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Gene Therapy and Tissue Engineering in Repair of the Musculoskeletal System

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Abstract Historically, surgeons have sought and used different procedures in order to augment the repair of various skeletal tissues. Now, with the completion of the Human Genome Project, many researchers have turned to gene therapy as a means to aid various ailments. In the orthopedic field, many strides have been made toward using gene therapy and tissue engineering in a clinical setting. In this review, several studies are outlined in different areas that gene therapy has or will influence orthopedic surgery. Gene therapy and tissue engineering can aid in fracture healing and spinal fusions by inducing bone formation, ligamentous repairs by increasing the production of connective tissue fibers, intervertebral disc disease by creating potential replacements, and articular cartilage repairs by providing means to improve cartilage. As we continue to see great contributions, such as the few mentioned here, this field will continue to mature and develop. J. Cell. Biochem. 88: 467–481, 2003. © 2003 Wiley-Liss, Inc.

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Historically, surgeons have sought and used different procedures in order to augment the repair of various skeletal tissues. Recently, researchers have brought together knowledge of molecular and cell biology, biochemistry, chemical engineering, genomics, and material science to provide clinicians with new modalities to aid in this repair: tissue engineering and gene therapy [Caplan and Goldberg, 1999].

Tissue engineering is the development and manipulation of laboratory-grown molecules, cells, tissues, or organs to replace or support the function of defective or injured body parts. Scientists have designed materials and cell material composites to enhance or aid the reconstruction or replacement of damaged or lost tissue. These biological structures can be grown on various scaffolds or different media in attempts to replicate basic biological processes [Caplan and Bruder, 1996; Caplan et al., 1998; Caplan and Goldberg, 1999]. Recently *tissue engineering* has been used in conjunction with gene therapy as a hybrid approach.

Gene therapy is the science of the transfer of genetic material into individuals for therapeutic purposes by altering cellular function or structure at the molecular level. By employing these techniques, genes can be used therapeutically to produce proteins to treat and potentially cure acute and chronic conditions [Salypongse et al., 1999]. There are two general ways that gene therapy can be performed: (1) a direct in vivo method and (2) an indirect ex vivo method. The direct method involves transferring the genetic material into the target somatic cells in vivo [Crystal, 1995; Oligino et al., 2000]. The indirect technique involves removal of cells from the patient followed by genetic modification of the cells ex vivo and return of the cells to the patient. Of the two approaches, the in vivo method is technically simpler to perform in a clinical setting giving it greater potential utility. The ex vivo techniques may be more complex, but they are relatively safer. Additionally, this method allows for selection of the cells that express the therapeutic gene at higher levels [Chen, 2001]. The choice of method requires one to take into account the disease to be treated, the gene to be delivered to treat the disease, and the vector used to deliver the gene [Oligino et al., 2000].

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In order for target cells to manufacture the protein products of the introduced gene, the exogenous genetic material must be delivered to the cell's nucleus. This process of transfection exists in two classes of vectors: viral and non-viral. The viral technique is associated with increased technical demands and increased risk of virus-associated toxicity [Salypongse et al., 1999]. However, viral vectors have been engineered for safety by making them replication incompetent [Robbins and Ghivizzani, 1998]. It is the viral ability to efficiently infect cells and in the process transfer DNA to the host without invoking an immune response that makes viruses attractive as vectors. These altered viruses can be propagated in cell lines specialized to provide the necessary absent viral functions [Graham et al., 1977; Krougliak and Graham, 1995; Zhou et al., 1996]. In general, retroviruses have been used for ex vivo gene therapy applications as they are unable to efficiently infect non-dividing cells [Danos and Heard, 1992; Robbins and Ghivizzani, 1998]. Adenovirus, herpes simplex virus, and adeno-associated virus, as well as the non-viral vectors may be used for either direct in vivo or ex vivo delivery [Oligino et al., 2000].

Retroviruses are RNA viruses that carry a gene for a reverse transcriptase that transcribes the viral genetic material into a double-stranded DNA intermediate. This DNA intermediate is then incorporated into the host DNA allowing the host cell machinery to produce all the necessary viral components. Additionally, because the viral genome is stabley integrated into the host DNA, any modification that has been made will be passed to all daughter cells that are derived from the transfected cell [Goff and Lobel, 1987; Oligino et al., 2000].

Currently, the most common retrovirus used is derived from the murine leukemia virus. The majority of clinical trials have utilized vectors based on the murine leukemia virus [Guild et al., 1988; Robbins et al., 1994; Marshall, 1995]. Murine leukemia virus has a number of characteristics that make it attractive as a gene therapy vector. It can be considered fairly safe, since Murine Leutemia Virus is non-pathogenic in humans. Additionally, because it has little homology with human retroviruses, the risk of recombination between the vector and any resident human viruses is low [Danos and Heard, 1992]. The MLV genome is relatively simple which allows for molecular exploitation and replication incompetent vectors can be easily created [Oligino et al., 2000]. Although, these vectors are capable of efficiently transducing target cells and integrating into the host genome, they are unable to infect non-dividing cells, a fact that lends MLV-based vectors more applicable to ex vivo use [Oligino et al., 2000]. A strength of retroviral vectors is that they can stably transduce target cells with long term transgene expression and no viral genes are expressed in transduced cells. Consequently, an immune response to the vector is not problematic with the use of retroviral vectors as it is with other vectors.

In contrast to retroviruses, adenovirus does not integrate its genome into the host genome. Instead, the adenoviral genome remains in the nucleus as an episomal element after infection of the host cell. Adenovirus consists of a large family of 47 known human virus serotypes of which serotype 2 and 5 are best characterized and most commonly utilized for gene transfer [Chroboczek et al., 1992; Hierholzer et al., 1988; Bramson et al., 1995]. There are three groups of replication incompetent adenoviral vectors based on the number of viral genes that have been inactivated: first generation, second generation, and gutless [Oligino et al., 2000]. The advantages common to all adenoviral vectors include the ease of purification and concentration and the high efficiency rate of host cell infection or various cell types, dividing or non-dividing [Oligino et al., 2000]. These advantages make adenoviral vectors a good candidate for direct in vivo gene transfer. Usefulness of the first generation vectors was limited by two factors. In most tissues, the duration of transgene expression is limited to a few days to a week [Hitt et al., 1997; Robbins and Ghivizzani, 1998]. There are two reasons for this short duration. The first is due to the fact that the incorporated DNA remains in the nucleus as an episome; therefore, during cell division, it is not maintained in the nucleus and is eventually degraded [Oligino et al., 2000]. The second reason is that viral genes are also transduced and expressed, which elicit an immune response to the transduced cells that ultimately results in their clearance [Engelhardt et al., 1993; Yang et al., 1994; Yang and Wilson, 1995].

The shortened timeframe of gene expression was the impetus to minimize the immune response to the vector, which resulted in the

second generation and gutless adenoviral vectors [Oligino et al., 2000]. The second-generation adenoviral vectors were products of attempts to reduce the immunogenicity by removing some or all of the open reading frames encoding transacting regulatory proteins [Wang and Finer, 1996; Christ et al., 1997]. This resulted in decreased immunogenicity and subsequently increased transgene expression duration; however, as duration did increase, there was also a decrease in overall expression for the second generation adenoviral vectors [Armentano et al., 1997; Christ et al., 1997; Lusky et al., 1999]. In another attempt to create a less immunogenic adenoviral vector, all the viral genes were removed with only the inverted terminal repeats and the packaging signal remaining [Kochanek et al., 1996; Hammerschmidt, 1999; Kochanek, 1999]. These gutless, or gutted, vectors have the advantage of not encoding viral proteins thus having reduced immunogenicity. In addition, these vectors can transfer as much as 30 kb of foreign genetic material [Clemens et al., 1996; Morral et al., 1999; Oligino et al., 2000]. However, without its viral genes, propagation of these vectors requires coinfection with first or second generation helper viruses, and because of this it is difficult to purify gutless vector particles away from the helper virus particles [Oligino et al., 2000]. There are other weaknesses to using adenovirus's: (1) transient expression, (2) very high expression transiently may be problematic, and (3) tissue tropism.

Adeno-associated virus, like gutless adenoviral vectors, requires a helper virus such as adenovirus or herpes simplex virus for replication. Purification is difficult, but recently systems have been designed that allow for production of large-scale, high titer helper free preparations. This is accomplished by cotransfection of a plasmid that expresses the complementing adenoviral genes [Gao et al., 1998; Xiao et al., 1998; Clark et al., 1999; Collaco et al., 1999]. Adeno-associated virus is actually a member of the parvovirus family of single-stranded DNA viruses. The small size of the genome allows for easy manipulation such that shuttle vectors carrying the entire genome have been constructed [Samulski et al., 1982]. Also, this virus is non-pathogenic and not associated with any known disease but can infect a wide variety of cells, dividing or non-dividing, although with varying levels of efficiency [Oligino et al., 2000]. In some tissues, transduction efficiency is high enough to achieve significant levels of gene expression after in vivo delivery [Oligino et al., 2000].

The ability to infect most cells including non-dividing cells is the major advantage of herpes simplex virus vectors [Oligino et al., 2000]. Another advantage is the large carrying capacity and ability to insert expression cassettes into specific loci of the genome that allows for the construction of vectors able to express multiple transgenes [Krisky et al., 1997]. The herpes simplex virus Type I is a human pathogen whose complex in vivo life cycle starts generally by invading the host through the skin or mucosal membranes. The virus then continues into the nervous system through axon terminals up the axon by retrograde transport to the nerve cell body in the sensory ganglia. At this point, the virus can follow the lytic pathway or the latent pathway [Hill, 1985]. If the virus proceeds down the lytic pathway, the virus becomes more infectious versus the latent pathway where viral gene expression is silenced except for a specific set of latency-associated RNA transcripts until the virus reverts to the lytic pathway [Hill, 1985; Spivack and Fraser, 1987]. The goal in order to produce a feasible Herpes Simplex Virus vector is to alter ability of the virus to replicate after infection so that the virus is forced into a pseudo-latent state and avoid lysing the infected cells. This complexity makes production of herpes simplex viral vectors more technically difficult and time consuming [Oligino et al., 2000].

Non-viral vectors are much cheaper and easier to produce in large amounts. These vectors have a limited immunogenicity, which allows for potential redosing and are considered safe, since there is no possibility of recombination that would result in a competent virus that could potentially cause disease [Oligino et al., 2000]. Non-viral vectors are, however, put at a severe disadvantage when compared to viral vectors when taking into account their markedly less-efficient gene transfer rate [Salypongse et al., 1999]. The use of non-viral vectors can be in the form of injections of naked DNA (usually plasmids), liposomes, or particle-mediated gene transfer ("the gene gun"). The genetic material can be placed in liposomes in order to increase DNA uptake in tissue culture. The last of these vectors uses a process by which the micro-projectiles (e.g., gold, tungsten) are

coated with DNA and then accelerated by either helium pressure or a high-voltage electrical discharge thus carrying enough energy to penetrate the cell membrane [Yang et al., 1990; Klein et al., 1992].

FRACTURE HEALING

Currently the preferred method of promoting bone formation is autogenous cancellous bone grafting, "the gold standard," whether for fracture healing, spinal fusion, or osseous defect filling. This process is plagued with significant morbidity and limited supply which directs research toward finding a biosynthetic alternative to autogenous grafting. These alternatives generally contain one or more of three critical components: the cellular component, an osteoconductive matrix, and osteoinductive factors [Lane et al., 1999]. Each of these alternatives is measured against the gold standard, which is still autogenous cancellous bone graft harvested from the iliac crest. Autogenous bone graft contains the three components necessary for bony formation and healing. The two key problems with autogenous grafts are the limited supply and associated surgical morbidity. For children and larger procedures where bone defects are larger, such as revision surgery, tumor resections, or multilevel spinal fusions, use of the iliac crest as a donor site does not provide sufficient material for adequate healing. The morbidity of graft harvesting includes donor site pain, paresthesia, and infection that can reach levels of 8–10% [Younger and Chapman, 1989]. One of the first alternatives was allografts, which yielded inferior results and were associated with increased inflammation and infection [Strong et al., 1996].

With the discovery of bone morphogenetic proteins (BMPs), a new direction for research in bone regeneration was possible [Urist, 1965; Urist and Strates, 1971; Wang et al., 1988]. However, there are still very few clinical studies that report positive results. Some reports suggest significant effects with superphysiological doses between 1.7 and 3.4 μg [Hollinger et al., 2000]. It has been suggested that the megadoses are required due to the poor healing environment of a fresh wound and the relatively short half-lives of the BMPs. Delivery methods have come under scrutiny in order to overcome these drawbacks. Potentially, a carrier system that will allow the BMPs to remain relatively localized and protected and then be able to have a sustained release would minimize required doses [Hollinger et al., 1996; Winn et al., 1999]. The format of a delivery system could have a drastic impact on the dosing requirements [Stocum, 1998]. This format can be described as the innate physical and chemical properties of the delivery substrate. Its surface size, surface shape, internal morphology, and whether it is monolithic or granular can have effects on the cell-matrix interaction. It has been established that cell shape and the surface–cell interface affect phenotype, which thereby affects the cell responsiveness to the signaling molecules [Folkman and Moscana, 1978; Berthiaume and Yarmush, 1995; Hubbell, 1995].

One of the ways that the dosing of the growth factors can be co-regulated is through gene therapy. Using vectors, one could be able to transfer to cells the gene for a particular growth factor. Those cells would then be able to produce the osteoinductive growth factor in the local environment. Gene therapy is amenable to osteoinductive applications using both the indirect ex vivo and direct invivo methods.

Using the ex vivo method and the rat femur critical-sized defect model, Lieberman et al. [1998, 1999] harvested autologous bone marrow cells and transduced the cells utilizing an adenoviral vector carrying the BMP-2 gene. The cells were combined with guanidine extracted-demineralized bone matrix. The mixture was then used to fill the 8-mm femoral defect. Because the guanidine extracted-demineralized bone matrix has minimal osteoinductive properties, when placed alone in the defect, it acted as a negative control. Other negative controls included marrow cells alone and marrow cells transduced with an adenoviral vector containing the lacZ marker gene [Oakes and Lieberman, 2000]. The positive controls were defects treated with recombinant human BMP-2. By radiographic criteria both the experimental group and positive control resulted in healing in a significant number of subjects, but the histological analysis of the healing showed a thicker rim of neocortex and coarser trabeculae of the cancellous bone in the samples healed using the transduced cells [Lieberman et al., 1998, 1999].

The ex vivo method was also evaluated through use of BMP-7 gene transduction of rabbit periosteal cells using retroviral vectors [Breitbart et al., 1999]. The BMP-7 transduced

periosteal cells demonstrated significant production of BMP-7. When these cells were loaded into polyglycolic acid scaffolds and used to repair critical-size rabbit calvarial defects, there was statistically higher bonerepair demonstrated by histology and radiography at 12-week time compared to control transduced cells. Although not in a long bone model, this research substantiates the conclusion that the ex vivo method of gene-enhanced tissue engineering augments the the bone healing mechanism. [Breitbart et al., 1999].

The direct in vivo method also showed promise in providing the osteoinductive factors necessary. Using a rabbit model, adenoviral vectors containing either BMP-2 or transforming growth factor-β (TGF-β) were diluted with saline and injected into the plate-fixed 1.3-cm femoral defect [Baltzer, 1999; Baltzer et al., 2000]. After the given time point, almost all of the experimental subjects showed healing, while none of the control defects healed. This reiterates the osteoinductive properties of BMP-2 and TGF-β, and also shows that direct in vivo method of gene therapy using adenoviral vectors is a feasible treatment option. This experiment is of particular interest because new bone was able to be generated within the large segmental defects in the absence of exogenous added osteoconductive matrix.

The in vivo method was also evaluated using non-viral vectors, Fang et al. [1996] employing a gene activated matrix (GAM), a collagen sponge loaded with naked DNA. After creation of the critical-size defect in the rat femur, the gene-activated matrix was inserted containing a marker gene (e.g., β-gal, firefly Luciferase) or a osteoinductive factor gene (e.g., BMP-4, PTH_{1-34}). Controlled by empty collagen sponges and collagen sponges containing only marker genes, it was shown that wound repair fibroblasts were capable of DNA uptake and expression of the recombinant gene [Fang et al., 1996; Oakes and Lieberman, 2000]. Results were highly significant using PTH_{1-34} as both sets of controls showed no bone formation or bridging of the gap defect while the PTH samples did.

Non-viral vectors were also employed using collagen sponges in different models of bone repair in canine tibias and femurs utililizing similar collagen sponges [Bonadio et al., 1998, 1999]. The three models were designed to investigate expression, retention, and dose response. The expression model showed that 30–50% of the fibroblasts in the area treated were transduced, the retention model showed the presence of DNA plasmids as long as 6 weeks, and the dose response model showed the direct relationship between the number of plasmids present in the gene activated matrix and the completeness of bone healing [Bonadio et al., 1998; Oakes and Lieberman, 2000].

SPINAL FUSION

Lumbar interbody fusions have been touted as a means of treating various conditions including, but not limited to, disc disruptions, instability, tumors, and failed posterolateral fusions [Sandhu et al., 1996; Goldstein, 2000; Patil et al., 2000]. Posterolateral fusion is the most common type of spine fusion performed in the US. However, 40% of patients with single level fusions fail to achieve solid union (nonunion); the failure is even higher with multiple level fusions [Farey et al., 1989; Steinmann and Herkowitz, 1992; Boden et al., 2000a]. This nonunion is frequently the cause of unsatisfactory resolution of clinical symptoms [Ferlic et al., 1975; Conaty and Mongan, 1981; Zoma et al., 1987]. Gene therapy has been studied as a means to augment the process of bone formation in spinal fusions.

BMPs as purified extract and as recombinant proteins have been used with some success in small animal models of spinal fusion [Johnson et al., 1988, 1992; Riew et al., 1998; Boden et al., 2000b]. However, the results have not yet been translated to human clinical trials secondary to the underestimation of the corresponding dosage and to the difficulty in identifying an appropriate carrier for the longer healing time of higher animals [Boden et al., 1995, 1996, 1998a, 2000b; Khan et al., 2000]. It has been suggested that gene therapy may be used as an alternative to administering a bolus of BMP [Boden et al., 2000a; Khan et al., 2000]. The cost of extracted or recombinant proteins is substantially more than the process of DNA replication, and gene therapy may provide the means to a more prolonged delivery [Boden et al., 2000a].

Direct in vivo gene therapy was investigated by Alden et al. [1999] using adenoviral vector constructs injected into the paravertebral musculature of athymic rats. The injections consisted of either adenoviral vectors carrying the BMP-2 gene or adenoviral vectors containing

the marker gene β-galactosidase [Alden et al., 1999]. Each rat received two injections, one on each side at the junction of the spinous process and lamina. The rats were divided into three groups, receiving two injections of Ad-BMP-2, two injections of Ad-β-gal, or one of each [Alden et al., 1999]. The rats were studied radiologically employing computed tomography at 3, 5, 8, and 12 weeks after injections when they were studied histologically. Computed tomography scans showed interval formation of bone at each site injected with Ad-BMP-2 and no bone formation at the control Ad-β-gal sites. These results were confirmed by the histological examination with extensive endochondral ossification within the paraspinal muscles at the Ad-BMP-2 sites [Alden et al., 1999]. Additionally, at the Ad-BMP-2 sites, there was well-developed vasculature, areas of cartilage, and the elements of cancellous bone (i.e., bone marrow elements and bony trabeculae) [Alden et al., 1999]. There was no evidence of distant bone formation or neural compromise suggesting that this direct in vivo model may be a safe approach to spinal fusion [Alden et al., 1999; Boden et al., 2000a].

An attempt using an indirect ex vivo method was performed by Wang et al. [1999, 2000]. Bone marrow cells were harvested from syngeneic rats and expanded. The cells were then transduced with an adenoviral vector containing BMP-2 and loaded onto a guanidine-extracted, demineralized bone matrix carrier. This was then implanted between the transverse processes of the lumbar spine. The results of this study at 4 weeks showed that bone marrow cells transfected with adenovirus-BMP-2 constructs can be implanted to produce complete fusion as well as recombinant human BMP-2 [Wang et al., 1999, 2000b].

Boden et al. [1998a, 2000a] studied the effects of liposomal-mediated transfer of a novel osteoinductive protein gene, the LIM mineralization protein-1. Expression of this gene is suspected to cause the secretion of a soluble factor that affects neighboring cells [Boden et al., 2000a; Chen, 2001]. LIM mineralization protein-1 cDNA was inserted in both the forward and reverse orientation into plasmids that were used to transfect bone marrow fibroblasts isolated from rat hindlimbs. The reverse orientation cDNA strand acted as a control, since the normal initiation code would be absent [Boden et al., 1998a]. The implants consisted of the fibroblasts transfected with the forward and reverse orientation LIM mineralization protein-1 cDNA loaded onto guanidine-extracted, demineralized bone matrix [Boden et al., 1998a].

In the pilot study, athymic rats received implants placed in the chest subcutaneously, active on the right and control on the left, and in the spine, active in the lumbar spine and control in the thoracic spine. The active subcutaneous implants showed complete bone formation with marrow and osteoblast lined spicules, while the control implants showed no bone formation. The spinal implants had similar results with the thoracic spine showing no bone formation and the lumbar spine showing complete fusion [Boden et al., 1998a]. In the pivotal study, the athymic rats received the active or control implants in the thoracic spine and the other in the lumbar region. All of the sites treated with marrow cells transfected with the active LIM mineralization protein-1 cDNA displayed fusion, while no evidence of fusion was observed at the sites treated with inactive LIM mineralization protein-1 cDNA [Boden et al., 1998a].

LIGAMENTS

Ligaments are dense bands of connective tissue that help maintain normal joint motion and joint stability. Injuries to these structures are particularly disruptive secondary to the fact that injuries to ligaments heal poorly. The amount and rate of healing also varies between ligaments. For example, the medial collateral ligament may heal reliably on its own without surgical repair, while the cruciate ligaments show little healing after isolated disruption [O'Donoghue et al., 1971; Hawkins et al., 1986; Weiss et al., 1991; Woo et al., 1997]. Novel ex vivo techniques in gene therapy and tissue engineering have provided promising clinical possibilities for the future. Current prospects include the application of growth factors, gene transfer techniques, and cell therapy.

In terms of protein synthesis rates, TGF-β1 was the only growth factor that significantly increased the production of Type-II collagen in both rabbits and in canine models. According to Hannafin et al. [1997] in their study of chemotactic effect of cytokines, Platelet derived growth factor, Epithelial growth factor, hepatocyte groth factor, BMP-2, and IL-1 each increased fibroblast migration across nucleopore membranes coated with Type I collagen.

Although, some have found that TGF-β1 may significantly affect healing by increasing collagen synthesis and cell proliferation in medial collateral ligament explants, others have observed that individual growth factors have little effects, but in combination growth factors do increase cell migration [Amiel et al., 1995; Lee et al., 1995; Spindler et al., 1996].

In the realm of gene therapy, there have been several studies that suggest gene transfer into ligaments and tendons can be a possible means of therapy in the future. Hildebrand et al. [1999] used in vivo adenoviral and ex vivo retroviral techniques to introduce and express the LacZ marker gene in the medial collateral ligament and anterior cruciate ligament of rabbits in both an injured and uninjured state. In this study, the period of expression was of a longer duration using the in vivo adenoviral technique but seemed to be unaffected by the presence or absence of injury or healing response. In both injured and uninjured states, lac Z gene expression could be detected from 10 days to 3 weeks time [Hildebrand et al., 1999]. In another study, decorin mRNA expression and protein synthesis were decreased when antisense decorin oligodeoxynucleotides were introduced using Haemagglutinating virus of Japan conjugated liposomes in vivo. Additionally, it has been determined that a direct injection into the ligament of HVJ-conjugated liposomes produced a greater number of transfected cells than an intra-arterial injection of liposomes [Nakamura et al., 1998].

Research in cell-based therapy for ligament healing is still in the early stages, however, mesenchymal stem cells are considered the most promising [Woo et al., 1999]. Mesenchymal stem cells can differentiate into the major cell types responsible for production of the substances required of wound healing, such as collagens, proteoglycans, cytokines, and tissue enzymes [Woo et al., 1999]. A study by Young et al. [1998] has shown that implanted mesenchymal stem cells have significantly improved the structural properties of injured tendons. One may be able to extrapolate these results as a basis for mesenchymal stem cell use in ligament repair [Woo et al., 1999].

INTERVERTEBRAL DISC DISEASE

Intervertebral disc disease is a consequence of the aging process in humans with degeneration starting as early as the first decade of life in males and the second decade in females [Frymoyer, 1993]. Disc degeneration is suggested as an important precursor to nucleus pulposus herniation, spinal stenosis, and segmental spine instability [Diwan et al., 2000]. Current therapy is directed toward the symptoms of these sequelae, instead of ameliorating the underlying condition. Interest is currently developing in the field of disc replacement and regeneration.

A study on the tails of 112 rats suggested that replacing a nucleus pulposus with fresh or cryopreserved nucleus pulposus after percutaneous nucleotomy for induced herniated nucleus pulposus resulted in a delay in the degeneration of the annulus fibrosus, vertebral end plate, and remaining nucleus pulposus [Nishimura and Mochida, 1998]. Researchers are currently investigating means of replacing the intervertebral disc and/or regenerating the disc. Replacement may involve using prosthetic devices to replace the entire disc [Steffee, 1992; Enker et al., 1993; Vuono-Hawkins et al., 1995; Lemaire et al., 1997] or just the nucleus pulposus [Ray, 1992; Schoenmayr et al., 1997; Eysel et al., 1999; Yuan, 1999]. Another possibility of replacement includes complete allograft intervertebral units which include disc and adjacent vertebrae.

The other possibility is to attempt regeneration or repair of the intervertebral disc using techniques of tissue engineering and gene therapy. In order to investigate the possibility of creating nucleus pulposus tissue, nucleus cells were extracted and cultured from young calves. These cultured cells were seeded onto three separated polymer scaffolds (polyglycolic acid, calcium alginate, and pluronic F127), and were then implanted along with empty controls subcutaneously in nude mice [Kusior et al., 1999]. When harvested and examined, it was noted that there was the greatest growth of nucleus pulposus cells and least fibrocytic ingrowth into the sample seeded onto the polyglycolic acid, which suggests that Polyglycolic Acid might be applicable as a medium for nucleus pulposus repair [Kusior et al., 1999]. Tissue cultures established for annular, nuclear, and transition zones were also exposed to various growth factors, including TGF-β1, EGF, Fibroblast growth factor, Insulin like growth factor-1, and BMP-7 [Thompson et al., 1991; Masuda et al., 1999; Nishida et al., 1999;

Takegami et al., 1999]. Results of these studies showed much greater incorporation rates compared to baseline of the implants, with the nuclear and transitional zones responding more than the annular region. Additionally in one study, it was noticed that TGF-β and EGF induced a better response than FGF [Ray, 1992].

In choosing a method for gene transfer, one must consider the unique characteristic of the nucleus pulposus. Because of its relatively avascular and encapsulated environment, there is a weak immune presence in the nucleus pulposus, and immunogenicity of the viral vector may not be of great concern [Nishida et al., 2000]. Also, transduction of non-dividing cells is a requirement due to the highly differentiated, quiescent quality of the cells. Therefore, it has been suggested that adenovirus-mediated transfer of exogenous genes is the most appropriate approach, taking into account the characteristics of the vectors as well as considering the nature of the target tissues [Nishida et al., 2000].

Credence is given to this theory by an investigation using an adenoviral construct containing the human TGF-β1 gene [Nishida et al., 1999]. The constructs, constructs containing luciferase marker gene, and saline were injected directly into the nucleus pulposus of three groups of rabbits. There was a fivefold increase in TGF-β1 in the rabbits injected with the TGF-β1 gene with respect to each of the control, which included intact discs. Additionally, there was a doubling of the production of newly synthesized proteoglycans, suggesting that adenoviruses are suitable vectors for this purpose [Nishida et al., 1999, 2000].

ARTICULAR CARTILAGE

Articular cartilage repair remains one of the most highly researched topics in orthopedic surgery and has drawn many researchers to use different modalities in order to augment this poor healing mechanism. Articular cartilage lesions are a very common problem in the field of orthopedics; they may occur acutely from trauma or may have a gradual onset from degenerative osteoarthritis or osteochondritis dissecans [Hunziker, 1999]. However, there has yet to be a completely satisfactory solution to these problems due to the poor intrinsic ability of chondral and osteochondral injuries for self-repair. Cartilage lesions often result in suboptimal healing with fibrocartilage.

It has been well determined that lesions that do not penetrate into the subchondral bone will fail to heal spontaneously [Meachim, 1963; Mankin, 1974; Kim et al., 1991; Hunziker and Rosenberg, 1996], but if the integrity of the subchondral bone is compromised then a spontaneous, partial repair reaction will occur due to the cell infiltration from the bone marrow and vasculature contained in the subchondral bone [DePalma et al., 1966; Harada et al., 1988; Masuda et al., 1999]. This partial repair is consistent in neither quantity nor quality and is mechanically incompetent. Current therapy for partial thickness or full thickness lesions includes no medications for anything other than palliation. If refractory to this medical management the next step in treatment is a surgical procedure.

The goal of the researcher is to provide the surgeon a means to deliver a high density of cells to the injury site to bring about the repair [Brittberg, 1999]. High cell density provides signals for primitive mesenchymal cells at the injury site to undergo a series of cellular changes leading to differentiation ultimately to the original hyaline cartilage phenotype. All types of mesenchymal cells have this common repair lineage [Caplan, 1991; Caplan et al., 1993]. Additionally, this repair mechanism is dependent on the local supply of chondrogenic cells. All types of chondral defect repair involve the prescence of chondrogenic cells at the site. The two possible cell types, chondroprogenitor cells (mesenchymal stem cells) or committed cells (chondrocytes), provide the basis for the main strategies for cell-based tissue engineering [Brittberg et al., 1994; O'Driscoll, 1999].

It has been suggested that one major drawback in the natural healing of articular cartilage defects is the lack of reparative response from the surrounding chondrocytes, unless the defect extends into the subchondral bone [Johnstone and Yoo, 1999]. This may be because of the migration of marrow progenitor cells into the defect. It has been recognized that cartilage precursor cells in postnatal humans can differentiate into chondrocytes under the influence of specific conditions, but Owen and Friedenstein [1988] were first to propose that lying resident in the bone marrow, are stem cells for all mesenchymal tissues. Mesenchymal stem cells actually can refer to periosteal,

perichondrial, perivascular, muscle, and bone marrow derived cells. These cells are regarded as more adaptable than mature chondrocytes as they may be encouraged into the bone or cartilage lineage by local environmental factors [Grande and Nixon, 2000]. Caplan et al. [1993] described the mesengenic process, whereby mesenchymal stem cells can enter the bone, cartilage, tendon, muscle, ligament, adipocytic, or hematopoietic support cell lineages.

Mesenchymal stem cells from bone marrow have been well investigated because of their known chondrogenic potential and their ease of acquisition [Owen and Friedenstein, 1988]. It has been postulated that bone marrow-derived stem cells may offer better repair of articular surface damage due to the cells ability to repair the articular cartilage as well as the subchondral bone. A study was performed using mesenchymal stem cells, harvested from the periosteum and bone marrow. The expanded cells were imbedded in collagen before use to fill full-thickness defects created in rabbit knees [Wakitani et al., 1994]. Compared with the empty defects and cell free collagen controls, the experimental defects showed improved healing whether the cells were derived from periosteum or bone marrow.

Another method of articular cartilage regeneration is to use whole periosteal explants. These explants can be grown in vitro from periosteal derived stem cells. With whole tissue, maintenance of the transplanted cells in the area of the lesion is less concerning, since retention of the graft can be anchored to the subchondral bone through suture tunnels that extend under adjacent joint surfaces [O'Driscoll et al., 1988a,b; Brittberg et al., 1994]. Another benefit of whole tissue grafts is the cells can be kept in their natural environment with the appropriate extracellular matrix. In this manner, the cells exhibit the behavior that they do in vivo, compared with cells that have been isolated and explanted to culture dishes, which may be altered by the process. Osteochondral defects in rabbit knees that were treated using this technique healed with predominantly hyaline cartilage with about 90% Type II collagen and the normal matrix constituents [Benya and Schaffer, 1982; O'Driscoll et al., 1988a].

Mature chondrocytes have advantages of their own. Because mature chondrocytes are terminally differentiated, they are able to synthesize molecules such as Type II collagen and aggrecan that are required for the native extracellular matrix molecules. Additionally, they can be harvested as pure populations and infected efficiently with viral vectors. Although, the isolated chondrocytes seem to dedifferentiate while being cultured in a monolayer system, the cells reclaim the chondrocytic phenotype when the cells become surrounded by their own newly formed matrix in a three-dimensional environment [Benya and Schaffer, 1982]. Mature chondrocytes also have some disadvantages. Three-dimensional chondrocyte grafts may be resistant to vascularization or mineralization. Vascular proximity and oxygen tension are strong factors in inducing osteogenesis, and fibrosis may drive differential formation of the osteochondral histology during full thickness repair [Wakitani et al., 1994].

Peterson et al. [1984] had used a rabbit patellar model [Grande et al., 1989; Brittberg et al., 1996], filled a premade patellar cartilage defect with mature chondrocytes cultured from the rabbit itself and was then covered by a flap of periosteum containing the bioactive chamber). This was shown to be significantly better than controls with only periosteal covering [Brittberg et al., 1996]. Autologous chondrocyte transplantation uses cells that are harvested autologously or as allografts from an uninjured area of the donor tissue. Isolated cells are expanded, and reimplanted into a defect at a much higher cell concentration. This represents one of the only cell-based therapies in clinical use today for cartilage repair.

There has been development and testing of a new modular apparatus for implantation of chondrocytes into an articular cartilage surface [Grande et al., 1999]. The device is composed of two parts, a polyglycolic acid head on which cells can be loaded and a polylactic acid stem used to anchor the device. The head of the device has a hexagonal shape to allow for multiple applications in order to resurface a larger defect area. One of the pitfalls of current cartilage repair, inadequate fixation resulting in slippage and leakage of material into the joint space, is overcome by the solid frame that includes a barbed anchor stem that attaches to the subchondral bone. This device is designed to be able to be inserted through an arthroscope [Grande et al., 1999].

Recently, gene therapy has been evaluated as a possible modality for permanent restoration of injured articular cartilage. Various

morphogens, transcription factors, and growth factors have shown promise in animal models for repair and regeneration of cartilage [Mankin and Buckwalter, 1996]. Gene therapy has been adapted to deliver genes to sites of regeneration where therapeutic factors can be locally expressed. To accomplish this, genes can be piggy-backed on a tissue engineering platform. Recently, a study was performed that represents this use of tissue engineering and gene therapy in cartilage repair [Mason et al., 2000]. In this study, 3-mm osteochondral defect were created in the patellofemoral grooves as described in previous studies [Grande et al., 1995; Mason et al., 1998]. Within these defects were placed one of three samples: nothing (i.e., no polyglycolic acid fibers and no cells); polyglycolic acid fibers loaded with periosteal derived chondroprogenitor cells transduced with only the neomycin resistance gene (G418) from the retroviral vector LNCX; and polyglycolic acid fibers loaded with periosteal derived chondroprogenitor cells transduced with a BMP-7 retroviral vector and selected by the G418 neomycin resistance gene [Miller and Rosman 1989; Mason et al., 1998; Breitbart et al., 1999].

The defects filled with BMP-7 transduced cells were superior to either of the other two categories of samples. Also, histological scores modified from O'Driscoll et al. [1985] for these samples approached the maximum of 24. In addition to demonstrating that BMP-7 gene enhanced periosteal-derived mesenchymal stem cells when placed on polyglycolic acid fibers could regenerate full thickness osteochondral defects in a matter of 8 weeks, one can also conclude from the study that optimal bone and cartilage regeneration occurs when cell-based tissue engineering is combined with genetic enhancement of cells with appropriate tissue regenerative genes such as BMP-7 [Mason 2000]. Another possible method for delivery of the gene to the proper site is to perform direct in vivo transfer. One example of this method used adenoviral vectors containing either green fluorescence protein gene sequence, lacZ sequence, or IGF-I cDNA. Part of this series of experiments consisted of transduction of synovial explants. Direct injection into the synovium of AD-IGF-I vector resulted in IGF-I secretion by transduced cells in the synovium [Nixon et al., 2000]. A second example of this was performed by Frisbie and McIlwraith [2000]. Using an equine model, adenovirus vectors were transduced with equine interleukin-1 receptor antagonist. These constructs were then introduced into the intercarpal joints of the horses. Synovial fluid levels of interleukin-1 receptor antagonists were significantly elevated, demonstrating that the adenoviral vectors transduced the synoviocytes and expressed the interleukin-1 receptor antagonist transgene. Significant changes in synovial fluid parameters were observed [Kang et al., 2000].

This gene was also used in the clinical trials using retroviral vectors harboring the interleukin-1 receptor antagonist to transduce harvested and expanded synovial tissue. The transduced cells were then injected into the metacarpophalangeal joints of female patients with rheumatoid arthritis [Kang et al., 2000].

Some success has been reported using liposomes for delivery of genes to chondrocytes, but direct in vivo transfer into chondrocytes remains equivocal [Tomita et al., 1997]. Ex vivo techniques may be required to transfect chondrocytes in adult articular cartilage due to the dense cartilaginous matrix. The matrix density and high negative charge results in a unique diffusion constraint, that perhaps restricts the access of vectors to the cells by in vivo delivery.

CONCLUSION

Completion of the Human Genome Project has encouraged researchers to employ this new bank of knowledge, while launching gene therapy into the forefront of medicine. Gene therapy has expanded beyond its original purpose of removing the deleterious effects of genetic derangements. It has forged its own place in almost every sphere of orthopedic research. In the near future, it may be possible for orthopedic surgeons to employ the principles of gene therapy in their daily practice. Research on fracture healing and bone formation will have impact in orthopedic trauma, spinal fusions, oncologic defects, and total joint replacements. The field of sports medicine may have new means to deal with anterior cruciate ligament reconstruction and Achilles tendon rupture. And as the average age of Americans continues to rise, better treatments for degenerative conditions such as osteoarthritis and intervertebral disc disease may be available.

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