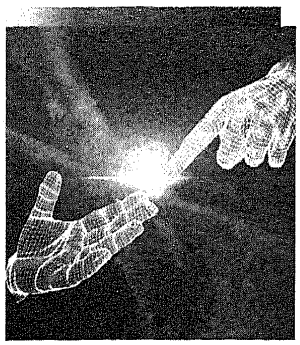

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Scaffold-Based Articular Cartilage Repair

Future Prospects Wedding Gene Therapy and Tissue Engineering

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Recent studies have demonstrated the potential of three-dimensional scaffolds for enhancing articular cartilage repair, based on the findings from in vitro and in vivo investigations. In this article, current clinical procedures for articular cartilage repair are reviewed in the context of the contributions that tissue engineering approaches can make in improving the outcome. Specific attention is directed toward the promising effects of growth factors and the potential advantages of employing gene therapy techniques in combination with three-dimensional (3-D) scaffolds. New data are presented to demonstrate the utility of nonviral transfection methods for the transfer of genes to cells in order to induce the synthesis of desired growth factors. The combination of genes and tissue engineering scaffolds for a sustained, localized production of growth factors at cartilage defects is a promising advance that could further improve the outcome of cartilage repair procedures in the future.

Overview

The clinical problem being addressed in this article is the loss or degeneration of articular cartilage as a result of trauma, disease, or chronic mechanical loading imposed on joints with aging. Breakdown of articular cartilage leads to pain, instability, and swelling. Depending on its location and size, left untreated, a focal defect in articular cartilage can extend itself until it leads to more extensive degeneration resulting in profound disability. The current methods for treating such focal defects include microfracture, autologous chondrocyte implantation, and osteochondral autografting. These procedures, however, have limited long-term follow-up. Current clinical experience questions the durability of the repair. Preclinical studies implementing tissue engineering approaches have yielded results that represent improvements over the currently employed cartilage repair procedures.

The term "tissue engineering" is currently used to describe a wide array of procedures employing cells, biomaterial scaffolds, and diffusible molecular regulators (e.g., growth factors) alone or in combination for the production of tissue in vitro or as implants to facilitate the regeneration of tissue in vivo. However, as originally proposed, a unique aspect of tissue engineering was the employment of porous, absorbable scaffolds as delivery vehicles for cells grown in culture. In fact, two of the enabling technologies underpinning tissue engineering are: 1) the methods for producing such scaffolds, and 2) the procedures for expanding the number of cells in culture in such a way as to preserve their phenotype, or allow it to be recovered when transferred to other environments in vitro or in vivo. One of the challenges facing tissue engineering approaches applied to selected tissues and organs relates to the relatively low number and mitotic and biosynthetic activity of the parenchymal cells. This may require the prolonged delivery of selected growth factors, a solution for which may lie in gene transfer.

Current Clinical Status of Articular Cartilage Repair Procedures

Traumatic injury to articular cartilage is a common orthopedic problem. Osteochondral defects that penetrate the underlying subchondral bone normally heal by partial or complete filling with tissue that contains fibrocartilaginous elements. This fibrocartilaginous tissue does not provide mechanical properties like those of articular cartilage, which is a form of hyaline cartilage required to support the applied loading and tribology. Chondral defects, which do not penetrate the bone, often show little filling with any type of reparative tissue. The lack of regenerative power of chondral defects may be attributed in part to the avascularity of the surrounding cartilage, and to the presence of relatively few cells of low mitotic activity. Left untreated, these defects may progress to osteoarthritis and eventually necessitate total joint replacement. Because articular cartilage shows little capacity for regeneration, the search for methods for resurfacing the joint is compelling.

The current methods for treating defects in the articular surface in the clinic include drilling, abrasion arthroplasty, microfracture, osteochondral autografts and allografts, and injection of cultured autologous chondrocytes under a periosteal cover. While promising results have been reported with all of these procedures, each has its shortcomings when implemented in its present form.

Methods focused on resurfacing damaged articular cartilage seek either to regenerate the damaged tissue or to replace it with autograft or allograft cartilage or a different tissue that will relieve symptoms and support joint function, at least temporarily. One set of surgical procedures deliberately penetrates the subchondral bone to allow blood and marrow cells to fill the defect in order to form a reparative tissue. In drilling,

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abrasion arthroplasty, and microfracture, only the extent and nature of the penetration vary. Because penetration of the subchondral bone has been found to increase the incidence of pain and may introduce changes in the bone, the consensus is to use a less-invasive procedure. Currently, that treatment is microfracture [1], which uses sharp picks or awls to puncture the surface, while leaving most of the bone intact. In microfracture, the defect is filled through punctate bleeding and infiltration of marrow from the small pick holes. The result of all of these procedures is generally a fibrocartilaginous repair tissue that often provides at least temporary symptomatic relief to the patient. However, the long-term success remains in question. The role of a scaffold in microfracture is addressed in a later section.

Osteochondral autograft procedures [2] performed arthroscopically or by arthrotomy raise questions about donor-site morbidity. Short-term follow-up has not revealed symptoms that can be related to the harvest site. However, recent studies have shown that the cartilage at locations used as harvest sites is load bearing. Other studies have indicated that cartilage distant from the sites of harvest may undergo changes in mechanical properties that predispose it to degradation.

One recent technique employs cultured autologous chondrocytes (CACs) held in place by an autologous periosteum cover [3]. In this technique, chondrocytes are obtained from the patient via a cartilage biopsy, isolated and proliferated in culture, and then returned to the patient. This procedure has generated a great deal of interest and controversy since publication in 1994. Disadvantages of this procedure relative to the microfracture method are the potential for donor site morbidity and cost. In our canine investigations of the efficacy of this procedure we found no significant effect of the treatment at 1.5, 12, and 18 months [4]. There was, however, more hyaline cartilage in three-month CAC-treated defects than in untreated control defects [5]. There was an indication that this tissue underwent degeneration after six months. In contrast to previous work in the rabbit [6], no significant effects of CAC versus the periosteum-alone control were found in our canine model. This may be explained by the species difference, relative age of the animals, unknown retention of the cells in the defect, and location of the defect (patella versus trochlea). The finding of degenerative changes in the reparative tissue in the canine model after one year suggests that improvements may be necessary to ensure long-term success for this procedure. Recent studies, reviewed in a later section, have demonstrated the benefits of implantation of a CAC-seeded scaffold.

Scaffold-Based Tissue Engineering Strategies

The use of 3-D scaffolds that mimic the natural in vivo environment (viz., extracellular matrix) of cells has been shown to facilitate the reparative process and result in the successful growth of various functioning tissues. The 3-D environment supplied by these porous matrices (see, for example, Figure 1) serves as a desirable structural support for seeded or migrating cells and allows for a much greater surface to volume ratio for increased cell attachment as compared to a 2-D surface. There are several requirements for a scaffold to be used as an implant for articular cartilage regeneration [7]. The scaffold needs to be biodegradable, nontoxic, able to be fixed to the defect site, facilitate cell attachment, regulate cell expression, and possess sufficient mechanical strength [8].

Various synthetic and natural materials have been employed for the fabrication of porous absorbable scaffolds for articular cartilage tissue engineering. Among the list of absorbable or partially absorbable materials used for cartilage repair are: collagen sponge-like matrices [9]-[12] and gels [13], hyaluronan [14], fibrin [15, [16], polylactic acid (PLA) and polyglycolic acid (PGA) [17]-[19], chitosan [20], devitalized cartilage [21], hydroxyapatite [22], [23], demineralized bone matrix [24], [25], and bioactive glass [8], [26]. There are advantages and disadvantages that come with using either natural or synthetic polymers as materials for these scaffolds. For example, some advantages of using synthetic polymers (such as poly-DL-lactic-co-glycolic acid, PLGA) include easy molding into specific shapes; accurate control of mechanical, dissolution and degradation properties; and reproducibility. However, most synthetic polymers possess a surface chemistry that does not promote cell adhesion (which plays an important role in signal transduction pathways). Further manipulation of these synthetic polymer matrices such as by coating or tethering adhesion peptides or proteins onto the surface is usually needed for sufficient cell attachment and enhanced tissue regeneration. In addition, these synthetic scaffolds (like PLGA) can produce high local concentrations of acidic by-products during degradation that can induce an adverse inflammatory response,change the rate of degradation of the implant, or create a local environment in the scaffold that may not favor the biological activity of cells being cultured for tissue engineering purposes [27].

Natural polymers such as collagen provide a more native surface to cells, since it is a major component of the natural extracellular matrix and possesses ligands that favor cellular attachment. Collagen substrates have also been shown to modify the morphology, migration, and in some cases differentiation of cells [28]. Moreover, prior studies have demonstrated that type I collagen-glycosaminoglycan (GAG) scaffolds produced by freeze-drying techniques (Figure 1) can facilitate the regeneration of dermis and peripheral nerve [29]-[31]. Other work has demonstrated the promise of type II collagen-GAG scaffolds for articular cartilage repair

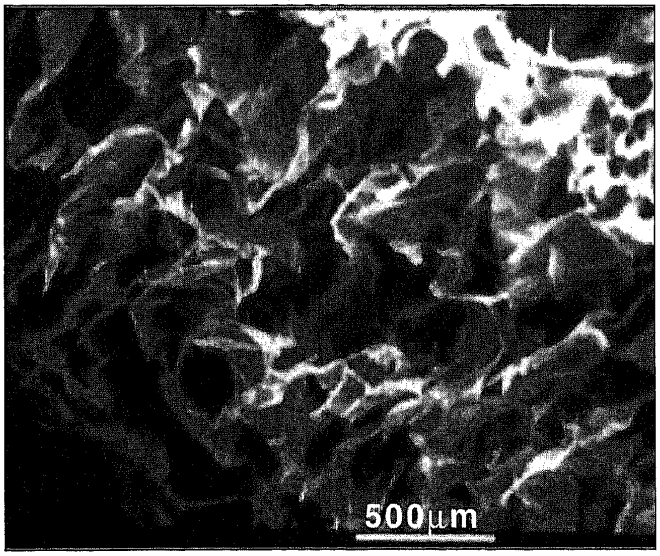

Fig.1. Scanning electron micrograph of a type I collagen-glycosaminoglycan porous absorbable scaffold employed for tissue engineering. The material is produced by freeze-drying a blended slurry of type I collagen and chondroitin sulfate as previously described in the literature (30).

While promising results have been reported with all of the current clinical procedures, each has its shortcomings when implemented in its present form.

[32]-[34]. One problem associated with natural polymer scaffolds, however, is reproducibility. Numerous investigations are still ongoing to improve the properties of scaffolds for articular cartilage tissue engineering.

Studies have confirmed that the addition of cells seeded within these 3-D scaffolds enhance matrix synthesis and increase type II collagen production in vivo [17], [18], [34]. There are various cell types that may be used to enhance cartilage synthesis when seeded into matrices. These include articular chondrocytes and chondroprogenitor cells derived from marrow, periosteum, or perichondrium [8]. The advantage of using chondrocytes obtained from articular cartilage as a cell source is that they already express the desired phenotype for articular cartilage repair and they have been shown to be able to synthesize matrix containing type II collagen and aggrecan [35]. Chondrocytes, however, are very limited in supply, especially for autologous transplantation, and expansion in culture is necessary to obtain a number sufficient for enhanced repair. Furthermore, the site from which autologous chondrocytes are harvested (usually taken from a minimal-load-bearing region of the joint) does not spontaneously regenerate and may pose as a potential problem. To alleviate this problem of donor-site morbidity, other cell sources such as bone marrow (for marrow-derived mesenchymal stem cells), which have natural regenerative capabilities and are readily accessible, have been investigated for articular cartilage repair [35], [36]. The chondroprogenitor cells from marrow have been shown to be able to differentiate into a cartilage lineage when exposed to the appropriate stimuli [8] and therefore may be a more feasible cell source for articular cartilage regeneration.

Contributions of Tissue Engineering Scaffolds to Cartilage Repair Procedures

As noted above there are several advantages afforded by the implementation of biomaterial scaffolds for tissue engineering. One example of a benefit derived through the use of a scaffold for selected cartilage repair procedures is the increase in the amount of reparative tissue in cases treated by microfracture or implanted with CACs. One recent study performed in adult dogs [33] investigated the implantation of a type II collagen-GAG scaffold in chondral defects [i.e., down to the tidemark (the calcified cartilage)] treated by microfracture. There was significantly more reparative tissue filling microfracture-treated defects implanted with the collagen scaffold compared to the microfracture-treated sites that did not receive implantation of the scaffolds [33]. A likely explanation for the findings was that the scaffold served to stabilize the blood clot that formed and provided a framework for the migration of cells from the marrow cavity (including mesenchymal stem cells), given access to the defect site through the microfracture holes. An important finding was that the majority of the reparative tissue in all of the microfracture-treated defects comprised fibrocartilage, as has been reported in biopsies of human cases [1]. Fibrocartilage, the tissue composing menisci, differs from hyaline cartilage (of which articular cartilage is a type) by collagen type (I for fibrocartilage and II for hyaline cartilage) and amount of aggregating proteoglycan (e.g., aggrecan). It is not yet clear as to how these differences affect joint tribology.

The finding of fibrocartilage in microfracture-treated defects contrasts with the reports of hyaline cartilage (and hyaline cartilage of the specific architecture of articular cartilage) in untreated chondral defects and chondral defects treated with the cell-based approach of implantation of CACs (see, for example, Figures 2(a) and 3). In the case of a cell therapy, a scaffold could serve as a delivery vehicle for the cells allowing for a greater number of adherent cells to be retained in the defect by virtue of the large surface area of the sponge-like matrix. Recent studies compared the reparative tissue in chondral defects in adult dogs implanted with CACs alone [5] and CAC-seeded type II collagen-GAG scaffolds cultured for 24 hours [33] and four weeks [34] prior to implantation. The cell-seeded scaffolds yielded a greater amount of reparative tissue than the sites implanted with the CACs alone (Figure 3). The cell-seeded scaffolds cultured for 24 hours induced more reparative tissue formation than the injection of cells alone, but this tissue was made up of fibrocartilage and fibrous tissue with virtually no hyaline cartilage. The question remains as to the relative importance of the amount versus make-up of the reparative tissue with respect to providing symptomatic relief for individuals with focal cartilage defects. Related to this point is the fact that the hyaline cartilage found at sites treated by CACs alone and in the collagen scaffolds did not display the architecture of articular cartilage. Of note was that the greatest amount of reparative tissue was induced by the CAC-seeded scaffold cultured for four weeks prior to implantation and that this group also demonstrated the same amount of hyaline and articular cartilage (Figure 3) as found in defects implanted with the cells alone [34]. While these studies demonstrate the promise of implementing tissue engineering scaffolds for cartilage repair, there are potential problems and significant expense associated with the need to culture a cell-seeded scaffold for four weeks prior to implantation. This focuses attention on the implementation of growth factors to accelerate cell proliferation and matrix synthesis.

Role of Growth Factors

Numerous studies have shown the effects of various growth factors on chondrogenesis in vivo as well as on chondrocyte proliferation, metabolism, and matrix synthesis in vitro. Among the most prominent growth factors investigated for articular carti-

lage tissue engineering are insulin-like growth factors (IGFs), bone morphogenetic proteins (BMPs), basic fibroblastic growth factor (bFGF or FGF-2) and transforming growth factor-ß (TGF-ß). The effects of growth factors in both monolayer and 3-D culture of chondrocytes have been shown to be significant. The complexity of choosing the correct combinations and doses of growth factors to obtain the optimal tissue engineered articular cartilage construct in vitro poses a major challenge. While there are numerous combinations of factors that can be investigated, relative to the type and dose of growth factor used, studies have already demonstrated the profound benefits of certain agents for engineered articular cartilage constructs. For example, supplementation of culture medium with IGF-1 alone has been shown to increase cell proliferation, proteoglycan synthesis, type-II collagen synthesis, and chondrogenesis, both in monolayer and in 3-D cultures [37]-[39].

In vivo, an improved histologic appearance and an increased proportion of type II collagen in full thickness cartilage defects in horses was shown using fibrin polymers laden with IGF-1 [15]. BMPs (specifically BMP-2 and BMP-7) have also been shown to increase proteoglycan and matrix synthesis, maintain the chondrocyte phenotype, and stimulate cartilage formation in vivo in a manner similar to endochondral ossification [40]. In vivo studies using New Zealand White rabbits have demonstrated that full-thickness femoral osteochondral defects treated with rhBMP-2-supplemented collagen sponges displayed a greatly accelerated formation of new subchondral bone, an improved histologic appearance of overlying articular cartilage, and more type II collagen and tissue filling in the defect as compared with controls [41]. BMP-7 (also called osteogenic protein-1, OP-1) has also been shown to stimulate cartilage formation and aggrecan synthesis in subchondrol defects in goats [42]. FGF-2 has demonstrated to be a potent mitogen for chondrocytes and a stimulator of matrix synthesis [40]. Furthermore, in vivo studies in rabbit models [43] have reported that full-thickness cartilage defects treated with intra-articular FGF-2 had enhanced differentiation of mesenchymal cells to the chondrocyte phenotype, increased proliferation of differentiated chondrocytes, and increased accumulation of type II collagen and proteoglycan.

TGF-ß has shown to be most effective in conjunction with other growth factors in eliciting its complex effects on cartilage metabolism [40]. Many findings, however, have shown contradictory affects of TGF-ß supplementation in culture (e.g., affects of cell proliferation and proteoglycan synthesis) due to its sensitivity to varying experimental conditions in the different studies. The more promising affects of TGF-ß supplementation include increasing collagen and proteoglycan production and inhibiting matrix breakdown [40]. It has also been shown that TGF-ß has a role in the regulatory network of growth factors that maintains articular cartilage in the differentiated phenotype [44] and is an important factor in inducing chondrogenesis in marrow-derived mesenchymal progenitor cells [36].

The effects of using combinations of growth factors have also been studied in monolayer and 3-D cultures and demonstrate the complex interactions and signaling events that can occur. Growth factor-supplemented media used for the expansion of chondrocytes in monolayer have been shown to directly influence the outcome of cell-seeded 3-D cultures grown in a different specified medium [45]. Not only does supplementation of expansion medium with specific growth factors affect chondrocyte proliferation, morphology, and phenotype, it also influences the chondrocytic potential or ability to redifferentiate back into a chondrocytic phenotype when re-introduced into a 3-D environment.

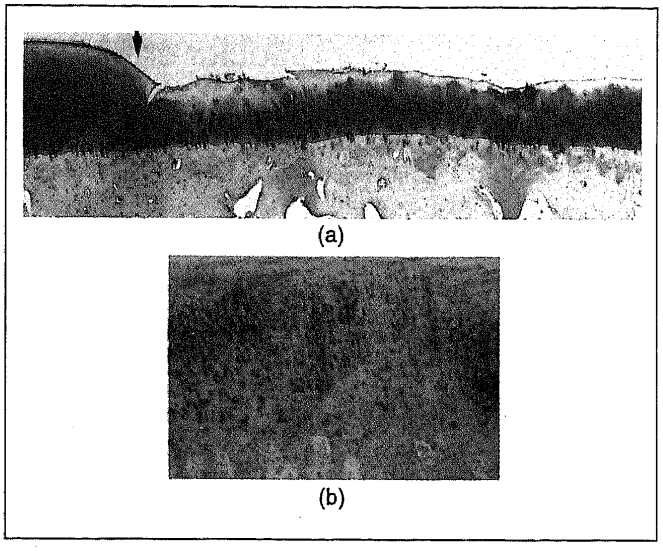

Fig. 2. Micrographs of defects in the articular surface of the adult canine knee joint approximately 15 weeks after being implanted with cultured articular chondrocytes alone (a) and with a chondrocyte-seeded type II collagen-GAG scaffold cultured for four weeks prior to implantation (b). The reparative tissue is comprised of hyaline cartilage with articular cartilage at the base of the defect. (a) Safranin O stain. (b) Immunohistochemical preparation stained with an antibody for type II collagen. (a) 50X. (b) 100X.

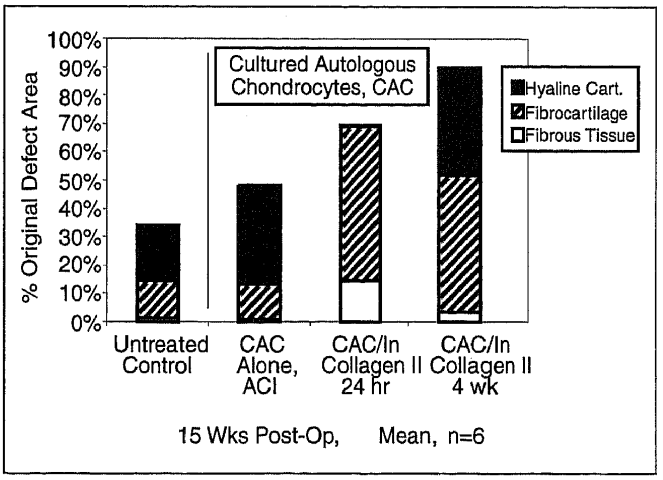

Fig. 3. Graph showing the areal percentage of the original adult canine articular cartilage defect filled with reparative tissue (the height of the column) and the percentages of specific tissue types comprising the reparative tissue (with articular cartilage combined with hyaline cartilage). ACI refers to autologous chondrocyte implantation. The analysis was made through a transverse section through the middle of the defect. In the third column from the left the chondrocytes were grown in the type II collagen-GAG scaffold for about 24 hours prior to implantation while in the fourth column the cells were grown in the scaffold for four weeks prior to implantation. The difference in the amount of reparative tissue between groups was statistically significant based on analysis by ANOVA.

The complexity of choosing the correct combinations and doses of growth factors to obtain the optimal tissue engineered articular cartilage construct in vitro poses a major challenge.

Rationale for Gene Transfer in Tissue Engineering

Growth factors can make significant contributions to cartilage repair procedures and tissue engineering by stimulating cell proliferation, migration, differentiation, and matrix synthesis. There are, however, major challenges faced in the direct application of human recombinant proteins in a clinical setting. Proteins are difficult to administer exogenously—in accurate, sustained, and therapeutically useful amounts—to sites of cartilage injury. Single bolus doses of growth factors alone in vivo have short half-lives as a result of degradation or diffusion from the defect site. Various strategies, including the use of polymers, pumps, and heparin, have been investigated as possible methods by which to achieve constant levels of growth factors at a given injured site; however, success remains limited [46]. Furthermore, although it is now possible to produce large quantities of these recombinant proteins for the purpose of treatment, the expense is still another unattractive feature. A different means of supplying proteins in a localized and sustained manner in vivo is therefore needed. Delivery of a gene that could be expressed within the wound is an attractive alternative to application of the recombinant protein. Gene transfer provides the DNA that encodes for the desired protein, so that infected cells can create higher and more sustained levels of the growth factor over extended periods of time, a likely requirement for effective articular cartilage regeneration. More than one gene can be transferred and independently regulated to supply multiple growth factors to the defect site. Some studies have suggested that endogenously expressed proteins, induced by gene transfer, may have a more positive and more potent effect on matrix synthesis and biological activity than exogenous recombinant proteins [47].

Gene Transfer into Cells for Cartilage Regeneration

Many questions are involved in deciding the best method of gene transfer for articular cartilage repair. Such variables include: a) the cell, or cells, to be targeted (e.g., chondrocytes, mesenchymal stem cells, synovial cells, etc.); b) the protein, or proteins, to be encoded; and c) the delivery vector to be employed (which is also dependent on the size of the DNA encoding the growth factor). The vectors used in gene transfer procedures applied to articular cartilage repair include: viral vectors such as adenoviruses; lipid-mediated reagents such as liposomes; and naked DNA alone.

Several studies have focused on adenoviral vectors for the transduction of cells due to high infection efficiencies and ease of manufacturing. Both in vitro and in vivo studies have proved this method of infection to be beneficial to cartilage regeneration. In monolayer studies, cultured articular chondrocytes infected with an adenoviral vector containing the IGF-1 coding sequence [48] found that at an optimal adenovirus-IGF-1 concentration (100 multiplicities of infection, MOI) gene expression was detected at therapeutic concentrations for at least 28 days. This prolonged expression resulted in an 8-fold increase in matrix products secreted in the medium and an increased resistance to de-differentiation over time under serum-starved conditions. Moreover, the cells maintained a normal chondrocyte molecular phenotype compared to controls. The significant effects of, and contrasting outcomes resulting from, using different genes separately or in combination can be seen in another monolayer study that used rabbit articular chondrocytes to compare the effects of adenoviral delivery of IGF-1, TGF-ß, and BMP-2 in the absence or presence of the inflammatory cytokine, interleukin-1 (IL-1) [47]. It was found that proteoglycan synthesis was significantly stimulated by the BMP-2 (~8-fold) and IGF-1 (2-3 fold) genes separately and the effects were additive upon co-transduction of chondrocyte monolayers. Furthermore, the IGF-1 gene most strongly stimulated collagen and noncollagenous protein synthesis. Although the addition of IL-1 decreased proteoglycan synthesis by 50-60%, IGF-1 and TGF-ß genes restored proteoglycan synthesis to control levels, and the BMP-2 gene further elevated proteoglycan synthesis beyond control levels. It can thus be seen that finding the right genes to use individually or in combination can significantly affect the success of the final regenerated tissue. Other studies have demonstrated the successful transduction of other cell types such as mesenchymal stem cells and synovial cells in monolayer and explant cultures, for the production of therapeutic proteins [49].

Although the use of viral vectors has proved to be very effective in enhancing the biosynthetic activity of cells in vitro, much caution has to be exercised when directly injecting viral particle solutions in vivo, due to the immunogenic nature of viruses and the possibility of the vector spreading into other tissues and organs. This issue has been addressed in an in vivo study comparing the direct injection of adenoviral vectors for IGF-1 or BMP-2 to transplantation of syngeneic fibroblasts infected ex vivo with the same vectors—with respect to virus spread, immune response, and cartilage formation [50]. Inadvertent spread of the adenoviral vector was observed in the liver, lung, and spleen in all mice that had received the vector directly, whereas spread rarely occurred in fibroblast-mediated gene transfer. Furthermore, administering the genes via injecting ex vivo-infected fibroblasts limited cartilage formation to only regions near the injected cells and also avoided the strong immune response that was elicited following direct ap-

plication of the vector. Ex vivo methods of gene transfer to harvested cells (such as chondrocytes, fibroblasts, bone marrow cells, or synovial cells) may therefore be safer than direct injection of viral particles alone.

Nonviral methods of gene transfer are also being developed to avoid the potential problems associated with adenoviral vectors. Nonviral vector systems offer the advantages of low immunogenicity, simplicity of vector design, and relative ease of large-scale production [51]. Although transfection efficiencies for nonviral vectors have been known to be much lower than that of viral vectors, significant advances in the development of more efficient nonviral transfection reagents are emerging. Lipid–mediated gene transfer has been shown to result in the transfection of articular chondrocytes and the maintenance of prolonged gene expression. One recent study implemented the lipid-mediated transfection reagent FuGENE 6 with a hyaluronidase treatment to transfect bovine articular chondrocytes with a plasmid vector containing the cDNA for human IGF-1. Transfection efficiencies were reported to be about 41% with gene expression lasting for over four weeks in vivo [52], [53]. Transplantation of the transfected chondrocytes onto the surface of articular cartilage explants led to the formation of a new tissue layer on the explant surface, which was characterized by the presence of type II collagen and proteoglycan and the absence of type I collagen, consistent with hyaline-like cartilage. Furthermore, the tissue formed by transfected chondrocytes was thicker and contained more cells than the controls. The overexpression of IGF-1 also increased DNA and glycosaminoglycan synthesis by the underlying explant cartilage chondrocytes [53].

Comparison of Viral and Nonviral Methods of Gene Transfer to Chondrocytes

We recently compared viral and nonviral methods of gene transfer in monolayer cultures of adult canine articular chondrocytes and for their effects on gene expression over the course of two weeks. For viral transductions, a recombinant adenovirus coding for the IGF-1 gene was used at 100 viral particles per cell (~$1x10^6$ cells/well in a 24-well plate). Nonviral transfections entailed using a lipid-mediated transfection reagent, GenePorter®, and a plasmid containing the IGF-1 gene at an 8:1 (v/w) ratio of transfection reagent to plasmid DNA. A serum-free medium was used in these cultures and was collected and changed every two to three days after infection. Figure 4 summarizes the amount of protein detected in the media by a sandwich ELISA kit for human IGF-1 protein. We found that there was a difference in the protein production kinetics comparing the two infection methods. The nonviral method of transfection demonstrated a higher production of IGF-1 protein at earlier times that tapered off over time to the control level [Figure 4(a)]. In contrast, the viral transduction method displayed an increase in protein production within five days of infection and then steadily produced IGF-1 protein at a constant level [Figure 4(a)]. Despite the difference in the kinetics of IGF-1 release, of significance is that the accumulated amount of IGF-1 reached a therapeutic level (e.g., 50-100 ng/ml [37]) for both methods of gene transfer [Figure 4(b)]. This demonstrated the promise of nonviral transfection techniques for the treatment of focal cartilage defects.

Tissue Engineering Approaches Incorporating Gene Transfer

A promising approach for enhancing gene transfer and retention of genes or expressed proteins within a defect site employs 3-D scaffolds. The combination of gene therapy and tissue engineering could provide the ultimate treatment for articular cartilage defects as it involves a supporting scaffold that can serve as a carrier for gene vectors or infected cells resulting in a sustained, prolonged, and localized delivery of therapeutic proteins in vivo. It has also been demonstrated that cells first seeded into 3-D scaffolds and then transfected show higher gene expression levels and longer expression times as

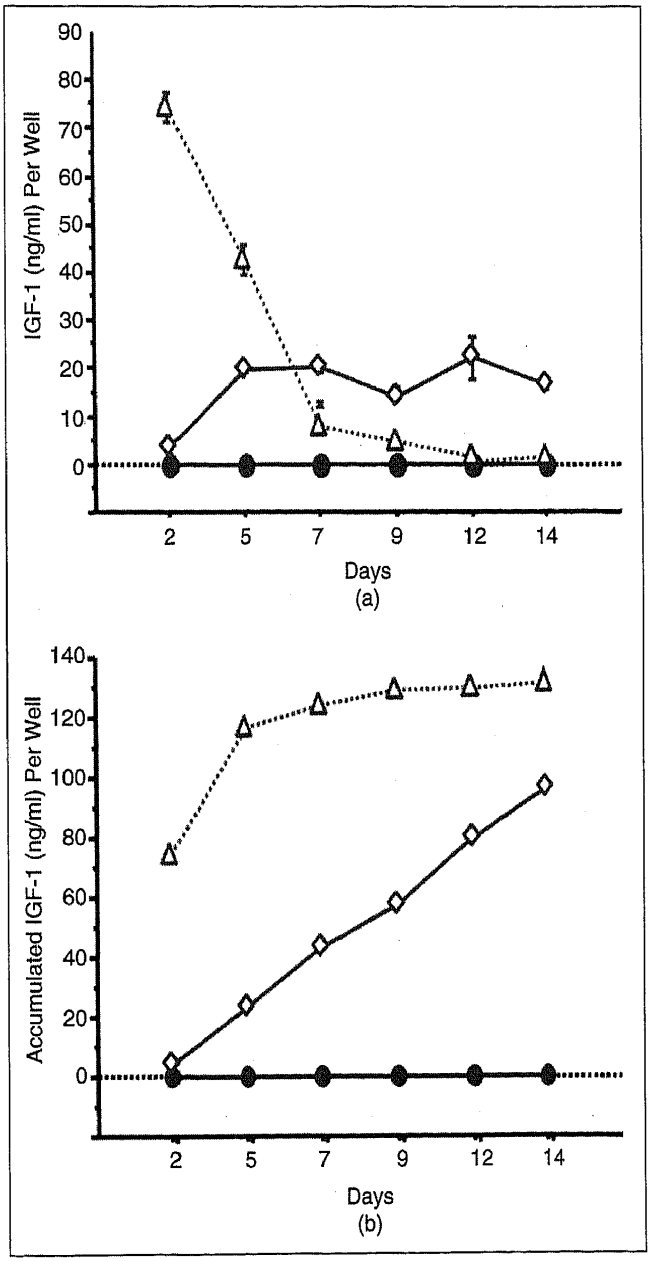

Fig. 4. IGF-1 protein production detected in the collected medium samples of adult canine chondrocyte monolayer cultures transduced with AdIGF-1 or transfected with the GenePorter® reagent and IGF-1 plasmid DNA. (a) Concentration of IGF-1 protein per well (24-well plate; ~$1x10^6$ cells/well). (b) Cumulative amount of IGF-1 protein produced over a two-week period. Closed circles indicate noninfected chondrocyte monolayers, open diamonds indicate viral transduced chondrocyte monolayers, and open triangles indicate nonviral transfected chondrocyte monolayers.

compared to 2-D transfection [54]. This observation is important in demonstrating how the 3-D matrix environment can influence cell behavior and processes.

Most studies using gene therapy and tissue engineering concepts for the regeneration of articular cartilage involve ex vivo infection of cells that are transduced [55], [56] or transfected [57] in vitro and then subsequently seeded into 3-D scaffolds (e.g., fibrin or polymer scaffolds). Several cell types and genes have been investigated for this application including: transfection of articular chondrocytes with the IGF-1 gene [57]; transduction of periosteal stem cells with the OP-1 gene [55]; and transduction of mesenchymal cells from rib perichondrium with the BMP-2 and IGF-1 genes [56]. In all of these cases, chondrogenesis and matrix synthesis were significantly enhanced in vivo.

The disadvantage of methods implanting cells tranfected or transduced ex vivo, as described above, is that there may be a decrease in expressed protein over time as the infected cells apoptose or migrate. It would be ideal if the scaffold could serve as a vehicle to immobilize gene vectors so that when implanted: 1) cells migrating into the scaffold and proliferating could take up the gene; and/or 2) surrounding cells could take up the genes released as the scaffold degrades. The DNA vector as well as the transiently expressed therapeutic protein would be retained within the defect site, thereby increasing the opportunity for a maximal therapeutic response, with a decreased likelihood of vector dissemination to surrounding tissue [58]. With time, more endogenous cells could become infected and a prolonged release thus maintained over the full duration of cartilage regeneration. These gene-supplemented scaffolds could be particularly beneficial in regenerative medicine applications because in vitro culture would not be required. A non-cell-seeded gene-supplemented scaffold could be implanted to induce regeneration in vivo.

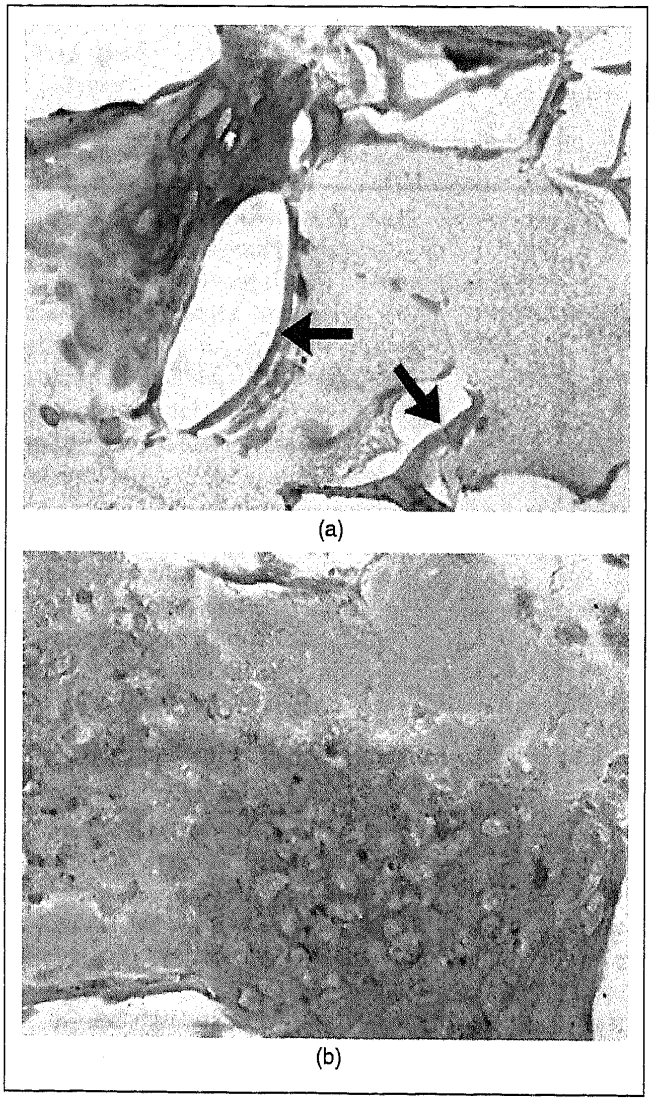

Fig. 5. Histology demonstrating the second passage adult canine chondrocytes and extracellular matrix synthesized after two weeks of growth in a type II collagen-GAG scaffold (a). The construct in (b) consists of a collagen-GAG scaffold with IGF-1 gene incorporation. Safranin O stain. 40X.

Several studies have synthesized gene-supplemented scaffolds for treatment of defects in tissues including bone [59], [60] and skin [61]. The variety of methods used to create these gene-supplemented matrices could be beneficial for articular cartilage repair. Matrices loaded with naked DNA (encoding for platelet-derived growth factor, PDGF) were shown to provide sufficient transfection for enhancement of tissue formation and vascularization when implanted subcutaneously in Lewis rats, compared to direct injection of plasmid [61]. High initial loading of plasmid DNA within the scaffolds, however, was needed to obtain sufficient transfection.

In another study, porous gene-supplemented collagen-glycosaminoglycan (GSCG) matrices were loaded with plasmid DNA coding for the luciferase reporter gene, and the effects of cross-linking and pH (during gene loading) on release kinetics and DNA integrity were determined [62]. The optimal conditions showed luciferase expression in chondrocyte-seeded GSCG constructs up to 28 days, demonstrating continuous transfection of articular chondrocytes throughout the culture period.

In a related (unpublished) study employing GSCG matrices, second passage adult canine articular chondrocytes were grown in a type II collagen-GAG scaffold into which the gene for IGF-1 was incorporated (~4 micrograms of DNA per matrix, 9 mm diameter x 3 mm thick). The results indicated that there was a greater amount of cartilaginous matrix synthesized in the IGF-1-gene incorporated cell-seeded constructs than in the control [Figures 5(a) and (b)]. These findings demonstrate the promise of accelerating growth factor-stimulated matrix synthesis in chondrocyte-seeded GSCG scaffolds.

Although direct loading of either naked plasmid DNA or adenoviral vectors into the scaffold by simple immersion in or injection of a vector solution is the simplest method for gene incorporation within 3-D matrices, a major problem is still rapid vector diffusion from the scaffold, where the majority of the vector is expelled from the matrix within the first 24 hours. Tissue engineering of cutaneous wound repairs may benefit more from these types of matrices since the healing time for these defects are on the order of weeks [58], [61]-[63]. While certain cartilage defects may also benefit from such scaffolds, in other cases a longer period of gene delivery (e.g., months) may be required. An improved means of retaining gene vectors within the scaffold to allow a steady, controlled, and more prolonged release of gene vectors would be advantageous.

One method of gene incorporation that has succeeded in retaining vectors within matrices is by assembly and subsequent fusion of plasmid DNA-loaded polymer microspheres using a gas foaming/particulate leaching process [64]. These scaffolds maintained DNA integrity and exhibited sustained and gradual controlled release for at least 21 days with the majority of the vector still remaining within the scaffold after 24

hours. Another method of gene retention within tissue engineering matrices involves mixing adenoviral vectors within collagen gels and injecting the gene-gel complex into a 3-D scaffold [65]. In one study [65], an adenovirus containing the gene encoding for platelet-derived growth factor-B (AdPDGF-B) was mixed with a collagen gel and injected into a poly-vinyl alcohol sponge (PVA). This construct was compared to injection of an aqueous solution of the gene or injection(s) of the recombinant protein PDGF-B within a PVA sponge for in vivo studies in ischemic excisional wounds. The collagen-immobilized AdPDGF-B/PVA complexes were shown to retain both vector and transgene products within delivery sites as late as 28 days. In contrast, the aqueous formulations allowed vector seepage from application sites, leading to PDGF-induced hyperplasia in surrounding tissues but not in wound beds. Furthermore, repeated applications of PDGF-B recombinant protein were required for neotissue induction approaching equivalence to a single application of collagen-immobilized AdPDGF-B, confirming the effectiveness and advantage of using gene transfer methods as a means to deliver therapeutic proteins as opposed to using exogenous recombinant proteins. It can be seen that the creation of scaffolds that more effectively control and retain gene vectors in a localized area can be very beneficial for articular cartilage repair. For nonviral transfection with naked plasmid DNA, gene retention within a scaffold could increase the opportunity for the plasmid to be taken up by seeded or surrounding cells over time. For viral transduction using scaffolds, undesirable diffusion of viral vectors to surrounding tissues or other parts of the body could be reduced or prevented.

Future Challenges

The complex effects of various proteins or combinations of proteins on articular cartilage regeneration still need to be examined to identify optimal conditions for articular cartilage repair. Furthermore, variables such as cell types and their sources, scaffold material, culture conditions, and type of gene vector have significant effects on gene transfer efficiencies and transgene expression. Therefore, these variables need to be further investigated to better understand the mechanisms of gene transfer and to determine the parameters that result in optimal infection efficiencies. One specific challenge is to develop more effective methods of gene retention within 3-D scaffolds in order to provide controlled, localized, and sustained release in vivo. Achieving this goal would not only benefit tissue engineering applications but also other gene therapy applications where prolonged delivery is desirable. Additionally, alternative methods to enhance gene transfer into cells, especially for nonviral vectors, need to be further investigated. For example, conjugating DNA vectors to a growth factor ligand (FGF-2) has shown to dramatically increase infection efficiencies by targeting vectors for cellular uptake via FGF receptors [63]. This method of increasing transfection efficiency may be more effective for in vivo applications, compared to using viruses or lipid-mediated reagents, since it involves a more natural method of cellular uptake.

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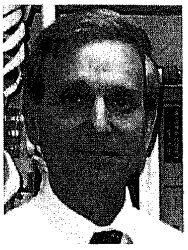

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References

[1] J.J. Rodrigo, J.R. Steadman, J.F. Silliman, and H.A. Fulstone, "Improvement of full-thickness chondral defect healing in the human knee after debridement and microfracture using continuous passive motion," *Am. J. Knee Surg.*, vol. 7, pp. 109-116, 1994.

[2] V. Bobic, "Arthroscopic osteochondral autograft transplantation in anterior cruciate ligament reconstruction: a preliminary clinical study," *Knee Surg. Sports Traumatol. Arthrosc.*, vol. 3, pp. 262-4, 1996.

[3] M. Brittberg, A. Lindahl, A. Nilsson, C. Ohlsson, O. Isaksson, and L. Peterson, "Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation," *N. Engl. J. Med.*, vol. 331, pp. 889-894, 1994.

[4] H.A. Breinan, T. Minas, H.-P. Hsu, S. Nehrer, C.B. Sledge, and M. Spector, "Effect of cultured autologous chondrocytes on repair of chondral defects in a canine model," *J. Bone Joint Surg.*, vol. 79-A, pp. 1439-1451, 1997.

[5] H.A. Breinan, T. Minas, H.-P. Hsu, S. Nehrer, S. Shortkroff, and M. Spector, "Autologous chondrocyte implantation in a canine model: Change in composition of reparative tissue with time," *J. Orthop. Res.*, vol. 19, pp. 482-492, 2001.

[6] M. Brittberg, A. Nilsson, A. Lindahl, C. Ohlsson, and L. Peterson, "Rabbit articular cartilage defects treated with autologous cultured chondrocytes," *Clin. Orthop.*, vol. 326, pp. 270-283, 1996.

[7] C.R. Lee and M. Spector, "Status of articular cartilage tissue engineering," *Curr. Op. Orthop.*, vol. 9, pp. 88-93, 1998.

[8] R.D. Coutts, R.M. Healey, R. Ostrander, R.L. Sah, R. Goomer, and D. Amiel, "Matrices for cartilage repair," *Clin Orthop.*, vol. 3915, pp. S271-9, 2001.

[9] D.P. Speer, M. Chvapil, R.G. Volz, and M.D. Holmes, "Enhancement of healing in osteochondral defects by collagen sponge implantation," *Clin. Orthop.*, vol. 144, pp. 326-335, 1979.

[10] D.A. Grande, M.I. Pitman, and H. Alexander, "The use of a collagen-chondrocyte composite allograft for the repair of articular cartilage defects," presented at Society for Biomaterials 15th Annual Meeting, Lake Buena Vista, Florida, 1989.

[11] S.R. Frenkel, B. Toolan, D. Menche, M.I. Pitman, and J.M. Pachence, "Chondrocyte transplantation using a collagen bilayer matrix for cartilage repair," *J. Bone Jt. Surg.*, vol. 79-B, pp. 831-836, 1997.

[12] S. Kawamura, S. Wakitani, T. Kimura, A. Maeda, A.I. Caplan, K. Shino, and T. Ochi, "Articular cartilage repair. rabbit experiment with a collagen gel-biomatrix and chondrocytes cultured in it," *Acta Orthop. Scand.*, vol. 69, pp. 56-62, 1998.

[13] S. Wakitani, T. Kimura, A. Hirooka, T. Ochi, M. Yoneda, N. Yasui, H. Owaki, and K. Ono, "Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel," *J. Bone Joint Surg.*, vol. 71-B, pp. 74-80, 1989.
[14] L.A. Solchaga, J.E. Dennis, V.M. Goldberg, and A.I. Caplan, "Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage," *J. Orthop. Res.*, vol. 17, pp. 205-13, 1999.
[15] A.J. Nixon, L.A. Fortier, J. Williams, and H. Mohammed, "Enhanced repair of extensive articular defects by insulin-like growth factor-I-laden fibrin composites," *J. Orthop. Res.*, vol. 17, pp. 475-87, 1999.
[16] G.A. Paletta, S.P. Arnoczky, and R.F. Warren, "The repair of osteochondral defects using an exogenous fibrin clot," *Am. J. Sports Med.*, vol. 20, pp. 725-731, 1992.
[17] H.P. von Schroeder, M. Kwan, D. Amiel, and R.D. Coutts, "The use of polylactic acid matrix and periosteal grafts for the reconstruction of rabbit knee articular defects," *J. Biomed. Mater. Res.*, vol. 25, pp. 329-339, 1991.
[18] C.R. Chu, R.D. Coutts, M. Yoshioka, F.L. Harwood, A.Z. Monosov, and D. Amiel, "Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): A tissue-engineering study," *J. Biomed. Mater. Res.*, vol. 29, pp. 1147-1154, 1995.
[19] L.E. Freed, J.C. Marquis, A. Nohria, J. Emmanual, A.G. Mikos, and R. Langer, "Neocartilage formation *in vitro* and *in vivo* using cells cultured on synthetic biodegradable polymers," *J. Biomed. Mater. Res.*, vol. 27, pp. 11-23, 1993.
[20] D.L. Nettles, S.H. Elder, and J.A. Gilbert, "Potential use of chitosan as a cell scaffold material for cartilage tissue engineering," *Tissue Eng*, vol. 8, pp. 1009-1016, 2002.
[21] K. M. Spangenberg, G.M. Peretti, C.A. Trahan, M.A. Randolph, and L.J. Bonassar, "Histomorphometric analysis of a cell-based model of cartilage repair," *Tissue Eng*, vol. 8, pp. 839-46, 2002.
[22] J.L. van Susante, P. Buma, G.N. Homminga, W.B. van den Berg, and R.P. Veth, "Chondrocyte-seeded hydroxyapatite for repair of large articular cartilage defects. A pilot study in the goat," *Biomat.*, vol. 19, pp. 2367-74, 1998.
[23] R.T. Chiroff, R.A. White, E.W. White, J.N. Weber, and D. Roy, "The restoration of articular surfaces overlying replamineform porous biomaterials," *J. Biomed. Mater. Res.*, vol. 11, pp. 165-178, 1977.
[24] R. Howes, J.M. Bowness, G.R. Grotendorst, G.R. Martin, and A.H. Reddi, "Platelet-derived growth factor enhances demineralized bone matrix-induced cartilage and bone formation," *Calcif. Tissue Int.*, vol. 42, pp. 34-38, 1988.
[25] A.H. Reddi, "Morphogenesis and tissue engineering of bone and cartilage: Inductive signals, stem cells, and biomimetic biomaterials," *Tissue Eng*, vol. 6, pp. 351-359, 2000.
[26] E. Suominen, A.J. Aho, E. Vedel, I. Kangasniemi, E. Uusipaikka, and A. Yli-Urpo, "Subchondral bone and cartilage repair with bioactive glasses, hydroxyapatite, and hydroxyapatite-glass composite," *J. Biomed. Mater. Res.*, vol. 32, pp. 543-551, 1996.
[27] D.A. Grande, C. Halberstadt, G. Naughton, R. Schwartz, and R. Manji, "Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts," *J. Biomed. Mater. Res.*, vol. 34, pp. 211-220, 1997.
[28] H.K. Kleinman, R.J. Klebe, and G.R. Martin, "Role of collagenous matrices in the adhesion and growth of cells," *J. Cell. Biol.*, vol. 88, pp. 473-85, 1981.
[29] I.V. Yannas, "Studies on the biological activity of the dermal regeneration template," *Wound Rep. Reg.*, vol. 6, pp. 518-524, 1998.
[30] I.V. Yannas, E. Lee, D.P. Orgill, E.M. Skrabut, and G.F. Murphy, "Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin," *Proc. Natl. Acad. Sci. USA*, vol. 86, pp. 933-937, 1989.
[31] L.J. Chamberlain, I.V. Yannas, H.P. Hsu, G.R. Strichartz, and M. Spector, "Near-terminus axonal structure and function following rat sciatic nerve regeneration through a collagen-GAG matrix in a ten-millimeter gap," *J. Neurosci. Res.*, vol. 60, pp. 666-677, 2000.
[32] S. Nehrer, H.A. Breinan, A. Ramappa, G. Young, S. Shortkroff, L. Louie, C.B. Sledge, I.V. Yannas, and M. Spector, "Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes," *Biomat.*, vol. 18, pp. 769-776, 1997.
[33] H.A. Breinan, S.D. Martin, H.-P. Hsu, and M. Spector, "Healing of canine articular cartilage defects treated with microfracture, a type II collagen matrix, or cultured autologous chondrocytes," *J. Orthop. Res.*, vol. 18, pp. 781-789, 2000.
[34] C.R. Lee, A.J. Grodzinsky, H.-P. Hsu, and M. Spector, "Effects of a cultured autologous chondrocyte-seeded type II collagen scaffold on the healing of a chondral defect in a canine model," *J. Orthop. Res.*, vol. 21, pp. 272-281, 2003.
[35] G.I. Im, D.Y. Kim, J.H. Shin, C.W. Hyun, and W.H. Cho, "Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow," *J. Bone Joint Surg. Br.*, vol. 83, pp. 289-94, 2001.
[36] B. Johnstone, T.M. Hering, A.I. Caplan, V.M. Goldberg, and J.U. Yoo, "In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells," *Exp. Cell Res.*, vol. 238, pp. 265-72, 1998.
[37] L.A. Fortier, G. Lust, H.O. Mohammed, and A.J. Nixon, "Coordinate upregulation of cartilage matrix synthesis in fibrin cultures supplemented with exogenous insulin-like growth factor-I," *J. Orthop. Res.*, vol. 17, pp. 467-474, 1999.
[38] A.A. Worster, B.D. Brower-Toland, L.A. Fortier, S.J. Bent, J. Williams, and A.J. Nixon, "Chondrocytic differentiation of mesenchymal stem cells sequentially exposed to transforming growth factor-beta1 in monolayer and insulin-like growth factor-I in a three-dimensional matrix," *J. Orthop. Res.*, vol. 19, pp. 738-49, 2001.
[39] A.M. Makower, J. Wroblewski, and A. Pawlowski, "Effects of IGF-I, rGH, FGF, EGF and NCS on DNA-synthesis, cell proliferation and morphology of chondrocytes isolated from rat rib growth cartilage," *Cell Biol. Int. Rep.*, vol. 13, pp. 259-70, 1989.
[40] W.J. O'Connor, T. Botti, S.N. Khan, and J.M. Lane, "The use of growth factors in cartilage repair," *Orthop. Clin. North Am.*, vol. 31, pp. 399-410, 2000.
[41] R. Sellers, D. Peluso, and E. Morris, "The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage," *J. Bone Jt. Surg.*, vol. 79-A, pp. 1452-1463, 1997.
[42] R.T. Louwerse, I.C. Heyligers, J. Klein-Nulend, S. Sugihara, G.P. van Kampen, C.M. Semeins, S.W. Goei, M.H. de Koning, P.I. Wuisman, and E.H. Burger, "Use of recombinant human osteogenic protein-1 for the repair of subchondral defects in articular cartilage in goats," *J. Biomed. Mater. Res.*, vol. 49, pp. 506-16, 2000.
[43] E. Fujimoto, M. Ochi, Y. Kato, Y. Mochizuki, Y. Sumen, and Y. Ikuta, "Beneficial effect of basic fibroblast growth factor on the repair of full-thickness defects in rabbit articular cartilage," *Arch. Orthop. Trauma Surg.*, vol. 119, pp. 139-45, 1999.
[44] J.S. Dounchis, R.S. Goomer, F.L. Harwood, M. Khatod, R.D. Coutts, and D. Amiel, "Chondrogenic phenotype of perichondrium-derived chondroprogenitor cells is influenced by transforming growth factor-beta 1," *J. Orthop. Res.*, vol. 15, pp. 803-807, 1997.
[45] M. Jakob, O. Demarteau, D. Schafer, B. Hintermann, W. Dick, M. Heberer, and I. Martin, "Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation in vitro," *J. Cell. Biochem.*, vol. 81, pp. 368-377, 2001.
[46] J. Huard, Y. Li, H. Peng, and F.H. Fu, "Gene therapy and tissue engineering for sports medicine," *J. Gene Med.*, vol. 5, pp. 93-108, 2003.
[47] P. Smith, F.D. Shuler, H.I. Georgescu, S.C. Ghivizzani, B. Johnstone, C. Niyibizi, P.D. Robbins, and C.H. Evans, "Genetic enhancement of matrix synthesis by articular chondrocytes: Comparison of different growth factor genes in the presence and absence of interleukin-1," *Arthritis Rheum*, vol. 43, pp. 1156-1164, 2000.
[48] B.D. Brower-Toland, R.A. Saxer, L.R. Goodrich, Z. Mi, P.D. Robbins, C.H. Evans, and A.J. Nixon, "Direct adenovirus-mediated insulin-like growth factor I gene transfer enhances transplant chondrocyte function," *Hum. Gene Ther.*, vol. 12, pp. 117-129, 2001.
[49] A.J. Nixon, B.D. Brower-Toland, S.J. Bent, R.A. Saxer, M.J. Wilke, P.D. Robbins, and C.H. Evans, "Insulinlike growth factor-I gene therapy applications for cartilage repair," *Clin. Orthop.*, pp. S201-13, 2000.
[50] K. Gelse, Q.J. Jiang, T. Aigner, T. Ritter, K. Wagner, E. Poschl, K. von der Mark, and H. Schneider, "Fibroblast-mediated delivery of growth factor complementary DNA into mouse joints induces chondrogenesis but avoids the disadvantages of direct viral gene transfer," *Arthritis Rheum.*, vol. 44, pp. 1943-53, 2001.
[51] H. Cohen, R.J. Levy, J. Gao, I. Fishbein, V. Kousaev, S. Sosnowski, S. Slomkowski, and G. Golomb, "Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles," *Gene Therapy*, vol. 7, pp. 1896-1905, 2000.
[52] H. Madry and S.B. Trippel, "Efficient lipid-mediated gene transfer to articular chondrocytes," *Gene Ther.*, vol. 7, pp. 286-91, 2000.
[53] H. Madry, D. Zurakowski, and S.B. Trippel, "Overexpression of human insulin-like growth factor-I promotes new tissue formation in an ex vivo model of articular chondrocyte transplantation," *Gene Ther.*, vol. 8, pp. 1443-1449, 2001.
[54] Y. Xie, S.T. Yang, and D.A. Kniss, "Three-dimensional cell-scaffold constructs promote efficient gene transfection: Implications for cell-based gene therapy," *Tissue Eng.*, vol. 7, pp. 585-598, 2001.
[55] D.A. Grande, J. Mason, E. Light, and D. Dines, "Stem cells as platforms for delivery of genes to enhance cartilage repair," *J. Bone Joint Surg. Am.*, vol. 85-A Suppl 2, pp. 111-116, 2003.
[56] K. Gelse, K. von der Mark, T. Aigner, J. Park, and H. Schneider, "Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells," *Arthritis Rheum.*, vol. 48, pp. 430-441, 2003.
[57] H. Madry, R. Padera, J. Seidel, R. Langer, L.E. Freed, S.B. Trippel, and G. Vunjak-Novakovic, "Gene transfer of a human insulin-like growth factor I cDNA enhances tissue engineering of cartilage," *Hum. Gene Ther.*, vol. 13, pp. 1621-1630, 2002.
[58] L.A. Chandler, D.L. Gu, C. Ma, A.M. Gonzalez, J. Doukas, T. Nguyen, G.F. Pierce, and M.L. Phillips, "Matrix-enabled gene transfer for cutaneous wound repair," *Wound Repair Regen.*, vol. 8, pp. 473-479, 2000.
[59] J. Bonadio, E. Smiley, P. Patil, and S. Goldstein, "Localized, direct plasmid gene delivery in vivo: Prolonged therapy results in reproducible tissue regeneration," *Nature Med.*, vol. 5, pp. 753-759, 1999.
[60] J. Fang, Y.Y. Zhu, E. Smiley, J. Bonadio, J.P. Rouleau, S.A. Goldstein, L.K. McCauley, B.L. Davidson, and B.J. Roessler, "Stimulation of new bone formation by direct transfer of osteogenic plasmid genes," *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 5753-5758, 1996.
[61] L.D. Shea, E. Smiley, J. Bonadio, and D.J. Mooney, "DNA delivery from polymer matrices for tissue engineering," *Nature Biotech.*, vol. 17, pp. 551-554, 1999.
[62] R.E. Samuel, C.R. Lee, S. Ghivizzani, C.H. Evans, I.V. Yannas, B.R. Olsen, and M. Spector, "Delivery of plasmid DNA to articular chondrocytes via novel collagen-glycosaminoglycan matrices," *Hum. Gene Ther.*, vol. 13, pp. 791-802, 2002.
[63] L.A. Chandler, J. Doukas, A.M. Gonzalez, D.K. Hoganson, D.L. Gu, C. Ma, M. Nesbit, T.M. Crombleholme, M. Herlyn, B.A. Sosnowski, and G.F. Pierce, "FGF2-Targeted adenovirus encoding platelet-derived growth factor-B enhances de novo tissue formation," *Mol. Ther.*, vol. 2, pp. 153-160, 2000.
[64] J.H. Jang and L.D. Shea, "Controllable delivery of non-viral DNA from porous scaffolds," *J. Control Release*, vol. 86, pp. 157-168, 2003.
[65] J. Doukas, L.A. Chandler, A.M. Gonzalez, D. Gu, D.K. Hoganson, C. Ma, T. Nguyen, M.A. Printz, M. Nesbit, M. Herlyn, T.M. Crombleholme, S.L. Aukerman, B.A. Sosnowski, and G.F. Pierce, "Matrix immobilization enhances the tissue repair activity of growth factor gene therapy vectors," *Hum. Gene Ther.*, vol. 12, pp. 783-798, 2001.