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Gene delivery strategies for cartilage tissue engineering[☆]

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Abstract

Tissue engineering is a multifaceted technology developed with a purpose of regenerating complex tissues and organs. Cartilage regeneration continues to challenge engineers and a new wave of efforts focus on developing strategies that provide sustained stimulation to cells by growth factors and other biological molecules to promote their differentiation into chondrocytes. Though significant research is dedicated to developing controlled release systems that deliver growth factors directly, a simpler approach to resolving this dilemma involves converting cells into protein producing factories. This is done through gene delivery. Gene Therapy studies published for articular diseases such as rheumatoid and osteoarthritis provide valuable information regarding different types of cells, gene delivery vectors and genes that can potentially be used to regenerate cartilage. Tissue engineering approaches provide the opportunity to combine two or more strategies used for Gene Therapy thus far and create a cohesive system that addresses both cartilage degeneration and synthesis simultaneously. Adopting gene transfer techniques for tissue engineering is a relatively novel approach, as non-viral gene delivery vectors are continually optimized for therapeutic purposes, and reservations about viral vectors have increasingly dampened their appeal. However, every element involved in gene transfection (i.e., the cell, vector and gene) is a variable which decides the physiological and biomechanical properties of the cartilage produced, and significant work still needs to be done in understanding the contribution of each of these factors to cartilage regeneration.

Keywords

Cartilage tissue engineering; Genes; Gene delivery; Gene Therapy; Gene delivery vectors; MSCs; Chondrocytes; Synovial cells

1. Introduction

Of all the organs targeted for tissue engineering, one could argue that cartilage would be one of the easiest to regenerate. Cartilage is one of the few avascular tissues in the body and is composed of a single cell type — the chondrocyte, which eliminates the need to address two critical problems in tissue engineering — angiogenesis and creation of multilayered tissues derived from cells of multiple lineages. Yet, cartilage tissue engineering is hardly dismissed as trivial and very few successful animal models for cartilage regeneration have progressed to clinical trials. According to the 2002 National Health Interview Survey conducted by the Center for Disease Control and Prevention (CDC), 43 million Americans have some form of arthritis of which 16 million report related limitations in activity [1,2]. More than 500,000 cartilage repair procedures are performed every year [3–5]. Perhaps nothing reflects the impact of

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cartilage related diseases to our society as clearly as the \$86 billion that the CDC estimates are annually lost in direct and indirect costs related to their treatment. “Treatment” of these disorders continues to rely heavily on analgesics, although the past few years have seen notable advances in surgical techniques. However, considering the limited regenerative capacity of cartilage, surgical interventions, at best, temporarily mask some of the symptoms without addressing the underlying pathology. This makes it a less viable option for younger patients. In recognition of this, clinicians are trying to incorporate cell transplantation and artificial matrices as treatment options, as they have been clinically proven to give better long term results [6,7]. Tissue engineering research continues to enhance the effectiveness of these treatments by including the use of growth factors and cytokines with the aim of decreasing damage to the surrounding tissue and regenerating tissue that more accurately resembles native cartilage.

Although various biological factors have been independently identified as necessary for reducing inflammation or promoting regeneration, it is becoming increasingly obvious that careful orchestration of the dose and type of numerous biological factors is essential to optimize this process. The challenge now lies in developing delivery systems that recognize this complexity in delivery of biological agents. The direct delivery of these agents either systemically or locally has numerous limitations. Biological agents have a half life in the order of minutes, which requires high doses of delivery and repeated administration. Furthermore, concentrations that are therapeutic to one organ may damage another. These problems related to targeting of multiple biological agents and their dose regulation can be solved by creating cells that are modified to transiently over-express certain proteins. Ex vivo transfection, where cells are initially isolated from a joint, transfected with the gene of interest and injected back into the joint, cleverly augments autologous cell transplant, a clinical procedure that is approved and widely used (Fig. 1). Though Gene Therapy for cartilage regeneration is still in its infancy, one can imagine that in the near future a treatment protocol might involve introduction of multiple cell populations at different time points, each expressing a different protein of interest working in synergy to control inflammation and encourage regeneration. This review article addresses studies related to gene delivery that have thus far been documented, with the aim of understanding the limitations and potential of gene delivery for cartilage regeneration.

2. Candidate cells for gene delivery

Cartilage defines the skeletal outline of a developing embryo. As the embryo develops most of the cartilage is converted to bone. However, cartilaginous tissue continues to subsist at the ends of bones and functions as a cushion, thus preventing friction between them. Fig. 2 is a schematic of the knee joint. Not all cartilage tissues are identical and there is just as much heterogeneity within the cartilage as there is between them. Cartilage is classified into three main categories — elastic cartilage found in the external ear, fibrocartilage that is found in the intervertebral discs and hyaline cartilage which is present between articular surfaces. As hyaline cartilage constitutes the most abundant type of cartilage, and is commonly affected in arthritic disorders, most tissue engineering models focus on regeneration of articular cartilage — which is a type of hyaline cartilage (Fig. 2). The mechanical and biochemical properties of the cartilage are defined by its extracellular matrix (ECM), all of which is secreted by cells that occupy 2% of the total volume. Consequently, the chondrocytes are immobilized within the thick ECM.

2.1. Autologous chondrocytes

An obvious choice for gene delivery would be chondrocytes, the cells that secrete cartilaginous matrix that defines the cartilage. Gene delivery approaches for tissue engineering of cartilage seldom target the chondrocytes in vivo, as these cells are inaccessible to vectors due to the rich matrix that surrounds them. Instead, autologous chondrocytes harvested from joints and

expanded in vitro in monolayers are preferably used for transfection. The primary drawback to using autologous cells is that they lose their chondrocytic phenotype to become fibroblastic when grown in vitro in monolayer as early as after the first passage of cells [8,9]. This limits the quantity of cells that can be used for transfection and implantation. In arthritic joints, this population of chondrocytes is further limited. Since cartilage is an aneural tissue, initial damage to the cartilage cannot be perceived by the patient. Focal cartilage injuries expand with time and use and lead to a more generalized cartilage loss that reaches the subchondral bone. Only when the damage is significant causing the patient to be immobilized do physicians seek surgical intervention. Furthermore, even during the very early stages of arthritis or other joint disorders, there is a significant change in the phenotype of the chondrocytes. Chondrocytes undergo significant changes in their cell surface markers as well as accelerate their production of extracellular matrix molecules to keep up with the damage caused to their surrounding tissue [10]. Gradually the biosynthetic machinery of the cell fails to keep up with the anabolic demands of the tissue and the chondrocytes undergo apoptosis causing loss of cartilage. Considering the circumstances which lead to the death of the chondrocyte, it would be impractical to further overburden the cells by forcing them to express additional genes.

2.2. Chondroprogenitor cells

Mesenchymal stem cells (MSCs) are increasingly being preferred to autologous chondrocytes as better alternatives for cell transplant. Large quantities of autologous MSCs can be extracted by minimally invasive techniques from the iliac crest of the patient, and other populations of MSCs are found in blood, and in the periosteum. MSCs are multipotent cells with the capacity to regenerate into numerous cell lines including chondrocytes although MSCs from different sources in the body may have different potentials of multipotency. Wakitani et al. pioneered the use of bone marrow derived MSCs for cartilage regeneration when they used cells from New Zealand white rabbits in full thickness cartilage defects [11]. The cells were saturated in collagen sponges that were embedded into the defect. Even in the absence of additional growth factors, the MSCs secreted a cartilaginous matrix that was converted to bone in subchondral regions of the defect. Although the regenerated cartilage had inferior mechanical properties and appeared to be discontinuous with the host tissue, the study established that cells other than chondrocytes could be used for cartilage regeneration [12]. Subsequent studies have emphasized the need to stimulate MSCs with appropriate chondrogenic factors or inductive material. BMP-2, BMP-7, IGF-1 and a variety of recombinant proteins have produced better results at regeneration than introduction of MSCs into joint spaces alone. A major drawback to most of these models, however, is the absence of joint pathology and most of the experimental models do not reflect the environment of a damaged cartilage [13]. Hence, determining which chondrogenic factors are required for MSCs to undergo true differentiation by imitating the pathways of embryonic development involves more work than mere extrapolation from developmental biology literature. Firstly, there is significant cross talk involved between inflammatory cells present in the joint space, the damaged chondrocytes, and the MSCs that are artificially introduced, and these interactions are absent during normal embryonic development. As a result, genes that stimulate cell differentiation in embryos may not respond the same way in a milieu of inflammatory cells. For example, IGF-1 in developing chondrocytes promotes the synthesis of cartilage specific matrix molecules [14]. However, in a milieu of inflammatory cells IGF-1 receptors are severely downregulated, and thus the required pathways stimulated through IGF-1 remain dormant [15]. Furthermore, there is significant inconsistency of response to biological factors between different animal models. For example, when collagen sponges embedded with recombinant BMP-2 and rabbit MSCs were implanted in rabbit knees, the regenerated cartilage was thin and irregular [16]. However, when similar sponges containing BMP-2 transfected perichondral cells were implanted in the knees of rats, the resultant cartilage had a rich collagen I component similar to fibrocartilage [17]. The above examples are not indicative of MSCs being poor

candidates for cartilage regeneration, but perhaps of the complexities involved in utilizing any multipotent cells in tissue engineering research. MSCs themselves have minimal disadvantages associated with them. MSCs have been shown to inhibit T-cell proliferation through immunosuppressive effects [18]. Although this delays immune rejection of transplanted MSCs, thus allowing for use of allogenic cells, it also raises the question of whether the transplanted MSCs can induce tumors in patients receiving them. Preliminary data suggest that such occurrences are rare although an extensive investigation is needed to further address this topic [19].

2.3. Cells within the synovial cavity

In addition to multipotent stem cells, synovial cells that line the joint cavity are also considered to be promising alternatives for Gene Therapy. The synovium lines the internal surfaces of a joint cavity and in contrast to the cartilage has a large cell population that covers a significant surface area. Hence, following either direct gene delivery or injection of transplanted cells into the synovial cavity, cell engraftment or transfection is exclusively observed in the synovial lining following simple probability parameters [20]. This opens the possibility of simplifying gene delivery to an intra-articular injection of gene delivery vectors or cells [21]. Studies by Gouze et al. showed that modified synovial cells continue to express significant amounts of gene product in vivo for up to 42 days after transfection by lentiviruses [22]. As compared to other models, gene transfer to synovial cells is significantly more advanced in clinical trials. A phase I clinical trial involving ex vivo modification of synovial fibroblasts derived from rheumatoid joints was based on extensive studies on rabbit knees that involved similar modification of synovial cells. In these studies synovial cells were transfected ex vivo with IL-1 receptor antagonist cDNA with retroviral vectors. When these cells were transplanted into arthritic rabbit knees it was observed that IL-1 RA was strongly chondroprotective and reduced the number of leukocytes entering the joint space [23]. Furthermore, independent studies by Makarov et al. showed that IL-1 RA delivered intra-articularly via Gene Therapy was four orders of magnitude more effective as an anti-arthritis than recombinant protein delivery systemically [24]. However, there is some contradiction in reports related to synovial gene transfer. While Evans et al. reported that no IL-1 RA was reported in the peripheral blood of the rabbits, Gouze et al. reported significant amount of protein in the all major organs. This discrepancy can be explained by the technique used for modifying synovial cells. While Evans et al. transfected the cells ex vivo and then introduced them into the synovium, Gouze et al. directly injected lentiviruses carrying IL-1 RA into the joint. Ex vivo gene transfer has been shown to be similarly successful using other plasmids including IGF-1, and TGF- β . However, current consensus from various studies is that ex vivo or direct gene delivery to synovial cells can be toxic resulting in joint fibrosis, osteophyte formation, extensive uncontrolled cartilage growth (in the presence of BMP-2), joint swelling and in some cases cartilage degeneration [21,25,26].

3. Candidate genes

Depending on the choice of cell used for gene delivery, there are a myriad of genes that can potentially be used for gene delivery. As mentioned above, the complexity lies in determining which gene or gene combinations are necessary and sufficient to induce chondrogenesis. Furthermore, the influence of these genes on different cell types in inflammatory environments remains to be determined.

3.1. Transforming growth factor β

Genes commonly used for cartilage formation include those from transforming growth factor (TGF- β) superfamily including TGF- β 1, which is responsible for initial cell-cell interaction between condensing progenitor cells [27]. In inflamed joints, TGF- β 1 has anti-inflammatory

properties and stimulates new matrix synthesis by chondrocytes [5,28]. TGF- β 2 mediates hypertrophic differentiation of chondrocytes by regulating Indian hedgehog (Ihh) and Parathyroid Growth Hormone (PTHrP) expression [29], however, it has not been used as extensively as TGF- β 1 for gene delivery. During development TGF- β 1-3 are involved in inhibiting formation of blood vessels in cartilage [30]. When Lee et al. transfected a monolayer of intervertebral disc cells with TGF- β 1 cDNA and grew them in 3 dimensional pellet cultures, they found a 375–475% increase in proteoglycan synthesis as compared to pellets containing cells not transfected with TGF- β 1 [31]. Irrespective of the number of cells used to grow these pellets, Lee et al. could not grow pellets larger than 5–7 mm in diameter. It is yet unclear whether the size of the pellet could be increased using different genes or a combination of genes including TGF- β 1. It is crucial to regulate the dose of TGF- β 1 secreted into the joints space. Mi et al. have shown that low doses of intra-articular injection of adenoviruses containing TGF- β cDNA have no therapeutic or harmful effect on arthritic joints [26]. However, high levels of TGF- β , when transfected into the joints, increase production of nitric oxide (indicating inflammation), muscle edema and reduced movement of the joint. These effects were absent in control joints that received the similar concentration of virus vector with luciferase cDNA.

3.2. Bone morphogenic proteins

Within the TGF superfamily are also included Bone Morphogenic Proteins (BMPs), BMP-2 and BMP-7 being most commonly used in gene delivery studies for cartilage. One of the first studies involved growing modified periosteal mesenchymal stem cells by transfection with BMP-7 cDNA [32]. When the modified cells were embedded in Poly(glycolic acid) (PGA) scaffolds and placed in full thickness defects within the rabbit knee, Grande, et al. found that transduced cells placed in PGA scaffolds did significantly better than non-transduced cells placed in scaffolds. They had a larger amount of hyaline cartilage, and quicker restoration of the subchondral bone (as early as six weeks) [32]. However, BMPs must be used with caution for cartilage regeneration as they are potent stimulators of ossification.

3.3. Insulin-like growth factor-1

IGF-1, a growth factor recognized to induce cartilage proteoglycan synthesis and collagen matrix production has also been studied in animal models with positive results. Since recombinant IGF-1 has a very short half life, researchers are keen upon developing a gene delivery system that can modify cells to synthesize their own IGF-1 for extended periods of time. Nixon et al. compared the effects of modifying pristine equine chondrocytes, bone marrow derived chondroprogenitor cells, and synovial cells with IGF-1 cDNA [15]. They found that these cells were capable of maintaining therapeutic levels of IGF-1 expression for up to 28 days in in vitro monolayer cultures. Transfected chondrocytes were able to maintain their morphology and secrete significantly greater amounts of proteoglycans and collagen II. However, independent experiments by Madry et al. found that when IGF-1 is transfected into articular chondrocytes, there is no significant increase in collagen II expression by these cells, although histologically the constructs score higher than untreated controls [2]. This discrepancy can be attributed to one or more of the differences in setup between the two experiments. While Nixon et al. were performing their studies in vitro, using adenoviruses with transfection efficiencies of 40–50%, Madry et al. were using FuGene 6, a non-viral vector in vivo, where transfection efficiency was at 35%. Transfection of rat articular chondrocytes ex vivo with IGF-1 cDNA containing adenoviral vectors and their subsequent implantation in partial thickness articular cartilage defects resulted in preservation of the chondrocytic morphology and formation of a structure that resembled hyaline cartilage 8 weeks into the study. Similar results were obtained when rabbit articular chondrocytes were modified after transfection. [33]. Further characterization of these constructs for mechanical properties is needed to enhance our understanding of the variables that dictate the outcome of long term expression

of IGF-1 on various cell lines. Table 1 lists the various delivery systems and their efficiencies at secrete IGF-1.

3.4. Other anabolic gene candidates

Although TGF- β 1, IGF-1 and BMPs are commonly targeted in experiments involving gene delivery for cartilage regeneration, up regulation of some other biological molecules such as transcription factors, intracellular signaling molecules and growth factors is also considered to be promising. These include Sox-9, Sox-5 and Sox-6, the first set of transcription factors that were identified as being essential and sufficient for cartilage formation [34,35]. Studies have also shown that fibroblast growth factor (FGF-3) receptor signaling is sufficient to induce chondrogenic differentiation [36]. Signal transduction molecules SMADs are also important intracellular regulators of chondrocytic differentiation. As these molecules function intracellularly, they cannot be delivered to cells in a soluble form. Gene Therapy is perhaps the only effective technique to utilize these molecules for cartilage regeneration [21]. The success of this strategy is limited by the transfection efficiency of the gene delivery vector and the survivability of the cells undergoing transfection.

3.5. Anti-inflammatory agents

Although cartilage regeneration mainly involves enhancing the anabolic activity of cells to either synthesize extracellular matrix molecules or imitate the differentiation observed in embryonic development, tissue engineering of damaged cartilage cannot be a complete success without addressing the inflammatory agents that overwhelm the joint space. Most of the work with anti-inflammatory agents has been performed in animal models where arthritis is induced using collagen or other stimulatory molecules. Studies have involved the use of IL-1 receptor antagonist, soluble TNF-alpha receptors, anti-inflammatory cytokines such as IL-10, IL-4 and IL-13, all of which have been successful at decreasing inflammatory response in animal models [23,37–39]. Other effective strategies involve using tissue inhibitors of matrix metalloproteinases (TIMPs). Following induction of arthritis, synovial cells secrete matrix metalloproteinase (MMPs) that cause severe destruction of the cartilage ECM. In addition, MMPs are also responsible for the cellular invasion of the joint by inflammatory cell. Tissue engineering strategies have been applied to curtail the destruction caused by MMPs by modifying arthritic synovial cells to over express TIMPs. Gelfoam sponges were seeded independently with synovial cells transfected with TIMP-1 and TIMP-3. The studies showed a 25% and 13% decrease respectively in the number of invading cells. Significant reduction in cell proliferation was also observed in addition to reduced levels of active MMPs. A possible extrapolation of this approach could involve the creation of a dual system, one containing synovial cells that overexpress anti-inflammatory agents and the other system containing chondrocytes or stem cells over expressing one or more anabolic factors — thus addressing both cartilage destruction and regeneration observed in articular diseases.

4. Candidate gene delivery vectors

4.1. Viral vectors

There are two main classes of gene delivery vectors: those involving viruses and those involving non-viral agents, such as polymers and liposomes. Viral vectors seem to be overwhelmingly preferred to non-viral vectors in most documented studies involving gene delivery for cartilage regeneration. The question of using viral versus non-viral vectors for any kind of gene delivery has caused intense debate since the inception of the idea of Gene Therapy. Viral vectors have been preferred in all avenues of gene delivery simply because they are better at successfully transfecting cells. While viral vectors can attain transfection efficiencies of around 80–90%, non-viral vectors can at most transfect 40–50% of the cell population. This continues to be a major obstacle in the use of non-viral vectors for gene delivery. However,

non-viral vectors are receiving increasing attention on the basis of their ease of synthesis, low immunogenicity and unrestricted plasmid size. The apprehension that envelopes the use of viral vectors for Gene Therapy has been justified based on many factors. Firstly, viral vectors induce an inflammatory response, which can cause a myriad of side effects ranging from mild edema to multi-system organ failure. The immune system's enhanced response to the now recognized virus makes it difficult to administer Gene Therapy repeatedly. At a more cellular level, transfection of a virus causes significant changes in cell surface markers of transfected cells, decreasing the possibility of reusing the same population of cells for a second round of gene delivery. Furthermore, cells that synthesize proteins encoded in the transgene express some viral markers and the duration of protein synthesis is limited by the ability of inflammatory cells to identify these cells and eliminate them. Modifications of virus genomes where minimal amounts of viral genes are retained have helped address many of these issues. Some of these modifications have extended expression of transgenes carried by the viruses for as long as 84 days [40].

However, direct injection of viral vectors is considered to be harmful as independent studies have shown dose dependent inflammatory response in joints of various animals [41]. Another disadvantage to intra-articular injection of viruses is that the viruses spread to other organs, however this dispersion of the virus has not been confirmed in larger animals such as rabbits and rhesus monkeys [42]. Ex vivo gene delivery is a novel method that circumvents most problems related to viral gene delivery while providing all of its benefits. The technique involves in vitro gene delivery to extracted cells after expanding the cells in culture. The transfected cells are then reintroduced into the body where they function as factories producing high quantities of the protein of interest. This technique, also coined cell mediated gene transfer, is ideal for introducing a variety cell populations transfected with different types of genes, at different time points without bringing the virus in direct contact with the body. There is some contention that viral genomes may be left behind in such ex vivo transfected cells, but that is currently being investigated. Studies prove that there is significant increase in the duration of protein expression with ex vivo gene delivery as compared to direct injection of viral vectors [42]. Nixon, et al. observed expression of marker Green Fluorescent Protein (GFP) beyond 60 days in chondrocytes transfected ex vivo with adenovirus. Rabbit synovial cells transfected ex vivo with a retrovirus maintained IL-1RA expression for up to 6 weeks [23]. Furthermore, recent work has shown that in the absence of immune recognition of transgene product cells lining the synovial cavity can transcribe the transfected cDNA at relevant levels for up to 6 months [22]. Other advantages of ex vivo gene delivery include the possibility to transfect cells other than those that line the synovial membrane. Articular chondrocytes as well as chondroprogenitor cells that are modified to secrete higher amounts of proteins can be directly injected within joints, where they are shown to preferentially adhere to damaged cartilage [42].

4.2. Non-viral vectors

FuGene 6 [2] and modified cationic liposomes [43] are two non-viral gene delivery methods that have so far been utilized for gene delivery for cartilage regeneration. FuGene 6 is a non-liposomal lipid formulation which has been shown to successfully transfect a variety of cell-lines. After transfection with FuGene 6, Madry et al. encapsulated the transfected articular chondrocyte into alginate spheres. The mean transfection efficiency was around 35% and 60% of the transfected chondrocytes expressed the transgene IGF-1 for as long as 6 weeks. IGF-1 expression peaked at day 5, and was sustained above therapeutic levels for 32 days. Transfection using the poly-L-lysine lipids designed by Goomer et al. involved a multi-step process where transfection was optimized by introducing cells in a detergent (lysolethicin) to permeabilize the cell membrane. Furthermore, transferrin was covalently attached to the polycationic backbone to promote electrostatic interaction with DNA. Transfection efficiency

by this method was reported at 71% in vitro. The transgene expression was maintained up to 13 days after transfection and no immune response was observed in vivo in animal models. Future studies will undoubtedly incorporate novel non-viral gene delivery vectors, including polymeric vectors that are currently being developed.

5. Scaffolds used for gene delivery

Scaffolds provide a way to localize cells to an area in the defect. Furthermore, since chondrocytes need a three dimensional configuration to preserve their morphology, scaffolds are ideal for cartilage regeneration as they provide spatial communication between cells while providing a infrastructure of fibers that provide mechanical support to the cells and healing tissue. Numerous natural and artificial materials have been used for cartilage regeneration including fibrin and collagen matrices, hydroxylapatite, synthetic hyaluronic acid sponges, and numerous polymers. However, scaffolds also introduce new limitations to the experimental design. When cells are seeded onto scaffolds, a good portion of them fail to attach to the scaffold fibers. The cells that eventually populate the scaffolds are produced from repeated cell duplications of the existing cells. Considering that in most practical scenarios transfection efficiency is significantly less than 100%, the actual number of transformed cells that continue to grow on scaffolds is very small. Furthermore, most viruses (and non-viral vectors) only transiently transfect cells and the expression of the transgene is lost after the cell replicates. Due to some of these reasons, previous experiments involving gene delivery to scaffolds have had limited success. When Baragi et al. used ex vivo transfected chondrocytes to populate collagen scaffolds and subsequently transplanted these scaffolds into articular cartilage defect, they noticed that over 90% of the cells were lost during the first 24 h after seeding [44]. Polyglycolic acid sponges have provided more promising results. Separate populations of periosteal stem cells that were transduced ex vivo with BMP-7 and sonic hedgehog respectively and seeded onto PGA scaffolds showed remarkable healing of full thickness cartilage defect as early as 6 weeks [32]. Similarly, Mason et al. transfected rabbit derived mesenchymal stem cells with BMP-7 and obtained complete osteochondral healing in defects implanted with cell seeded PGA scaffolds at 8 and 12 weeks [4]. There are limited accounts in literature involving scaffolds for growing modified cells, which allows for endless opportunities to try new variables in an effort to induce cartilage regeneration.

6. Concluding remarks

Table 2 summarizes the various cells, scaffolds and gene delivery vectors that have thus far been used for cartilage tissue engineering. Although the cartilage appears to be a simple structure at first, it soon becomes obvious that its complexity lies in the fact that it is designed like no other tissue of the body. It is relatively acellular, and completely avascular and aneural. The rich extracellular matrix made up of proteins (collagen II) glycoproteins (hyaluronic acid) and proteoglycans (aggrecan) serves as cushions between bones, thus preserving our joints through five to six decades of jumping, walking, dancing and jogging. Once damaged, either from disease or trauma, the cartilage cannot repair itself like some other tissues in the body, mainly because it lacks a steady blood supply that can provide the necessary stem cells for regeneration. Instead the chondrocytes undergo apoptosis and the cartilage undergoes degeneration. Hence, repairing damaged cartilage is a multi step approach. Initially it involves stopping cartilage degradation and further regenerating lost cartilage. Gene delivery, in general, had come to a standstill for a while after its safety became a subject of contention. However, the utility of successful gene delivery had never been under question. With recent advances in polymeric and liposomal gene delivery vectors, interest in gene delivery has a renewed vigor. This continues to be complemented by advances in molecular and cell biology following the Genome Project. As more and more genes and their functions are elucidated following sequencing of various genomes, it provides gene therapy researchers and tissue engineers

interested in gene delivery with tools to understand the results they see in their studies and to use this knowledge to device better gene delivery systems.

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Abbreviations

BMP-2

Bone Morphogenic Protein 2

BMP-7

Bone Morphogenic Protein 7

CDC

Center for Disease Control and Prevention

cDNA	complementary Deoxyribonucleic Acid
ECM	Extracellular Matrix
GFP	Green Fluorescent Protein
IGF-1	Insulin-like Growth Factor 1
IL-4	Interleukin 4
IL-1RA	Interleukin 1 Receptor Antagonist
IL-10	Interleukin 10
IL-13	Interleukin 13
MMP	Matrix Metalloproteinase
MSC	Mesenchymal Stem Cell/ Marrow Stromal Cell
PGA	Poly(glycolic acid)
SMADs	Smooth Muscle Actin (SMA) and Mitotic spindle Assembly checkpoint (MAD) proteins
TGF-β	Transforming Growth Factor β
TGF-β1	Transforming Growth Factor β 1
TGF-β2	Transforming Growth Factor β 2
TGF-β3	Transforming Growth Factor β 3
TIMP	Tissue Inhibitors of Matrix Metalloproteinase
TIMP-1	Tissue Inhibitor of Matrix Metalloproteinase 1
TIMP-3	Tissue Inhibitor of Matrix Metalloproteinase 3
TNF-α	

Tumor Necrosis Factor α

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Fig. 1.
Schematic for ex vivo gene delivery.

Fig. 2.
Schematic of knee joint.

Table 1

Comparison of secreted IGF-1 after gene delivery

Gene of interest	Method of transfection, cells and vectors employed	Concentration of protein secreted	Duration protein was detected	Reference
IGF-1	Ex vivo synovial cells adenovirus	Maximum: 246 $\pm$ 43 ng/ml	All 8 days evaluated	[14]
IGF-1	Ex vivo chondrocytes FuGENE 6	123 $\pm$ 22 ng/10 ⁷ cells	All 36 days evaluated	[2]
IGF-1	Ex vivo chondrocytes, MSCs synovial cells adenovirus	<i>Chondrocytes</i> : max: 70 ng/ml <i>MSCs</i> : max:115 ng/ml <i>Synovial cells</i> : max: 246 ng/ml	All 28 days evaluated	[15]

Table 2

Cells, gene delivery vectors and genes thus far used for cartilage regeneration

Cells	Vectors	Genes	Scaffolds	Reference
<i>In vitro transfection chondrocytes</i>				
Rabbit	Retrovirus	LacZ	Collagen	[45]
Horse	Adenovirus	IGF-1 GFP	—	[15]
	Adenovirus	IGF-1	—	[14]
Rat	Baculovirus	GFP		[46]
Lapine	FuGENE6	IGF-1 LacZ	Alginate beads	[2]
Cow	Adenovirus	GFP	Alginate beads	[47]
Human	Adenovirus	B-galactosidase	Alginate beads	[48]
<i>Chondroprogenitor cells</i>				
Rabbit MSCs	Retroviruses	BMP-7	Polyglycolic Acid	[4]
Rabbit periosteal cells	Retroviruses	BMP-7 Shh	Polyglycolic Acid	[32]
	Cationic lipids	TGF- β 1 PTHrP B-galactosidase	—	[43]
<i>Synovial cells</i>				
Horse	Adenovirus	IGF-1	—	[15]
<i>Other Cells</i>				
NIH/3T3	Retrovirus	IL-1 RA	—	[39]
Rat fibroblasts	Adenovirus	IGF-1	—	
		BMP-2	—	
Rat muscle cells	Retrovirus	LacZ	Collagen	[45]
<i>In vivo transfection</i>				
Rat	Lentivirus	B-galactosidase IL-1 RA	—	[22]
Rabbit	Adenovirus	TGF- β 1	—	[26]
	Adenovirus	IL-10	—	[37]
Cow	Adenovirus	TGF- β 1	—	[25]