Chapter 7
Engineering Gene-Activated Matrices for the Repair of Articular Cartilage Defect

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Abbreviations

AAV	Adenovirus-associated virus
BMP	Bone morphogenetic protein
FGF	Fibroblast growth factor
GAM	Gene-activated matrix
IGF	Insulin-like growth factor
iPS	Induced pluripotent stem cells
MSC	Marrow mesenchymal stem cells
PEI	Polyethyleneimine
PLGA	Poly(lactic-co-glycolic acid)
TGF-β	Transforming growth factor-β
3D	Three dimensional

7.1 Introduction

Cartilage is a slightly elastic connective tissue unique to humans and vertebrates, which is mainly present in the articular surface of bones, costal cartilage, the trachea, the ear, spinal disks, etc. [1]. Cartilage tissue is widely distributed in fetuses and young children, and it is gradually replaced by bone tissue over time. Cartilage is composed of chondrocytes, fibers, and a matrix. Depending on the matrix, cartilage can be categorized into hyaline cartilage, elastic cartilage, or fibrocartilage [2]. Articular cartilage is a type of hyaline cartilage that covers the surfaces of moving joints and disperses the load exerted on the subchondral bone by acting as a buffer. Articular cartilage mainly consists of thick and large

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chondrocytes and a rich cartilage matrix, and it does not contain blood vessels, lymphatic vessels, or nerves [3]. No structure can be observed in articular cartilage under a light microscope. The main components of the cartilage matrix are water, proteoglycan, and collagen; other components include fat, protein, inorganic salts. Water and small molecules can flow freely within the cartilage matrix, imparting elasticity and compressibility to the cartilage. Exerting a load on a joint compresses the articular cartilage, initiating water flow to provide nutrition to the articular cartilage. Articular cartilage is a highly specialized tissue that can protect the bones in the joint area from the effects of load and impact, such that activities can be performed without friction between the articular surfaces [4].

Articular cartilage, especially in moving joints, can be damaged by various causes, such as physical injury or osteoarthritis [5, 6]. Cartilage defects often cause joint swelling and pain and limit activity, producing symptoms similar to those of meniscal tear. Cartilage damage can be classified in terms of the extent of the damage: matrix damage, partial cartilage defects, and full-layer cartilage defects [7]. Following body tissue or organ damage, the newly formed cells and matrix in the surrounding area recover and replace the defect cells and the matrix. The repair process for damaged cartilage tissue is similar to that for other tissues, but cartilage has poor repair ability, and its original cartilage structure and function are not restored in most cases [8–11]. The histological and biological characteristics of cartilage limit its response to damage for the following reasons: (1) Cartilage tissue does not have undifferentiated cells to repair damage and defects; (2) unlike other tissues, cartilage defect sites other than full-layer cartilage defects do not contain blood vessels, making it difficult for undifferentiated mesenchymal cells to enter the damaged site and enable the healing process; and (3) the chondrocytes embedded in the dense collagen—proteoglycan matrix hinder the proliferation and migration of cells. For all of these reasons, articular cartilage damage is a common clinical disease. More than 400,000 articular cartilage repair surgeries were performed in the United States in 2007 alone, at a total cost of $50 to $60 million [12].

Traditional articular cartilage defect repair methods can be divided into two main categories: articular surface reconstruction and biological grafting. The first category mainly consists of arthroscopic grinding, drilling into the articular surface, and microfracture. Microfracture is an extremely important method that is used to repair a full-layer defect by puncturing the subchondral bone [13–16]. Bone marrow and blood ooze from the hole and form blood clots. The clots provide a scaffold into which bone marrow mesenchymal stem cells can migrate and differentiate into chondrocytes and bone cells. The body itself responds to microfracture as a damage event and produces a new alternative cartilage for patients. However, the limitation of this surgery is that the efficacy depends on the patient's age and weight and the size of the defect [17]. In addition, studies have shown that the microfracture technique does not completely heal the cartilage damage because the resulting cartilage is fibrocartilage rather than hyaline cartilage [18]. Fibrocartilage has less mechanical strength and is denser than hyaline cartilage; therefore, it breaks more easily under the pressure of repeated daily activities [19]. Biological grafting mainly includes osteochondral transplantation, periosteal/cartilage membrane

transplantation, and bone cell transplantation. Autologous chondrocyte transplantation is currently the most commonly used biological grafting technique. This technique has been increasingly attracting attention since its initial introduction by Brittberg because the transplanted chondrocytes can secrete and synthesize a new extracellular matrix at the defect site, which has the potential to form hyaline cartilage tissue [20]. Autologous chondrocyte transplantation is mostly applied to local traumatic articular cartilage defects. The transplanted chondrocytes proliferate, thereby further regulating the repair process via signal transduction. Follow-up results have shown that autologous chondrocyte transplantation has been able to repair cartilage defects completely for most patients [21, 22]. The drawback of the technique is that the treatment results are sensitive to joint mechanical instability, excessive loads, and the patient's age [23]. The technique also needs to be developed further to ensure that the transplanted chondrocytes are confined to the defect site for matrix secretion.

7.2 Application of Tissue Engineering to Cartilage Repair

Developments in tissue engineering have advanced cartilage construction techniques [24]. Tissue-engineered cartilage is constructed as follows. Seed cells are extracted, isolated, and cultured in vitro [25]. Growth factor(s) are added to induce considerable proliferation. The seed cells are then transplanted onto biocompatible, biodegradable, and resorbable tissue engineering scaffolds to form a cell—scaffold complex, which is subsequently transplanted into the body at the joint defect sites. As the cells grow and proliferate, an extracellular matrix is formed and the scaffold is degraded and absorbed; until finally, new tissue with cartilage function forms to repair the tissue structure and restore its function. Developments in modern biology have spurred the increasing use of growth factors to treat articular cartilage defects [26, 27]. Studies have shown that various growth factors, such as transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP), promote articular cartilage repair [28–31].

The combined use of cell culture, biomaterials, and growth factors in tissue-engineered cartilage has been experimentally successful [32]. Tissue engineering techniques for repairing tissue defects use an ideal matrix scaffold with two components: a growth factor release carrier and a scaffolding structure for cell proliferation and repair, which effectively reconstructs the tissue. Many studies have shown that an appropriate growth factor can be used for chondrocytes that are cultured in vitro to promote chondrocyte proliferation and maintain the cell phenotype; a three-dimensional (3D) scaffold provides the structure for chondrocyte proliferation [33, 34]. The growth factor must be continuously present over the requisite time period to facilitate tissue reconstruction [35]. Therefore, the growth factor is embedded in a biodegradable 3D scaffold and is continuously released as

the scaffold gradually degrades. Lee et al. embedded TGF-β1-coated microspheres in a scaffold, which effectively controlled the growth factor release to enhance cartilage formation in an in vitro culture [36]. Holland et al. studied the release rate of a TGF-β1 composite scaffold for repairing human articular cartilage damage in a simulated environment [37]. The results showed that the TGF-β1 complexed with an acidic gel under normal pH conditions in the body. The complex disappeared under acidic conditions (which would prevail in cases of tissue damage and inflammation, for example), significantly accelerating the release rate of the uncoated TGF-β1. This phenomenon can be prevented by combining the growth factor with a scaffold in experiments to release TGF-β1 at a specified constant rate. The cross-linking reaction time and the degree of cross linking of the 3D scaffold can be controlled to form scaffolds with different pore diameters and therefore release different rates of the embedded growth factor. All of these results show the considerable application potential of the growth factor composite scaffold in the application of tissue engineering techniques to repair cartilage defects. However, considerations of the large molecular weight of the growth factor and its short half-life in the body have continued to delay practical clinical studies on the application of the recombinant growth factor composite scaffold [38, 39]. The process of coating the growth factor onto microspheres requires a large amount of organic reagent, surfactants, and a high-temperature reaction [40, 41]. These conditions can degrade the growth factor, ultimately reducing its biological activity and increasing its immunogenicity instead. In addition, the difficulty in controlling the growth factor dose has also limited the use of the growth factor in clinical trials.

7.3 Gene-Activated Matrix

Several research initiatives are currently dedicated to developing a controlled release system to directly transfer the growth factor; however, a simpler technique is to convert the seed cells at the production site of the related proteins using gene transfer technology. A biological matrix containing genetic components is called a gene-activated matrix (GAM). A GAM combines the advantage tissue engineering and gene therapy to act as a cell growth scaffold in tissue engineering, provide a site and space for the growth and proliferation of seed cells, and act as a gene transfer carrier itself. A GAM can also enable the local transfer of a therapeutic gene [42]. In the defect area, the seed cells or cells in the tissue surrounding the GAM can capture the released genetic components or adhere to the matrix surface that has adsorbed the plasmid DNA. The therapeutic gene fragment is then obtained via endocytosis, such that the aforementioned cells act as local bioreactors for the expression and synthesis of the growth factors, which play autocrine and paracrine roles for the surrounding cells to ultimately realize the repair of the defect site.

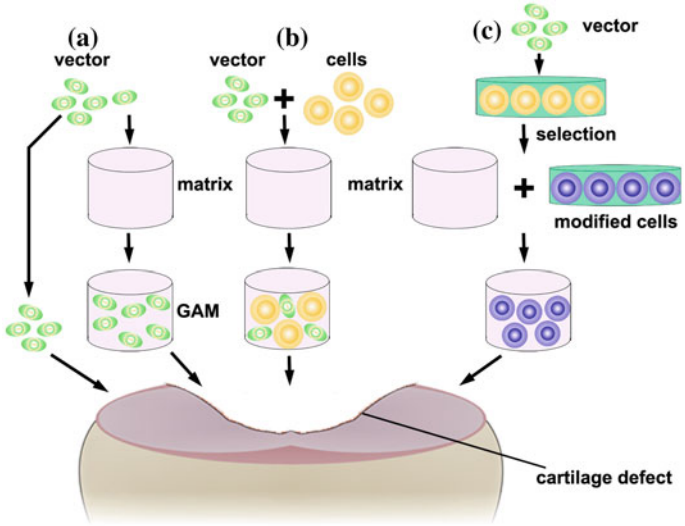

Fig. 7.1 Two different modes for gene transfer in articular cartilage regeneration: the direct in vivo method and the indirect ex vivo method

7.3.1 Gene Transfer Method to Repair Articular Cartilage

There are two main modes for gene transfer in articular cartilage repair, (as shown in Fig. 7.1): the direct in vivo method and the indirect ex vivo method. In the in vivo method, the gene vector (Fig. 7.1a) or the gene vector and the cells (Fig. 7.1b) are directly injected into the joint cavity; in the ex vivo method, the gene modification of seed cells is carried out in vitro, and these cells are then retransplanted into the body (Fig. 7.1c). Selecting one of the methods depends on many conditions including the gene and vector to be used. Recently, the ex vivo method has been most commonly used: The genetically modified cells are cultured on an in vitro matrix or embedded in a gel matrix, before being transferred to the cartilage defects for in situ repair [43]. This method can increase the cellular structure of the defect site; the gene is only transferred in situ, away from other non-specific tissues, thereby reducing edge effects.

7.3.2 Vector Types for Cartilage Gene Transfer

7.3.2.1 Viral Vectors

In recent years, viral vectors and non-viral vectors have been commonly used for cartilage gene transfer. The adenovirus vector is most commonly used for its high

transfection efficiency [44]. This vector can facilitate the stable and efficient transfection of a variety of cells but has a high immunogenicity in in vivo repair. The adenovirus vector also exhibits high toxicity at high doses. The toxicity of the adenovirus-associated virus (AAV) is milder than that of the adenovirus and has not been found to cause disease in humans thus far [45]. There is no viral protein expression in infected cells, but the transfection efficiency is not ideal. Adachi et al. used a retrovirus to transfect chondrocytes and stem cells in vitro, which were then embedded in a type I collagen gel and transplanted into the cartilage defect [46]. After 4 weeks, good histological results were obtained for the tissue repair in both groups. Nixon et al. used an adenovirus to transfect an IGF-1 gene that promotes secretion of a cartilage matrix into chondrocytes, bone marrow stem cells, and synovial cells, which were then filled into an articular cartilage defect [47]. The results showed that the aforementioned cells successfully prolonged the IGF-1 gene expression time and promoted the secretion of the cartilage matrix; however, a high initial virus titer was required, which had adverse long-term effects on the cartilage tissue. Note that in the aforementioned studies, all of the vectors used for gene transfection were viral vectors, which were chosen for their high transfection efficiency and ease of manipulation; however, the high immunogenicity of the viral vectors is as yet unresolved, which has limited their application to the clinical cartilage gene transfer system [48].

7.3.2.2 Non-viral Vectors

A technique has been developed that successfully uses non-viral vectors to elucidate gene structure, function, and expression. The preparation of a safe and efficient non-viral vector can profoundly impact the future development of gene therapy and biotechnology. Several polymers have been used for DNA transfer since the early 1970s; liposome is the most remarkable example of these polymers [49]. In 1987, Felgner et al. synthesized a cationic liposome, lipofectin [50]. The lipofectin/DNA complex is easy to manipulate and was the first vector used for in vivo chemical gene transfer. The peptide vector, in the form of the polylysine peptide vector, also has a strong affinity to DNA [50]. Consequently, the polylysine peptide vector/DNA complex can enhance the rate of cellular uptake. Polyethyleneimine (PEI) can inhibit lysosomes; in the acidic environment of endocytosis, PEI is protonated with an increased positive charge, which provides greater protection for DNA and facilitates plasmid escape from the lysosomes [51]. Consequently, PEI is widely used as a DNA transfer vector. Non-viral vectors are easy to manipulate for transfection and have a low immunogenicity and a high safety level; however, these vectors have a low transfection efficiency, and there target gene is only transiently expressed (typically for less than a week) [52]. Therefore, non-viral vectors are generally used only for in vitro mesenchymal stem cell differentiation and are difficult to use in vivo. Currently, an improved approach is being used in which the scaffold itself serves as a plasmid DNA transfer vector. The application of GAMs was developed to improve the poor efficiency of

non-viral vector via maintaining the high local gene concentration and sustainingly delivering therapeutic DNA to surrounding cells. The first report to be described for bone used scaffold comprising a collagen sponge impregnated with plasmid DNA encoding for the BMP-4 gene with or without another plasmid encoding a portion of the parathyroid hormone gene, PTH1-34. It was designed to deliver DNA to infiltrating reparative cells when implanted into an osseous defect. By expressing the transgene, the infiltrating cells generate an autocrine and paracrine osteogenic environment. Satisfactory therapeutic effect was observed in experimental defect models in rats and dogs [53, 54].

Another example is that poly-cationic polymers (chitosan and gelatin) can bind to the negatively charged plasmid DNA; this matrix is itself biodegradable, and its degradation products can form complexes and coat plasmid components to form a DNA/polymer complex. Meanwhile, the plasmid DNA attached to the matrix surface is also continuously released as the matrix degrades, thereby improving the transfection efficiency and ensuring the continuous expression of the target growth factors. Guo et al. prepared a gene-activated chitosan/gelatin scaffold embedded with a TGF-β1 plasmid to effectively promote the proliferation of rabbit articular chondrocytes in vitro while maintaining the cartilage phenotype [55]. This gene-activated scaffold has the potential to become a new cartilage repair scaffold. Building Guo et al. work, Diao et al. transplanted bone marrow mesenchymal stem cells into the aforementioned scaffold to promote the differentiation of directional mesenchymal stem cells and the synthesis of a cartilage extracellular matrix; the active cartilage repair matrix, which had been constructed in vitro, was then transplanted into rabbit articular cartilage defects to repair the cartilage defects in vivo; favorable therapeutic results were obtained [56].

Chen et al. used two plasmids, TGF-β1 and BMP-2, together for the bidirectional differentiation of bone marrow mesenchymal stem cells into chondrocytes and osteoblasts; an osteochondral transplantation complex was constructed on the same scaffold. The authors simulated a bone and cartilage-like tissue for both bone repair and cartilage function in vitro, which was subsequently used to repair a full-layer osteochondral defect; the tissue in the surface hyaline cartilage and the subchondral bone were simultaneously repaired successfully [57]. The schematic diagrams of constructing the bilayered GAM and the therapeutic effect are shown in Fig. 7.2.

7.3.3 Cell Types in the GAM

The cells used with a GAM for cartilage repair must have a stable source and a specified tissue repair potential. Currently used cells include adult chondrocytes, bone marrow mesenchymal stem cells, embryonic stem cells, newly discovered inducible pluripotent stem cells.

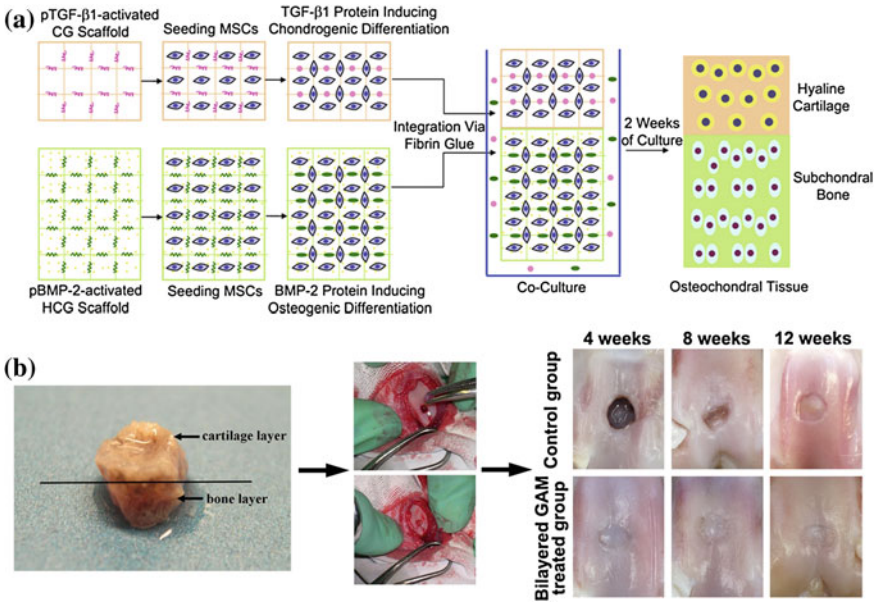

Fig. 7.2 **a** Diagrammatic representation of the procedures for the construction of the bilayered gene-activated composite osteochondral graft using mesenchymal stem cells loaded into TGF-β1-activated CG scaffold layer and BMP-2-activated HCG scaffold layer. pTGF-β1 plasmid TGF-β1; pBMP-2, plasmid BMP-2; *MSC* mesenchymal stem cell; *CG* chitosan–gelatin; *HCG* hydroxyapatite/chitosan–gelatin. **b** Osteochondral defects were created in the middle of each patellar groove of adult rabbits with a cylindrical drill. The bilayered gene-activated composite osteochondral graft was filled in the contralateral defect. Macrophotographs of the osteochondral repair in vivo were taken at 4, 8, and 12 weeks after the operation. Reproduced from [57]

7.3.3.1 Adult Chondrocytes

Adult chondrocytes are relatively simple to isolate and cultivate; these cells can be used to directly synthesize a cartilage-specific extracellular matrix [58]. A primary monolayer culture of chondrocytes can express a specific extracellular matrix, such as type II collagen and proteoglycans, which can be maintained for several weeks after passage. However, there is a limited source of adult chondrocytes, which tend to lose their phenotypes after multiple passages and culturing in vitro and to differentiate into fibroblasts, which cannot secrete a cartilage matrix. Consequently, the synthesis and secretion of type I and type III collagen increase, and the adult chondrocytes gradually lose their originally well-differentiated phenotypes, i.e., the tendency to dedifferentiate. The loss of phenotype has limited the large-scale in vitro proliferation of chondrocytes, making it difficult to obtain cartilage tissue with normal function after in vivo transplantation. Adult chondrocytes are usually used in conjunction with a scaffold or cell carrier, the surface features of which are used to maintain the normal matrix-secreting function of the chondrocytes. Autologous chondrocyte transplantation has been successfully carried out in clinical practice,

and satisfactory early treatment results have been obtained [59]. Animal experiments have shown that transplanting a chondrocyte/scaffold complex, which has been constructed in vitro, into large rabbit cartilage defects can promote the processes of repair and reconstruction [60]. The emergence and development of a 3D culture technique have enabled an extracellular matrix microenvironment to be simulated in the body. Chondrocytes, which have been cultured in vitro, can maintain a stable phenotype with a well-differentiated state and can even transform dedifferentiated chondrocytes in a monolayer culture into a well-differentiated state.

7.3.3.2 Bone Marrow Mesenchymal Stem Cells

Bone marrow mesenchymal stem cells (MSCs) are precursors to various mesenchymal cells such as osteoblasts, chondroblasts, and bone marrow stromal fibroblasts [61]. MSCs have a multi-directional differentiation potential with a high degree of evolutionary conservation. For over two decades, studies on the growth and differentiation of bone marrow MSCs have shown broad applications for stem cells that have been isolated and cultured from bone marrow by cartilage tissue engineering [62]. Currently, the isolation and application of MSCs is an important research subject in tissue engineering worldwide; experiments have shown that MSCs have a strong in vitro proliferative capacity and can be induced to differentiate into chondrocytes and form cartilage tissue in vivo. MSCs can be easily obtained via a simple bone marrow puncture: A couple dozen millimeters are sufficient to extract the number of cells needed in clinical trials. MSCs can be introduced into cartilage defects by two methods. The first method is the direct transplantation of MSCs into joints. Wakitani et al. were the first to transplant a complex of autologous bone marrow MSCs (which were cultured in vitro) and a type I collagen gel to repair rabbit articular cartilage full-layer defects [63]. The hyaline cartilage formed after only 2 weeks; by week 24, the articular cartilage and the subchondral bone defects were repaired, but the repaired tissue was thinner than the healthy tissue, and there was a gap between the repaired tissue and the healthy cartilage tissue. In the second method, chondrocytes that are induced in vitro or genetically modified chondrocytes are retransplanted into the defect area. Butnariu-Ephrat et al. used a high-density in vitro culture to induce MSCs into chondrocytes, which then formed a chondrocyte/2 % high molecular weight hyaluronic acid complex that was autologously transplanted to repair sheep articular cartilage defects [64]. A hyaline cartilage-like tissue similar to the normal articular cartilage structure formed after only 3 months.

7.3.3.3 Embryonic Stem Cells

Embryonic stem cells have an unlimited proliferative capacity and versatile differentiation; consequently, embryonic stem cells have a higher potential than adult stem cells to become new tissue engineering seed cells [65]. Embryonic stem cells

have been successfully induced to differentiate into chondrocytes in vitro and have even been used in attempts to construct cartilage tissue [66]. However, embryonic stem cell lines are difficult to obtain and establish. There are many challenges associated with the use of embryonic stem cell lines including safety, ethical, and immune rejection issues [67]. Therefore, there is currently only limited application of embryonic stem cells in tissue engineering.

7.3.3.4 Induced Pluripotent Stem Cells

Induced pluripotent stem (iPS) cells is a newly developed stem cell technology in which differentiated adult cells (such as skin cells) are introduced into a series of genes (Oct-3, Sox2, c-Myc, Klf4, and Nanog) and are then re-encoded into stem cells with multi-directional differentiation potential [68]. In this technique, isolated autologous adult differentiated cells are first re-encoded into stem cells, which differentiate into specific tissue cells under certain culture conditions. The specific tissue cells are then used for tissue engineering. All of the methods for obtaining induced pluripotent stem cells reported before March 2009 used a virus to transplant various genes into skin cells to promote cell transformation [69]. Both the viral vector and the transplanted gene pose cancer risks, which has greatly limited iPS application. Recently, breakthroughs have continued to be made with the iPS technique, such as bypassing the use of dangerous viral vectors to reduce the number of types of introduced genes and clean up the transplanted genes after the "usage time," thereby avoiding the various risks introduced by foreign genes [70]. It has been reported that iPS has been successfully induced in chondrocytes [71]. Thus, a series of dangerous or potentially dangerous risks has been circumvented, and we anticipate that the unlimited potential of iPS will be tapped for cartilage damage repair.

7.3.4 Cartilage Tissue Engineering Scaffold

An ideal scaffold is crucial for the successful construction of tissue-engineered cartilage. An ideal scaffold should meet the following criteria: (1) Good biocompatibility, which is required for seed cell adhesion, proliferation, growth, and differentiation; (2) A 3D structure with an optimal porosity of over 90 %; (3) Good biodegradability, with non-toxic degradation products that can be absorbed by the human body; (4) An effective matrix–cell interface for cell adhesion and growth, which, more importantly, can activate cell-specific gene expression and maintain the normal cell phenotype; (5) Plasticity and a prescribed mechanical strength to support new tissue. According to different sources, biologically active materials in tissue engineering can be classified into natural and synthetic materials. Natural biological materials generally have cell signal recognition capabilities; can promote cell adhesion, proliferation, and differentiation; generally have no toxic side effects; and possess good biocompatibility and biodegradability [72]. Polysaccharides and

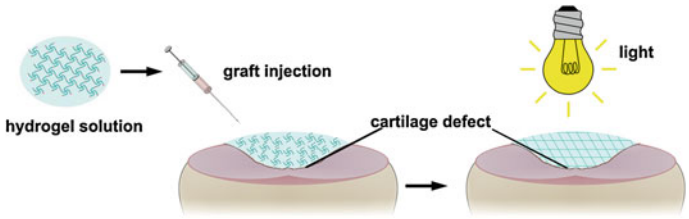

Fig. 7.3 The hydrogel solution is injected into the cartilage defect and photopolymerized in situ with light.

protein materials are commonly used because they are the main components of the extracellular matrix and can effectively simulate the microenvironment necessary for cell growth. Common natural polysaccharide materials include chitosan, chitin, chondroitin sulfate, and hyaluronic acid [73]. The proteins used as biological materials mainly include collagen, gelatin, and fibrin. These materials offer the advantage of carrying considerable biological information that enables cells to produce or maintain various functions [74]. These materials are directly derived from plants and animals and thus have good biocompatibility. The microstructure, the mechanical properties, and the degradation time of synthetic polymer materials can be predesigned and controlled [75]. Currently, poly(lactic-co-glycolic acid) (PLGA), which exhibits good biocompatibility, controlled degradation ability, etc., has been approved by the United States. FDA is a tissue engineering scaffold and is widely used [76]. However, PLGA also has many disadvantages for practical applications, such as insufficient hydrophilicity, weak cell adhesion, and the potential for inflammatory reactions of the acidic degradation products [77]. Composite materials are currently being intensively researched in tissue engineering: Two or more types of biological materials with complementary characteristics are combined in specified proportions following a particular method to produce a 3D material with an optimal structure and properties that compensate for the drawbacks of the individual materials themselves. Continuing advances in molecular biology, material science, and other disciplines have produced new materials such as electroplating chitosan/polyethylene oxide ethylene, fibrin polyurethane, and fiber bacterial cellulose. Experiments have shown that these scaffolds can act as artificial cartilage scaffold [78]. However, an ideal material has not been found thus far, and the search continues for a scaffold with enhanced cell compatibility, a controllable degradation rate, and a prescribed mechanical strength that can be used in current articular cartilage tissue engineering.

7.3.5 GAM Advantages for Cartilage Repair

GAMs offer several advantages for cartilage repair. (1) A GAM can be directly applied inside the articular cavity, which prevents excessive degradation of the genetic components by nucleic acid enzymes in the body's circulatory system; the

resulting locally high DNA concentration enhances the transfection efficiency. Local application also avoids ectopic transfection and is therefore safer. (2) The GAM functions as a targeted drug delivery system by directly acting on and targeting the cartilage repair cells; therefore, gene drug delivery into the joint cavity is maximized, and the genetic components are concentrated in the target area to several times or even hundreds of times the concentration of the systemic administration [79]. Huang et al. complexed BMP-4 plasmid and PEI, and the nanocomplex was encapsulated in a PLG scaffold. Researchers observed that this delivery strategy allows gene expression for periods of up to 18 weeks and achieved better therapeutic effect than blank scaffolds in a rat critical-sized defect [79, 80]. Therefore, the interaction between the DNA and the target cells is prolonged, thereby significantly improving the treatment results and reducing the amount of DNA used in the body and the rate of ectopic transfection. A GAM functions efficiently at low toxicity and therefore promotes safety. (3) The articular cartilage tissue cannot easily access large doses of cytokines from the blood circulation system, and it is therefore especially important to maintain a suitable concentration of cytokines in the defect area. In addition to facilitating the adhesion, proliferation, and differentiation of chondrocytes, the genetic components carried by the GAM can be locally expressed to secrete highly active therapeutic agents and promote repair. (4) The cartilage repair process consists of a single stage and does not require the long-term expression of the gene product. The DNA in the GAM has specific release kinetics to meet the requirements of the treatment window of the growth factor, thus avoiding excessive DNA dosing [81]. (5) A GAM may be incorporated into a 3D scaffold to provide filling support for the cartilage defect area; the scaffold is not affected by the range, size, and depth of the defect area and can be cut into any desired shape for direct injection into the articular cavity. Injectable in situ cross-linkable gels are highly desirable clinically as they can be performed using an arthroscopy, a convenient and less invasive procedure (Fig. 7.3). A recent study demonstrated that an alginate hydrogel containing BMP-2 plasmid and MSC could secrete biologically active BMP-2 protein 6 weeks after implantation. The protein levels were effective in inducing osteogenic differentiation as demonstrated by the production of collagen I and osteocalcin [82]. Injectable hydrogels containing plasmid encoding growth factors appear to be a promising new strategy for minimal-invasive delivery of growth factors in cartilage regeneration. (6) A variety of therapeutic genes can be composited together to synergistically affect cartilage defect repair. Our studies using gene therapy for cartilage repair applications have utilized growth factors such as TGF-β and BMP-2, which can promote the regeneration of both cartilage and subchondral bone [57]. From the results of H&E staining and immunohistochemical staining of collagen I and Alcian blue staining in Fig. 7.4, it is observed that the GAMs containing TGF-β or BMP-2 alone showed weakness in the repair of either subchondral bone or cartilage and need more wound healing time. Another study reported a combination of anabolic (IGF-1) and catabolic (IL-1 antagonist) to regulate tissue homeostasis using gene therapy. The catabolic proteins inhibit expression of genes related to catabolic tissue response, while

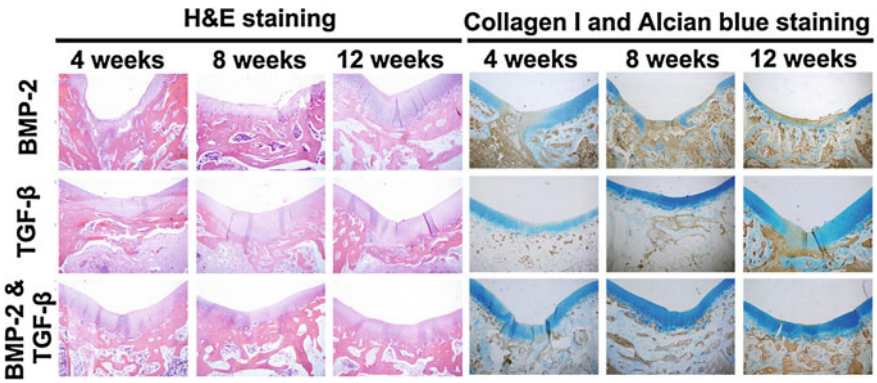

Fig. 7.4 H&E staining and immunohistochemical staining of collagen I and Alcian blue staining for hyaline cartilage at the indicated times during the osteochondral repair. Reproduced from [57]

anabolic proteins stimulate matrix production [83]. These studies indicate that multiple gene therapies have great potential in cartilage defect repair applications.

7.3.6 Issues in Using a GAM to Treat Cartilage Damage

GAMs have broad applications in the treatment of cartilage damage, but several challenges still remain. (1) Safety considerations show that both viral vectors and non-viral vectors pose risks of possible insertional mutations during the transfection process, which may cause cancer and auxiliary virus production. Conducting research and development to identify a safe vector without side effects is a daunting task in gene therapy. (2) The target gene has a low transfection efficiency and a short in vivo expression time. (3) Gene therapy for cartilage repair should develop in the direction of multi-gene transfection. Existing gene therapy studies mainly focus on single-gene diseases, whereas the complex pathological mechanism of cartilage damage is often a synergy of multiple factors. (4) Excessive and insufficient gene expressions are both harmful; therefore, further research is required to regulate gene expression such that the target gene products are expressed at an appropriate concentration and at an appropriate time and site.

7.4 The Prospects

Currently, many studies have demonstrated the effectiveness and feasibility of using GAMs for repairing tissue damage. Further development of these studies on the pathological mechanisms of tissue damage and the eventual resolution of the aforementioned issues can enable the GAM technique to become an effective and important method in the clinical treatment of orthopedic tissue damage.

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