators of bone formation. The use of BMP2/7 and BMP4/7 heterodimers has demonstrated increased bone induction and compared with that of their homodimer counterparts (Israel et al., 1996). Zhu et al. reported that supernatants from the culture media of a producer cell (A549) co-transfected by AdBMP-2 and AdBMP-7 induced much higher osteocalcin expression and alkaline phosphatase activity in both C2C12 and MC3T3-E-1 cells than the supernatants from the cells transfected either AdBMP-2 or AdBMP-7 alone even though the concentration of the produced BMPs are much lower in the co-transfected group. They further demonstrated that co administration of AdBMP-2 and AdBMP-7 resulted in a significantly greater number of mechanically stable fusions ans also 2-fold higher mineralization rate and bone volume in the fusion mass versus single BMP gene transfer (co-administrated group; 73% fusion, AdBMP-2 group; 8% fusion and AdBMP-7 group 16% fusion (Zhu et al., 2004).

### 6.5 LIM

Although the exact mechanism of LMP-1 remains unclear, solid spinal fusions have been achieved in rat and rabbit models using *ex vivo* LMP-1-transduced bone marrow cells and buffy-coat blood cells.

Boden et al investigated the feasibility of achieving lumbar spine fusion by liposomal-mediated transfer of a different osteoinductive protein gene, the LIM mineralization protein-1 (LMP-1) gene. LMP-1 is an intracellular protein that plays a key role in the BMP-6 stimulation of osteoblasts. Because LMP-1 is an intracellular signaling molecule, the technique of gene therapy is best suited to deliver the LMP-1 cDNA within the cell. Marrow fibroblasts were isolated from the hind limbs of rats and transfected with a plasmid containing LMP-1 in the forward orientation (study group) or the reverse orientation (control group) using lipofection. Once the cells were transfected, they were soaked in a devitalized bone matrix carrier. 14 athymic rats were used. Implants were composed of a devitalized bone matrix carrier loaded with bone marrow cells transfected with either the LMP-1 gene (active) or the reverse LMP-1 (control). In the pilot phase, two rats received subcutaneous implants on the right (active) and left (control) sides of the chest. The same rats received implants to the lumbar (active) and received implants to the posterior thoracic (control) spine. In the experimental phase, 12 rats received active and control implants in the thoracic (T11-12) and lumbar (L5-6) spine. Rats that received bone marrow cells transfected with the active gene in the thoracic region and vice versa. Four weeks postoperation, all rats were sacrificed. The samples of the rats that received subcutaneous implants underwent high-definition radiographs and undecalcified histology. All thoracic and lumbar spines in the experimental group underwent assessment of fusion by manual palpation, radiography, and undecalcified histology. Examination of subcutaneous implants from the two pilot rats revealed complete bone formation with marrow and osteoblast-lined trabeculae on the active side (carrier plus marrow cells with active LMP-1 cDNA) with no bone formation on the control side (carrier plus marrow cells with reverse LMP-1 cDNA). The two lumbar spines of the pilot rats that were implanted with the active LMP-1 cDNA were completely fused, whereas the two thoracic control fusion sites that had been implanted with reverse LMP-1 cDNA failed to show new bone formation. In the experimental group, three of the 12 rats died of perioperative anesthetic complications. In the remaining nine rats, complete arthrodesis was shown manually, radiographically, and histologically in nine of nine (100%) sites receiving bone marrow cells transfected with inactive LMP-1 cDNA were fused. The authors concluded that the local delivery of LMP-1 cDNA to bone marrow cells was feasible and efficacious for enhancing spine arthrodesis.

Boden et al. reported solid posterior fusion in an athymic rat model using bone marrow cells transduced for two hours with AdLMP-1 in a demineralized bone matrix carrier (Boden et al., 1998). Viggeswarapu et al. showed bone marrow or buffy-coat cells transduced with AdLMP-1 for 10 minutes combined with demineralized bone matrix or collagen-ceramic-composite sponges induced posterolateral lumbar fusion in rabbits (Viggeswarapu et al., 2001).

### 6.6 Nell-1

Lu et al reported the osteoinductive properties of Nell-1 in rat spinal fusion model. Demineralized bone matrix (DBM) carriers containing either adenovirus coding for Nell-1 (AdNell-1) or Lac Z (AdLacZ) were implanted at the intertransverse process of L4-5 in athymic rats. After six weeks, direct application of adenoviral Nell-1 in a DBM carrier

achieved significantly higher rates of spinal fusion over Lac Z controls: 60% Nell-1 versus 20% Lac Z by manual palpation and 70% Nell-1 versus 20% Lac Z by micoroCT and histology. Histological assessment of bone quality and maturity revealed more mature, higher quality bone in all the Nell-1 treated specimens relative to Lac Z at six weeks. They concluded that Nell-1, which is regulated by the master bone regulatory gene Runx2, may exert its effects more specifically in osteoblastic cells than BMPs which affects multiple cell types.

## 7. Conclusion and future direction

Spinal fusion will continue to be an important part of the surgical treatment of spinal pathology for the foreseeable future. Promising research in progress involves the understanding of the biology of bone formation (White et al., 2004) and the use of gene

| Authors | Vector | Gene | cell | animal | Fusion rate | F/U weeks | carrier |
|---|---|---|---|---|---|---|---|
| Riew et al | adenovirus | BMP-2 | Rat BMC | rabbits | 20% | 7 | collagen |
| Boden et al | plasmid | LIM-1 | Ratfibloblast | Athymic rats | 100% | 4 | DBM |
| Peterson et al | adenovirus | BMP-2 | Human BMC | Athymic rats | 100% | 12 | CS |
| Wang et al | adenovirus | BMP-2 | Rat BMC | rats | 100% | 8 | DBM |
| Hsu et al | adenovirus | BMP-2 | Human ADSC | Athymic rats | 100% | 12 | CS |
| Miyazaki et al | lentivirus | BMP-2 | Rat BMC | rats | 100% | 8 | CS |
| Viggeswarapu et al | adenovirus | LIM-1 | BCC | rabbits | 100% | 5 | DBM |
| | | | PBC | | | 4 | CCC |
| Hidaka et al | adenovirus | BMP-7 | Rat BMC | rats | 70% | 8 | allograft |
| Dumont et al | adenovirus | BMP-9 | Human BMC | Athymic rats | N/A | 8 | none |
| Riew et al | adenovirus | BMP-2 | Pig BMC | pigs | 100% | 6 | none |
| Miyazaki et al | adenovirus | BMP-2 | Rat BMC | rats | 100% | 8 | CS |
| | lentivirus | | | | | | |
| Miyazaki et al | adenovirus | BMP-2 | Human ADSC | Athymic rats | 100% | 8 | CS |
| | | | Human BMC | | | | |
| Lu et al | adenovirus | Nell-1 | none | Athymic rats | 70% | 6 | DBM |

BMC: bone marrow cell, ADSC: adipose derived stem cell, CCC: collagen-ceramic-composite, CS: collagen sponge, Buffy-coat cell: BCC, Peripheral blood cell: PBC

Table 1. Animal studies on *ex vivo* gene therapy for spinal fusion.

therapy or tissue engineering in facilitating the natural processes of a spinal fusion. A viral vector can be used to transfer a gene into human cells to produce various recombinant growth factors that may modify the fusion cascade both qualitatively and quantitatively. Viral vectors, particularly the adenovirus, may serve as transports for the material encoding osteogenic factors. However, significant advances need to be made in vector design for low immunogenetic potential, controlled gene expression techniques like tetracycline-regulated system, the way of preventing insertional mutagenesis, and tissue targeting before human clinical trials can be safely and successfully conducted.

## 8. References

Alden TD, Pittman DD, Beres EJ, et al. (1999) Percutaneous spinal fusion using bone morphonenetic protein-2 gene therapy. *Journal of Neurosurgery* 90 (1 Suppl), pp.109-114

Alden TD, Varady P, Kallmes DF, et al. (2002) Bone morphogenetic protein gene therapy. *Spine* 27 (165), pp.S87-S93

Augello A, Kurth TB, De Bari C. (2010) Mesenchymal stem cells: a perspective from *in vitro* cultures to *in vivo* migration and niches. *European Cells and Materials* 1 (20), pp.121-133

Bai Y, Soda Y, Izawa K, et al. (2003) Effective transduction and stable transgene expression in human blood cells by a third-generation lentiviral vector. *Gene Therapy* 10, pp.1446-1457

Baltzer AWA, Lieberman. (2004) Regional gene therapy to enhance bone repair. *Gene Therapy* 11 (4), pp.344-350

Benn SI, Whitsitt JS, Broadley KN, et al. (1996) Particle-mediated gene transfer with transforming growth factor bata 1 cDNA enhances wound repair in rats. *Journal of Clinical Investigation* 98, pp.2894-2902

Boden SD, Titus L, Hair G, et al. (1998) Lumbar spine fusion by local gene therapy with a cDNA encoding a novel osteoinductive protein (LMP-1). *Spine* 23 (23), pp.2486-2492

Bragdon B, Moseychuk O, Saldanha S, et al. (2011) Bone morphogenetic proteins: a critical review. *Cell Signal* 23(4), pp.609-620

Carlisle E, Fischgrund JS. (2005) Bone morphogenetic proteins for spinal fusion. *The Spine Journal* 5, pp.240S-249S

Cha CW, Boden SD. (2003) Gene Theray Applications for Spine Fusion. *Spine* 28 (15S), pp.S74-S84

Cowan CM, Jiang X, Hsu T, et al. (2007) Synergistic effects of Nell-1 and BMP-2 on the osteogenic differentiation of myoblasts. *Journal of Bone and Mineral Research* 22 (6), pp. 918-930

Desai J, Shannon ME, Johnson MD, et al. (2007) Nell-1 deficient mice have reduced expressiion of extrcellular matrix proteins causing cranial and vertebral defects. *Human Molecular Genetics* 15, pp.1329-1341

Dull T, Zufferey R, Kelly M, et al. (1998) A Third-Generation Lentivirus Vector with a Conditional Packaging System. *Journal of virology*, 72(11), pp. 8463-8471

Dumont RJ, Dayoub H, Li JZ, et al. (2002) *Ex vivo* bone morphogenetic protein-9 gene therapy using human mesenchymal stem cells induces spinal fusion in rodents. *Neurosurgery* 51 (5), pp.1239-1244

Evans CH, Ghivizzani SC, Robbins PD. (2004) Orthopaedic Gene Therapy, *Clinical Prthopaedics and related research* 429, pp.316-329

Evans C, Goins WF, Schmidt MC, et al. (1997) Progress in development of herpes simplex virus gene vectors for treatment of rheumatoid arthritis. *Advanced Drug Delivery Reviews* 27, pp.41-57

Fang J, Zhu YY, Smiley E., et al. (1996) Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. *Proceedings of the National Academy of Sciences* 93, pp.5753-5758

Feeley BT, Conduah AH, Sugiyama O, et al. (2006) *In vivo* molecular imaging of adenoviral versus lentiviral gene therapy in two bone formation models. *Journal of Orthopaedic Research*, pp.1709-1721

Gottfried ON, Dailey AT. (2008) Mesenchymal stem cell and gene therapies for spinal fusion. *Neurosurgery* 63 (3), pp.380-392

Gugala Z, Olmsted-Davis EA, Gannon FH, et al. (2003) Osteoinduction by *ex vivo* adenovirus-mediated BMP-2 delivery is independent of cell type. *Gene Therapy* 10. Pp.1289-1296

Hammerschmidt DE. (1999) Development of a gutless vector. *Journal of Laboratory and Clinical medicine* 134 (3), C3

Helm GA, Alden TD, Sheehan JP, et al. (2000) Bone morphogenetic proteins and bone morphogenetic gene therapy in neurological surgery : a review. *Neurosurgery* 46 (5), pp.1213-1222

Hidaka C, Goshi K, Rawlins B, et al. (2003) Enhancement of Spine Fusion Using Combined Gene Theray and Tissue Engineering BMP-7 Expressing Bone Marrow Cells and Allograft Bone. *Spine* 28 (18), pp.2049-2057

Hsu WK, Wang JC, Liu NQ, et al. (2008) Stem cells from human fat as cellular delivery vehicles in an athymic rat posterolateral spine fusion model. *Journal of Bone and Joint Surgery* 90, pp.1043-1052

Israel DI, Nove , Kerns KM, et al. (1996) Heterodimeric bone morphogenetic proteins show enhanced activity *in vitro* and *in vivo*. *Growth Factors* 13, pp.291-300

Kaito T, Myoui A, Takaoka K, et al. (2005) Potentiation of the activity of bone morphogenetic protein-2 in bone regeneration by a PLA-PLG/hydroxyapatite composite. *Biomaterials* 26(1), pp.73-79

Khan SN, Hidaka C, Sandhu HS, et al. (2000) Gene therapy for spine fusion. *Tissue Engineering in Orthopedic Surgery* 31 (3), pp.473-484

Khan SN, Sandhu HS, Lane JM, et al. (2002) Bone morphogenetic proteins : relevance in spine surgery. *The Orthopedic Clinics of North America* 33, pp.447-463

Khan SN, Lane JM. (2004) Spinal fusion surgery : animal models for tissue-engineered bone constructs. *Biomaterials* 25, pp.1475-1485

Kim HS, Viggeswarapu M, Boden SDD, et al. (2003) Overcoming the immune response to permit *ex vivo* gene therapy for spine fusion with human type 5 adenoviral delivery of the LIM mineralization protein-1 cDNA. *Spine* 28 (3), pp.219-226

Kootstra NA, Verma IM. (2003) Gene therapy with viral vectors. *Annual Review of Pharmacology and Toxicology* 43, pp.413-439

Kotin RM. (1994) Prospects for the use of adeno-associated virus as a vector for human gene therapy. *Human Gene Therapy* 5, pp.793-801

Lai CM, Lai YK, Rakoczy PE, et al. (2002) Adenovirus and adeno-associated virus vectors. *DNA and Cell Biology* 21, pp. 895-913

Lee K, Chan CK, Patil N, et al. (2008) Cell therapy for bone regeneration-Bench to bedside. *Journal of Biomedical Material and Research* Part B, pp. 252-263

Li JZ, Li H, Sasaki T, et al. (2003) Osteogenic potential of five different recombinant human bone morphogenetic protein adenoviral vectors in the rat. *Gene Therapy* 10, pp.1735-1743

Li S, Ma Z. (2001) Nonviral gene therapy. *Current Gene Therapy* 1, pp.201-226

Li Z, Dullmann J, Schiedlmeier B, et al. (2002) Murine Leukemia induced by retroviral gene marking. *Science* 296, pp.497

Lieberman JR, Le LQ, Finermn GA, et al. Regional gene therapy with a BMP-2-producing murine stromal cell line induces heterotopic and orthotopic bone formation in rodents. *Journal of Orthopaedic Research* 16 (3), pp.330-339

Lu SS, Zhang, X, Soo C, et al. (2007) The osteoinductive properties of Nell-1 in a rat spinal fusion model. *The Spine Journal* 7, pp,50-60

McCoy RD, Davidson BL, Roessler BJ, et al. (1995) Expression of human interleukin-1 receptor antagonist in mouse lungs using a recombinant adenovirus: effects on vector-induced inflammation. *Gene Therapy* 2 (79, pp.437-442

Miller DG, Adam MA, Miller AD. (1990) Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. *Journal of Molecular Cell Biology* 10, pp.4239-4242

Miyazaki M, Sugiyama O, Tow B, et al. (2008) The effects of lentiviral gene therapy with bone morphogenetic protein-2-producing bone marrow cells on spinal fusion in rats. *Journal of Spinal Disorder and Techniques* 21 (5), pp.372-379

Miyazaki M, Sugiyama O, Zou J, et al. (2008) Comparison of Lentiviral and Adenoviral gene Therapy for Spinal Fusion in Rats. *Spine* 33 (13), pp.1410-1417

Miyazaki M, Zuk PA, Zou J, et al. (2008). Comparison of Human Mesenchymal Stem Cells Derived From Adipose Tissue and Bone Marrow for *Ex Vivo* Gene Therapy in Rat Spinal Fusion Model, *Spine* 33 (8), pp.863-869

Monahan PE, Samulski RJ. (1999) AAV vectors: Is clinical success on the horizon? *Gene Therapy* 6, pp.904-912

Mroz TE, Wang JC, Hashimoto R, et al. (2010) Complications related to osteobiologics use in spine surgery: a systematic review. *Spine* 35 (9 Suppl), pp.S86-104

Nishida K, Suzuki T, Kakutani K, et al. (2008) Gene therapy approach for disc degeneration and associated spinal disorders. *European Spine Journal* 17 (Suppl 4), pp.S459-S466

Peterson B, Iglesias R, Zhang J, et al. (2005) Genetically modified human derived bone marrow cells for posterolateral lumbar spine fusion in athymic rats. *Spine* 30 (3), pp.283-290

Pola E, Gao W, Zhou Y, et al. (2004) Efficient bone formation by gene transfer of human LIM mineralization protein-3. *Gene Therapy* 11, pp.683-693

Oakes DA, Lieberman JR. (2000) Osteoinductive applications of regional gene therapy: *ex vivo* gene transfer. *Clinical Orthopaedics and Related Research* 379 Suppl, pp.S101-112

Okubo Y, Bessho K, Fujimura K, et al. (2000) Osteoinduction by bone morphogenetic protein-2 via adenoviral vector under transient immunosuppression. *Biochemical and Biophysical Research Communications* 267, pp.382-387

Phillips FM, Bolt PM, He TC, et al. (2005) Gene therapy for spinal fusion. *The Spine Journal* 5, pp250S-258S

Reddi AH. (1998) Role of morphogenetic proteins in skeletal tissue engineering and regeneration. *Nature Biotechnology* 16, pp.247-252

Reddi AH. (2001) Bone morphogenetic protein :from basic science to clinical applications. *Journal of Bone and Joint Surgery* 83-A, pp.S1-1-S1-6

Riew KD, Wright NM, Cheng SL, et al. (1998) Induction of bone formation using a recombinant adenoviral vector carrying the human BMP-2 gene in a rabbit spinal fusion model. *Calcified Tissue International* 63. Pp357-360

Riew KD, Lou J, Wright NM, et al. (2003) Thoracoscopic intradiscal spine fusion using a minimally invasive gene-therapy technique. *Journal of Bone and Joint Surgery* 85-A, pp.866-871

Truong S, Zhang X, Pathmanathan D, et al. (2007) Craniosynostosis-associated gene Nell-1 is regulated by Runx2. *Journal of Bone and Mineral Research* 122, pp.7-18

Vaccaro AR, Chiba K, Heller JG, et al. (2002) Bone grafting alternatives in spinal surgery. *The Spine Journal* 2, pp.206-215

Veillette CJH, Mckee MD. (2007) Growth factors – BMPs, DBMs, and buffy coat products :are there any proven differences amongst them ? Injury, *International Journal of the Care of the Injured* 38S1, pp.S38-S48

Viggeswarapu M, Boden SD, Liu Y, et al. (2001) Adenoviral delivery of LIM mineralization protein-1 induces new-bone formation *in vitro* and vivo. *Journal of Bone and Joint Surgery* 83-A, pp.364-376

Wang JC, Lanim LE, Campbell RA, et al. (2003) Effect of regional gene therapy with bone morphogenetic protein-2-producing bone marrow cells on spinal fusion in rats. *Journal of Bone and Joint Surgery* 85-A (5), pp.905-911

White AP, Weinstein MA, Patel TC, et al. (2004) Lumbar arthrodesis gene expression. *Clinical Orthopaedics and related research* 429, pp.330-337

Yoon ST, Boden SD. (2004). Spine fusion by gene therapy, *Gene therapy* 11, pp.360-367

Zhang X, Kuroda S, Carpenter D, et al. (2002) Craniosynostosis in transgenic mice overexpressing Nell-1. *Journal of Clinical Investigation* 110, pp.861-870

Zhao M, Zhao Z, Koh JT, et al. (2005) Combinatorial Gene Therapy for Bone Regeneration : Cooperative interactions between adenovirus vectors expressing bone morphogenetic priteins 2,4, and 7. *Journal of Cellular Biochemistry* 95, pp.1-16

Zhu W, Rawlins BA, Oachie-Adjei O, et al. (2004) Combined bone morphogenetic protein-2 and -7 gene transfer enhances osteoblastic differentiation and spine fusion in a rodent model. *Journal of Bone and Mineral Research* 19, pp.2021-2032

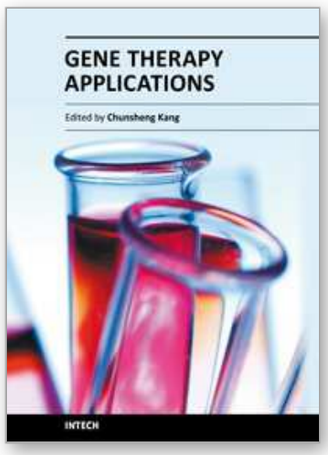
Zufferey R, Nagy D, Mandel RJ, et al. (1997) Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nature Biotechnology* 15, pp.871-875

Zufferey R, Dull T, Mandel RJ, et al. (1998) Self-Inactivating Lentivirus Vector for Safe and Efficient *In Vivo* Gene Delivery. *Journal of Virology*, pp.9873-9880

Zuk PA. (2001) Multilineage cells from human adipose tissue : implications for cell-based therapies. *Tissue Engineering* 7 (2), pp.211-228

Zuk PA. (2010) The adipose-derived stem cell : looking back and looking ahead. *Molecular Biology of the Cell* 21, pp.1783-1787

Zuk PA. (2011) Viral transduction of adipose-derived stem cells. *Methods in Molecuar Biology* 702,pp.345-357

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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.



**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
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Phone: +86-21-62489820
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