Cell and Tissue Transplant Strategies for Joint Lesions

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Abstract: Articular cartilage lesions that do not disrupt the integrity of subchondral bone are not capable of spontaneous repair. The asymptomatic nature of these lesions leads to articular cartilage degeneration and development of the osteoarthritic process. To avoid joint replacement surgery, several cellular therapies have been developed. These therapies focus on the regeneration of a new tissue, whose structure, biochemistry composition and function should be the same as those of endogenous articular cartilage.

Current approaches for interrupting the osteoarthritic process produce a fibrocartilaginous tissue, not articular cartilage. The implantation of autologous chondrocytes and autologous mosaicplasty induces a better quality of articular cartilage; however, both techniques damage the existing cartilage because of the need to harvest large numbers of chondrocytes or to extract an osteochondral cylinder for implantation. While stem cells are a promising tool for repairing articular cartilage, their use is in an early experimental stage at this time. Although studies of cell therapy have shown clinical and functional improvement in joints, the ability to regenerate articular cartilage that resists the degeneration process remains elusive.

Key Words: Articular cartilage, cell therapy, focal cartilage lesions, osteoarthritis, chondrocyte, stem cell.

INTRODUCTION

Articular cartilage, which is aneural and relatively avascular, receives its nourishment through diffusion from the synovial fluid. The capacity for the self-repair of articular cartilage is limited. Modalities of cellular therapy to repair focal articular cartilage defects include the implantation of cells with chondrogenic capacity and creating access to the bone-marrow. The overall objective is not only to heal the chondral defect (repair), but to generate new tissue identical to native articular cartilage in structure, biochemical composition and functional behaviour (regeneration). This review summarizes the options for treatment of articular cartilage defects from both the experimental and clinical perspective (Fig. **1**).

PERFORATION OF THE SUBCHONDRAL BONE

When the defect affecting the cartilage penetrates to the bone and bone marrow spaces (osteochondral injury), mesenchymal cells from the bone marrow migrate with the hemorrhage and remain in the blood clot filling the defect, and are thus responsible for the repair of the defect (Fig. **2**) [1]. Because this repair is limited to the size of the defect, no capacity exists for the spontaneous repair of larger osteochondral injuries. The opening of subchondral vascular spaces is utilized for several surgical strategies, such as arthroscopic abrasion [2], subchondral drilling [3], spongialization [4] and microfracture (which produces the best results) [5].

Experimental studies in rabbits [6, 7] and dogs [8] have shown that the repair tissue generated by these processes is fibrocartilaginous in nature, differing from hyaline articular cartilage in biochemical composition, structural organization, durability and biomechanical properties, and degenerates over time [1, 7]. The co-expression of types I and II collagens in repair tissue does not occur until one year following subchondral penetration [9]. Clinical results, to some degree, contradict the findings relating to the quality of the repair tissue. For example, the treatment of knee osteochondral defects by microfracture has provided good clinical results after two years [10]. This longevity, however, seems to be age-dependent, with the most persistent repair cartilage in patients under the age of 40 [11]. Although the initiation of a degenerative process for tissue repair has been described at 18 months after microfracture [12], and seven to 17 years after microfracture, improvement in articular function and pain relief were preserved [13].

IMPLANTS OF PERIOSTIUM AND PERICHONDRIUM

These membranes contain mesenchymal stem cells that are capable of chondrogenesis [14, 15]. The periostial or perichondrial implant forms a fibrocartilaginous repair tissue that does not seem to mature over time [16, 17]. However,

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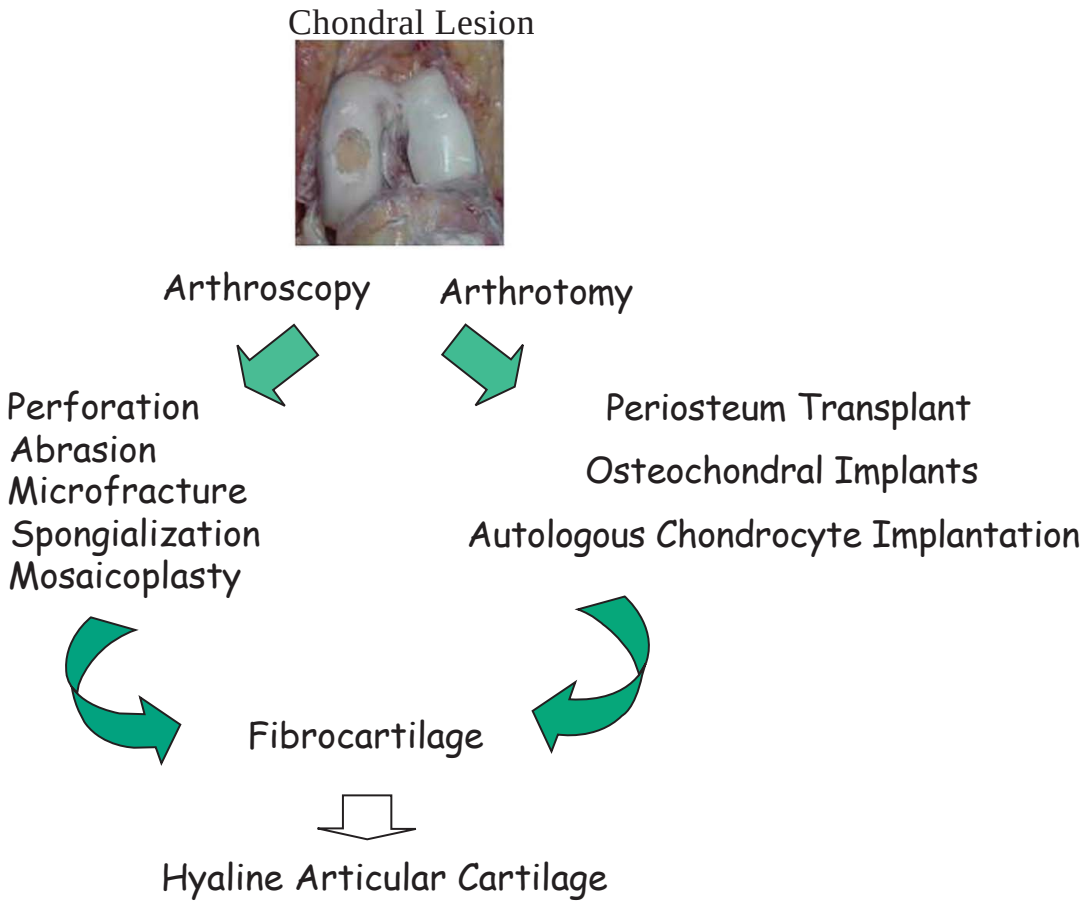

Fig. (1). Different options to treat articular cartilage lesions.

the clinical effects of a perichondrium implant are similar those of subchondral perforation. At 10 years following either procedure there were no significant differences observed between their outcomes [18].

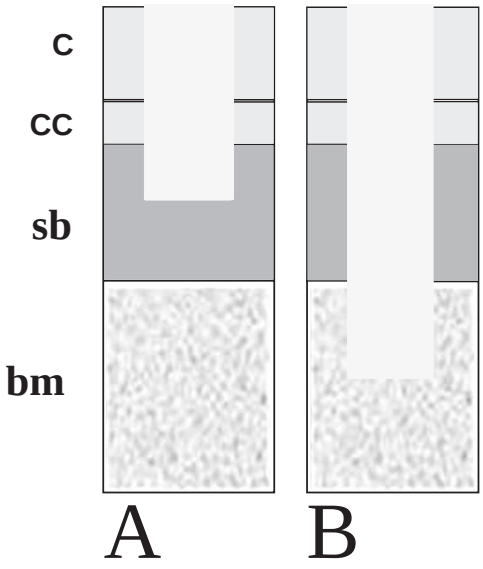

Fig. (2). Types of defects of articular cartilage. **A**). Partial defect. The lesion includes cartilage tissue and part of the subchondral bone. **B**). Deep defect. Subchondral bone is perforated to the bone marrow. c: cartilage; cc: calcified cartilage; sb: subchondral bone; bm: bone marrow.

OSTEOPERIOSTEAL IMPLANTS

Osteoperiosteal plug transplantation (bone plugs with a covering periosteal layer) into a mosaicplasty donor-site defect produces new fibrous tissue in animal models [19] that can acquire fibrocartilaginous characteristics by use of chondrogenic inductors [20]. Bleeding from bone marrow spaces from the injury probably interferes with the repair action of the periostium germ layer. In rabbits, nearly 67% of repair tissue cells was derived from the bone marrow after osteoperiosteal implantation [21]. Although this repair tissue appears to be of low histological quality, improved articular function and pain relief have been observed following this procedure [22].

MOSAICPLASTY

Autologous mosaicplasty is considered to be a promising alternative for treatment of small to medium-sized focal chondral and osteochondral defects [23]. This technique involves the translocation of osteochondral cylinders, or plugs, from a low-weight-bearing normal site to a high-weight-bearing diseased site. The donor sites spontaneously repair with mesenchymal stromal cells from the bone marrow to promote a new fibrocartilaginous tissue.

Arthroscopic evaluations at five [24] and 10 years [25] after osteochondral cylinder implantation showed survival of the transplanted articular cartilage, congruency between opposing (treated and untreated) joint surfaces and fibrocartilaginous repair of the donor sites. However, if the osteo-

chondral cylinders protrude above the surface, joint problems can arise. At four months post-surgery, patients with protruding cylinders experienced a "catching sensation" and some of these patients reported joint pain. Arthroscopic examinations of these cases revealed fissures in the osteochondral cylinders and fibrillation around the recipient site [26].

The use of autologous mosaicplasty is limited by the defect size, which determines the number of osteochondral cylinders required. The articular cartilage produced by this technique exhibits topographical variations in morphological, biochemical and physical properties [27, 28]. Because the implanted tissue is harvested from a low-weight-bearing area, the cartilage is thinner and differs in histological structure from cartilage from high weight-bearing areas [29, 30].

OSTEOARTICULAR ALLOTRANSPLANTATION

The use of osteoarticular allotransplantation may address some of the limitations of mosaicplasty where injury to the low-weight-bearing area of the cartilage is avoided, particularly if a large number of osteochondral cylinders are required. The advantage of the shell allograft is that it is obtained from a donor site that matches the precise location of the recipient and thereby in more likely to have a similar architecture. Furthermore, immunological problems are rare due to the avascular nature of cartilage and the encapsulation of chondrocytes in the extracellular matrix. In practice, shell osteochondral allotransplant in the knee is well integrated and provides a consistent functional improvement and pain relief after two years [31]. Graft requiring larger portions of bone for traumatic defects also do well. After 10 years, 85% of the implants proved durable [32].

AUTOLOGOUS CHONDROCYTE IMPLANTATION

A therapeutic alternative offering more effective repair of focal articular cartilage defects is autologous chondrocyte implantation (ACI) [33-36]. This procedure is also used for patients with osteochondritis dissecans [37], but not for osteoarthritis joints. Because the results of this technique are highly age-dependent, the use of this procedure is recommended for patients younger than 55 years of age. The technique involves obtaining, by arthroscopy, articular cartilage explants from low-weight-bearing areas (Fig. **3**). Chondrocytes are then isolated and grown *in vitro* to obtain a sufficient number of cells for implantation. In a second surgical intervention, the cultured chondrocytes are injected into the defect cavity, which is then closed with periosteal membrane from the same patient.

Autologous chondrocyte implantation has several shortcomings: *a)* obtaining cartilage explants requires an additional surgical intervention, adding to the articular cartilage damage that increases the osteoarthritic process; *b) in vitro* chondrocyte proliferation must be limited because the capacity to produce stable cartilage *in vivo* is gradually reduced when cell divisions are increased [38]; *c)* aging reduces the cellular density of the cartilage, which impacts chondrocyte proliferation capacity *in vitro* [39] and the chondrogenic potential of the periostium [40], and *d)* cell culture procedures take too long (three to six weeks) and increase the risk of contamination.

The first article about ACI in humans appeared in 1994 [33]. Clinical and arthroscopic evaluations of femoral implants showed good results after two years. Early studies demonstrated the durability of the implant, with good clinical results observed at five to 11 years post-procedure [37]. Histological analysis of the *de novo* formed tissue revealed some heterogeneity in the quality of the repair tissue. Of the 41 biopsies obtained one year following implantation, 10% consisted of hyaline cartilage; 24% consisted of a mixture of hyaline cartilage and fibrocartilage; 61% were entirely fibrocartilage and 5% consisted only of fibrous tissue [41].

Other studies at one year after implantation have shown that fibrocartilaginous morphology regions and hyaline morphology regions coexist in the same biopsy; both types having proteoglycans and type II collagen [34, 36]. Furthermore, aggrecanase activity was higher than metalloprotease activities in the fibrocartilaginous regions although both enzymes were found [36]. The expression of type IIA and IIB collagen mRNA was also detected [42]. These mRNA expressions seem be characteristic of the prechondrocytic state (type IIA) and differentiated chondrocytes (type IIB) [43]. These results suggest that ACI induces the regeneration of articular cartilage, probably by the turnover and remodeling from an initial fibrocartilaginous matrix using enzymatic degradation and synthesis of type II collagen [36]. It is believed that this process continues for more than 24 months following the implantation [35, 44] and takes place in three specific stages: cell proliferation (the first six weeks), transition (seven to 26 weeks) and remodeling (beyond 27 weeks) [45].

Doubt has been cast upon the use of periosteal membranes for retaining the chondrocyte suspension because the procedure requires a large surgical incision and can lead to peripheral graft hypertrophy and potential ectopic calcification. To obviate this problem, the use of a type I/III collagen patch has been proposed [46-48]. Any differences between the results of using periostium or the collagen patch remain controversial. A comparative study of these two techniques at two years post-operative has revealed no statistically significant differences between the clinical outcomes of ACI with collagen membranes versus ACI with periostium. However, a substantial number of patients who had ACI with periostium required subsequent shaving of an hypertrophied graft [48].

Matrix-associated autologous chondrocyte implantation (MACI) is another procedure that uses a cell-seeded collagen matrix for treatment of cartilage defects. A prospective clinical investigation carried out in 38 patients with localized cartilage defects for a period of up to five years after surgery, showed that MACI represents a viable alternative for treatment of local cartilage defects of the knee [49].

ALLOTRANSPLANTATION AND XENOTRANSPLANTATION OF CHONDROCYTES

Other therapeutic alternatives include allotransplantation [50-52] and xenotransplantation of chondrocytes [53, 54]. Allotransplantation is constrained by the necessity for compatible donors and limitations on storage of cartilage or chondrocytes because cryopreservation reduces survival and proliferation of chondrocytes. Xenotransplantation may resolve some of these problems, but this therapeutic alternative has rarely been investigated. The immune barrier is an important objection to the use of both of these procedures. Iso-

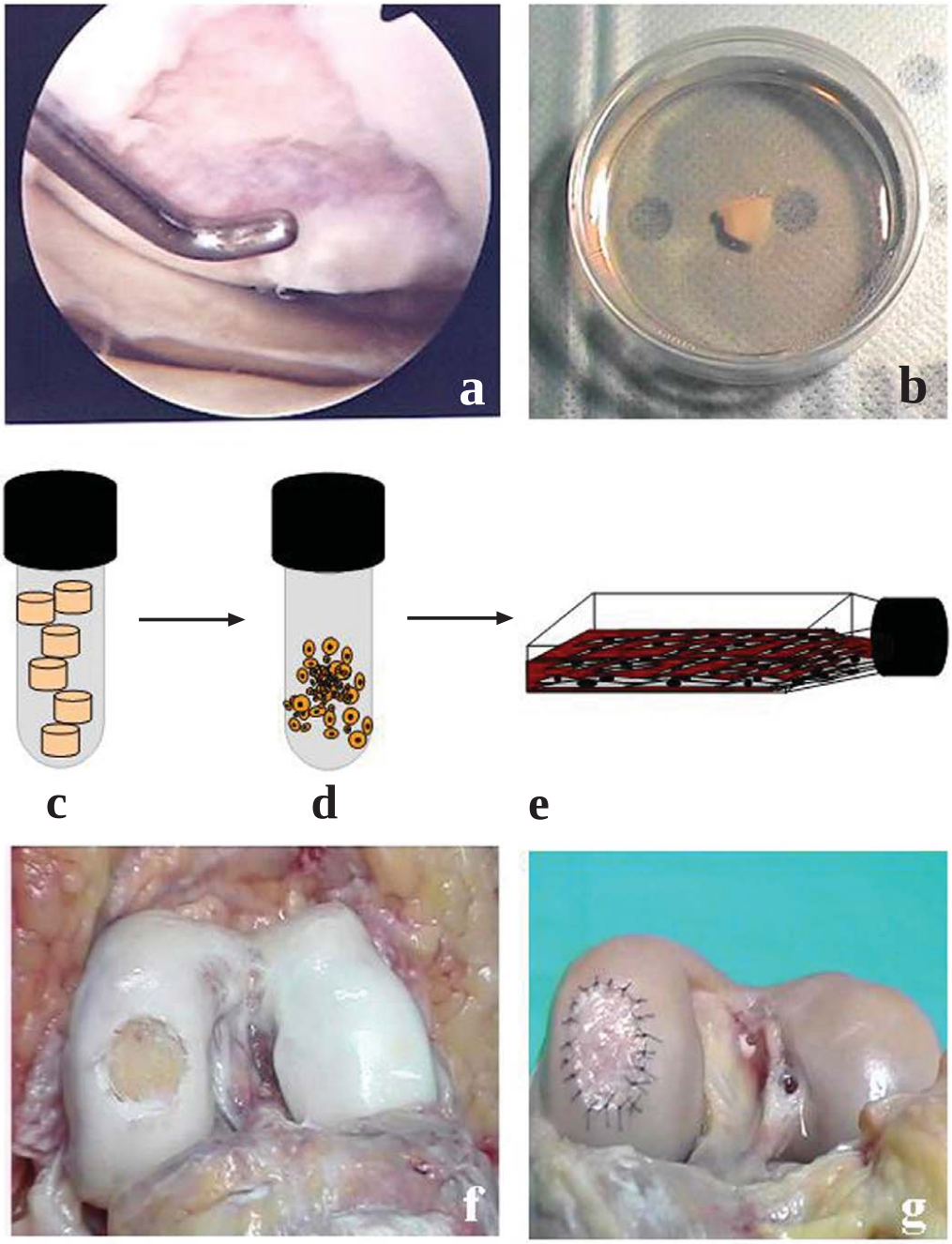

Fig. (3). Implant of autologous chondrocytes. **A** and **B**: biopsy. **C** and **D**: chondrocytes are obtained by by enzymatic digestion (trypsin and type IV collagenase). **E**: cells proliferate in a culture flask at 37°C. **F**: femoral condyle with chondral lesion. **G**: The defect cavity is closed with a patch (periosteum, type I/III collagen), then the chondrocyte suspension is injected.

lated chondrocytes result in immunogenic reaction, but allo-implantation of chondrocytes encapsulated in their extracellular matrix [52] or embedded in collagen gel or agarose [50, 51] resulted in few or no rejection reactions. Notably, xeno-transplantation *in vivo* of cultured pig chondrocytes into rabbit chondral defects closed with periosteal membrane no signs of infiltration by immune cells [54].

MESENCHYMAL STEM CELLS TRANSPLANTATION

Mesenchymal stem cells (MSCs) can differentiate *in vitro* into a number of cell types, including adipocytes, chondrocytes and osteoblasts [55]. This procedure uses cells isolated from small tissue samples, proliferated in culture, to obtain the appropriate number for clinical applications. They can be implanted in the donor patient, obviating rejection problems. Mesenchymal stem cells may be a tool for tissue repair that has the advantage of avoiding the problem of immunological rejection of the allotransplant and the ethical conflict of using embryonic stem cells.

MSCs are wide distributed *in vivo*, being found in bone marrow [56], periostium [57], perichondrium [58], synovial membrane [59], connective tissue of dermis and skeletal muscle [60], adipose tissue [61], umbilical cord blood [62],

peripheral blood [63, 64], amniotic membrane [65] and even in articular cartilage [66]. Although bone marrow is the usual source of MSCs, umbilical cord blood is emerging as an important reservoir for stem cells capable of differentiation into many cell types and possessing the advantages of immune status and relatively unshortened telomere length [67].

Because there is no specific marker for MSCs, the principal criteria for identification are adherence to the plastic of the tissue culture flask, fibroblast-like morphology [68], the prolonged capacity for proliferation in supportive media and the capacity to differentiate *in vitro* into cells of mesodermal origin (chondrocytes, adipocytes, osteoblasts). Furthermore, characteristics of MSCs are the absence of expression of typical hematopoietic antigens like CD34 and CD45, and the expression of surface markers like Stro-1, CD44, CD73, CD90, CD105 y CD166 [69].

The low number of MSCs isolated from a tissue sample requires growth *in vitro*. However, the number of mitotic divisions of MSCs in culture must be limited because MSCs age during *in vitro* culture, causing a reduction in their proliferative capacity [70, 71] and gradual loss of the potential for multiple differentiation [70, 72]. The conservation of phenotype and differentiation capacity of MSCs are proportional to telomerization [73]. Telomeres are normally shortened in successive cell divisions, however, in embryonic stem cells the telomere length is restored by telomerase enzyme activity. On the other hand, MSCs lack [74] adequate levels of telomerase activity to achieve telomeric restoration [72, 75, 76]. Patient age also influences the characteristics of MSCs because their proliferative capacity is reduced by aging [77].

Several studies have indicated that the MSC population obtained from a tissue sample contains cell subsets with distinct differentiation stages. Furthermore, MSCs from different tissue sources can have biologic distinctions. For example, MSCs derived from bone marrow show a higher potential for osteogenic differentiation [78], while MSCs of synovial origin show a greater tendency toward chondrogenic differentiation [79]. Under identical culture conditions for differentiation, MSCs isolated from the synovial membrane show more chondrogenic potential than those derived from bone marrow, periostium, skeletal muscle or adipose tissue [80].

Studies of cartilage injury repair in animal models using MSCs embedded in collagen gel [50] or injected into defects closed with periosteal membrane [81] indicate that MSCs can differentiate *in vivo* into a number of cell types in different biologic environments.

Several studies have recently reported the migration of intraarticularly injected MSCs to the site of a cartilage injury to repair chondral defects. In a caprine model for osteoarthritis in which OA is induced by the complete excision of the medial meniscus and resection of the anterior cruciate ligament, the intraarticular injection of MSCs produced meniscus repair after six weeks; however, there was no evidence of cartilage or ligament repair [82]. This suggests that the injected MSCs migrated to the injured meniscus, but not the damaged cartilage. The intraarticular injection of MSCs into rat knees, however, showed mobilization of these cells towards all injured tissues, including articular cartilage; the MSCs contributed to tissue regeneration [83, 84].

Studies of treatment with MSCs for human chondral lesions are rare. In osteoarthritic knees, MSCs embedded in collagen gel were implanted into chondral defects and closed with periosteal membrane. After 42 weeks, arthroscopic and histological results were better than in osteoarthritic patients without implants, although there was no statistically significant improvement in clinical results [85]. The use of MSCs to treat chondral lesions clinically has not been established, in part because the stages of chondrogenic differentiation of MSCs are not sufficiently defined. In addition, there are currently no protocols that ensure direct differentiation to the desired phenotype; the plasticity of the cells differentiated from MSCs can lead to undesirable phenotypic alterations [86, 87].

SCAFFOLDS

Scaffold-guided tissue regeneration involves differentiated or mesenchymal stem cells, scaffolds and bio-active factors. Requirements for the biomaterials used as a scaffold include biocompatibility, suitable ligands for implanted cell attachment, integration with native cartilage and biodegradation into non-toxic products that can be replaced by host cells. Other important factors in the design of a scaffold are pore size, porosity, adaptive shape, mechanical integrity, the ability to be retained at the implantation site and cost efficiency.

Natural biomaterials, such as fibrin, collagen, agarose, alginate, hyaluronic acid or chitosan [88-92] and synthetic biomaterials, such as poly-lactic glycolic acid (PLGA) [93] and a polymeric nanofiber [94], are used alone or in different combinations to make scaffolds.

Mechanical and biological properties of biomaterials significantly influence chondrogenesis and the long-term maintenance of the structural integrity of the neo-formed tissue. The three-dimensional nature of the scaffolds promotes maintenance of a rounded cell morphology and the elevated expression of glycosaminoglycans and type II collagen [92, 95]. However, scaffolding biomaterials have differing influences on the metabolism of host cells and, consequently, the quality of the tissue-engineered cartilage [90, 96]. For example, the use of chitosan, compared to PLGA, for cartilage tissue engineering produces a superior maintenance of structural integrity because the expression of type II collagen protein and mRNA became weaker over time in the PLGA group [96]. Scaffolds using hyaluronic acid are also being used with excellent clinical and histological results [97].

GENE THERAPY

The introduction of genetic manipulations into the field of tissue damage repair can enhance the process of articular cartilage restoration. This process involves the determination of the appropriate gene and type of cell (chondrocytes, chondrogenic cells and cells of the synovial membrane) for the gene transference, as well as the determination of the optimal vector to incorporate the cDNA [98]. Several anabolic factors [members of the transforming growth factor-β (TGF-β) super-family, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) induce chondrogenesis and synthesis of extracellular membrane components. Anti-inflammatory molecules, such as

interleukin-4 (IL-4), IL-10, IL-1 receptor antagonist (IL-1Ra), and tumor necrosis factor soluble receptor (TNFsR) act as inhibitors to articular cartilage degradation [99].

The synovial membrane seems useful as a target for chondroprotective therapies [100]. The viral transfection *in vivo* with the IL-1Ra gene in rheumatoid arthritis joints reduces the severity of the disease process in animal models [101]. Furthermore, this technique makes possible the safe intraarticular expression of the IL-1Ra gene [102, 103]. Chondrocytes and MSCs are the preferred targets for the induction of chondrogenesis. Using animal models, the transplantation *in vivo* of MSCs transfected with bone morphogenetic protein-2 (BMP-2) cDNA produces improved chondral lesion repair with a higher production of proteoglycans and type II collagen compared to controls [104].

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