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DEGRADATION AND REPAIR OF ARTICULAR CARTILAGE

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1. ABSTRACT

Approximately 95,000 total knee replacements and 41,000 other surgical procedures to repair cartilaginous defects of the knee are performed annually in the United States (1). The response of normal articular cartilage to injury or arthritic degeneration is often a sub-optimal repair; the biochemical and mechanical properties of the new tissue differ from the native cartilage, resulting in inadequate or altered function. It is believed that the chondrocytes from the surrounding areas, although perhaps capable of some limited migration at the damaged site, are not able to proliferate and produce the macromolecules necessary to create an organized matrix characteristic of normal articular cartilage (2,3). Current therapeutic options for articular cartilage injuries and degeneration have resulted in repair tissue which may be hyaline-like, but does not approximate the durability and function of the normal articular surface. Numerous studies have been performed to increase our understanding of the normal repair process of articular cartilage and its limitations, and to devise methods and materials to regenerate the joint surface.

2. NORMAL ARTICULAR CARTILAGE

Articular cartilage contains chondrocytes embedded in an extracellular matrix composed primarily of type II collagen, proteoglycans, and other collagens and noncollagenous proteins (4). The tissue is divided into three zones, distinguished by the shape of the resident cells and the arrangement of the type II collagen fibers (5). Chondrocytes in the most superficial or tangential zone are flattened, with collagen fibrils arranged parallel to the surface. The collagen network in the thicker intermediate zone is oriented in vertical columns perpendicular to the surface, with round chondrocytes arrayed between the collagen fibers. Chondrocytes are also round in the basal or deepest zone. A microscopically distinct line, the tidemark, separates the basal zone from underlying calcified cartilage; deep to the calcified region lies the subchondral plate of bone.

The collagen architecture contributes to the tensile strength and stiffness of cartilage (6); its compressibility is due to its proteoglycan component (7). While type II collagen predominates (comprising about 90-95% of total collagen), smaller amounts of types V, VI, IX, X, and XI collagen are also present (8,9). Cartilage proteoglycans (PG) include hydrodynamically large, aggregating PG, with covalently linked sulfated glycosaminoglycans, as well as hydrodynamically smaller nonaggregating PG such as decorin, biglycan, and lumican. Among the noncollagenous proteins are thrombospondin and cartilage oligomeric matrix protein; their functions continue to be elucidated. The chondrocyte elaborates

matrix components, degradative enzymes, and the activators and inactivators of these enzymes. This synthetic function is influenced by a variety of cytokines and growth factors.

The repair response in vascularized tissues is triphasic, consisting of necrosis, inflammation, and repair (10). The avascular nature of articular cartilage allows it to undergo necrosis in response to injury, but the inflammatory phase is largely absent. Therefore, if the damage is limited to the cartilage layer and does not involve the subchondral bone, there is no recruitment of undifferentiated cells to effect a repair. Chondrocytes in mature articular cartilage have little intrinsic potential for replication (11,12,13); lesions may attempt repair by an "extrinsic" mechanism which depends on metaplasia of mesenchymal cells from para-articular connective tissues (14). However, the population of mesenchymal stem cells declines with increasing age (4). When the subchondral bone is penetrated, its vascular supply allows a triphasic repair to take place. The resulting tissue is usually mechanically sub-optimal fibrocartilage (10).

3. OSTEOARTHRITIC DEGRADATION

Arthritic change in the articular surface is usually first demonstrable as fraying and fibrillation of the surface (15). Loss of PG from the matrix is characteristic. Early in the course of osteoarthritis (OA), the tissue mounts an attempt at repair. Chondrocytes may proliferate and form clusters, and there is an increase in matrix synthesis. However, in the face of chronic mechanical degenerative forces, degradative enzymes overwhelm the synthetic capability and the repair fails. Where the cartilage is damaged, there may be duplication of the tidemark. In the absence of a traumatic or destabilizing insult to the joint, it is difficult to identify the cause which sets the degeneration in motion. It has been suggested that there is a genetic component; indeed, inheritance of a mutant allele for the Type II procollagen gene (COL2A1) has been identified in some individuals and families with OA (16).

As the surface fibrillation progresses, the defects pentrate deeper into the cartilage, and cartilage is lost. The subchondral bone thickens, is slowly exposed, and may appear eburnated, or polished and ivory-like. Bone cysts may form deep to the eburnated areas; the cysts communicate with the articular surface via a small crack in the subchondral plate. Bony nodules or osteophytes often form at the periphery of the cartilage surface. These may occasionally grow over the adjacent eroded areas. If the tidemark cracks, vascular ingrowth may occur and cause the formation of tissue plugs conaining fibrocartilage.

4. TRAUMATIC DEGRADATION

Traumatic injuries to cartilage fall into three categories: microdamage or blunt trauma, chondral fractures, and osteochondral fractures.

4.1. Microdamage

Microdamage to chondrocytes and cartilage matrix may be caused by a single impact or by repetitive blunt trauma; it does not obvioiously disrupt the articular surface (4,10). Radin *et al.* showed that repetitive loading of rabbit cartilage caused a loss of superficial PG which resulted in increased metabolic activity of the chondrocytes (17). These changes are consistent with metabolic and biochemical changes seen in the early stages of degenerative joint disease. Repetitive loading of articular cartilage can result in cellular degeneration or death, disruption of collagen ultrastructure, increased hydration, fissuring of the articular surface, and thickened subchondral bone (2,4,18). These phenomena are again similar to the course of early osteoarthritis. Osteoarthritic cartilage contains elevated levels of degradative enzymes; these enzymes and cytokines (such as TNF and IL-1) may cause further degradation of the surrounding matrix (17). Accumulated microdamage eventually becomes irreversible. As in OA, chondrocytes may be able to restore lost PG and matrix components at a basal rate; however, with concurrent damage to the collagen network, the rate of loss is greater and results in irreversible degeneration (4).

4.2. Chondral fractures

In this type of injury, the articular surface is disrupted without violation of the subchondral plate. Chondrocyte necrosis at the site is followed by increased mitotic and metabolic activity of the surviving chondrocytes bordering the defect, within a few days of injury (10,19). Fibrous tissue forms a lining of clefts in the surface. As above, there is increased synthesis of extracellular matrix components and type II collagen (10,19,20). This anabolic response is observed for approximately two weeks after injury, after which the metabolic rate returns to normal levels.

Despite the transitory increase in mitotic and metabolic activity, the repair response after superficial injury remains suboptimal since no bleeding occurs, with no subsequent inflammatory response in the avascular cartilage to promote tissue repair. The newly synthesized matrix is nearly always insufficient to fill the defect and restore the surface (4,21). Animal studies of the natural history of partial-thickness defects report little evidence of repair even in immature animals (10,20,22). By six months, the newly formed matrix deteriorates, and the repair tissue reverts to a markedly irregular appearance (22,23).

4.3. Osteochondral Fractures

An osteochondral fracture is a lesion which crosses the tidemark, penetrating the underlying subchondral plate. In this case, the presence of subchondral vasculature elicits the three-phase repair response typically encountered in other tissues. The defect fills with blood; the resultant fibrin clot activates an inflammatory response. The clot then becomes a fibrovascular repair tissue (10,20), and the various cellular components release growth factors and cytokines including transforming growth factor beta (TGF-beta), platelet-derived growth factor (PDGF), bone morphogenic proteins, and insulin-like growth factors (4). These factors stimulate further repair.

Recruitment, proliferation, and differentiation of undifferentiated cells into chondrocyte-like cells occurs within the first two weeks (4). Mesenchymal stem cells are deposited in the fibrin network, which eventually forms a fibrocartilaginous zone (23,24). These stem cells originate in the underlying bone marrow rather than the adjacent articular surface (20,23). The cells progressively differentiate into chondroblasts and chondrocytes; at six to eight weeks, the repair tissue contains chondrocyte-like cells in a matrix of proteoglycans and predominantly type II collagen, with some of type I collagen (25,26,27). However, the matrix will degenerate, with replacement of the chondroid tissue by more fibrous tissue and fibrocartilage and a shift in the synthesis of collagen from type II to type I (19,21,23,24,27). Early degenerative changes include surface fibrillation, followed by depletion of PG, chondrocyte cloning and death, and vertical fissuring from the superficial to deep layers (23,25). At one year, the repair tissue resembles a mixture of fibrocartilage and hyaline cartilage, with a substantial component of type I collagen (25).

Clinically, this fibrocartilaginous repair tissue may function satisfactorily for a certain length of time; however it does not approximate the material properties of normal articular cartilage. There is a more random array of collagen fibers with a lower elastic modulus in this repair tissue as compared to normal cartilage (28). The permeability of the repair tissue is also elevated, thus reducing the fluid-pressure load-carrying capacity of the tissue (2). The changes result in increased viscoelastic deformation, making the repair less able to withstand repetitive loading than normal articular cartilage (28). GAG levels in the cartilage adjacent to osteochondral defects have been reported to be reduced by 42% of normal values, indicating that the injury led to degeneration beyond the defect itself (29). Thus, it appears that osteochondral lesions may result in osteoarthritic changes.

The size of the osteochondral defect seems to be a factor in the quality of repair. In the equine femur, articular defects smaller than three millimeters in diameter show complete repair after nine months; however, larger defects do not repair completely (30). Others have also shown that the repair of larger defects is much less predictable and effective than that of smaller defects (2).

5. TREATMENT OPTIONS

5.1. Continuous Passive Motion

In 1970, as a result of many studies showing the deleterious effects of immobilization on synovial joints and the benefits of intermittent motion (31), Salter *et al.* hypothesized that constant motion should be beneficial to joints. (32-34) However, because of the fatigability of muscle, constant motion would have to be passive rather than active (32). They investigated the biological effect of continuous passive motion (CPM) on healing of full-thickness defects in rabbit knees and found that CPM produced healing by hyaline articular cartilage in 52% of the rabbits at three weeks compared to <10% in rabbits that were either immobilized or permitted intermittent active motion (33). The regenerated cartilage was maintained at six months and at one year in 50% of the defects (33,34). It was also noted that the results obtained with only 1 week of CPM were similar to those with 3 weeks of CPM; thus, the first week was deemed the most important (32,34), with a minimum of eight hours per day (35). These studies suggested that the cells responsible for the improved repair originated as mesenchymal cells within the subchondral bone, as the chondrocytes on the intact edges of the defect remained virtually unchanged (32,33).

The biological mechanism of CPM enhancement of cartilage regeneration has not been fully elucidated. Local factors such as compaction and low oxygen tension have been shown to be conducive to selective differentiation of stem cells into chondrocytes rather than fibroblasts and osteoblasts (32). One possible explanation is that the cyclic intra-articular pressure variations created by the pumping action increase the volume and biochemical constituents of synovial fluid and result in enhanced nutrition of the articular cartilage with subsequent increased survival and metabolic activity of chondrocytes (32,36,37). Another hypothesis is that physical transduction mechanisms such as cell and matrix deformation, physiochemical changes, and hydrostatic pressure gradients favor chondrocyte biosynthesis (38). Healing of partial-thickness defects in rabbits was unaffected by CPM (32).

5.2. Surgical Options

For many years, debridement of the joint was a widely used procedure for degenerative processes of the knee. Pridie and Insall described debridement along with drilling in the exposed subchondral bone to encourage fibrocartilagnous repair (39). Ficat *et al.* introduced "spongialization" in which all diseased cartilage was resected en bloc to expose the underlying cancellous bony bed completely (40). With the advent of arthroscopy, modifications in the procedures described above have been made, retaining the basic concepts of joint debridement and subchondral drilling to promote three phase tissue healing.

Chondral shaving of partial-thickness defects provides symptomatic relief for a brief period, but does not stimulate regeneration of cartilage (38,41,42). At the surface, the cartilage instead had become fibrillated with occasional cracks and fissures evident in the surrounding cartilage (43). This lack of regeneration has been attributed to the lack of induction of an inflammatory response since chondral shaving does not violate the tidemark or subchondral bone. Therefore, abrasion arthroplasty and subchondral drilling may be employed; this does result in fibrin clot formation and the ensuing inflammatory response (38,42,44).

Abrasion arthroplasty yields variable results in the repair tissue produced, ranging from fibrous tissue with little type II collagen to hyaline-like cartilage with some type II collagen (38,42). When compared with subchondral drilling, abrasion arthroplasty appears to have worse long-term results with increasing breakdown of the repair and subsequent degeneration (45). Subchondral drilling

seems to promote excellent early repair , with evidence of hyaline articular cartilage formation and increased chondrocyte mitotic and metabolic activity and increased proteoglycan staining (23,24,45,46). However, long term results in rabbits (8-12 months after treatment) showed degeneration and loss of cartilaginous structure with significant PG staining. The surface layer was more typical of fibrocartilage and the tangential orientation of collagen was lost (24,42).

5.3. Treatment Modalities Utilizing Transplantation

5.3.1. Soft Tissue Grafts

Fascia, tendon, muscle, periosteum, and perichondrium have all been used as graft material in soft-tissue athroplasties (4). Perichondrial and periosteal grafts have had the best results. In fracture healing and embryonic limb development, mesenchymal progenitor cells present in the cambium layer of periosteum are capable of differentiation and proliferation (47,48). Local low oxygen tension and nutrient accessibility encourage mesenchymal differentiation into chondrocytes (47,48) Rib perichondrial grafts for full-thickness defects in rabbits have been reported to result in neo-cartilage that is morphologically and biochemically similar to hyaline cartilage (49,50). However, this tissue begins to degenerate after eight to twelve months of normal joint function (51) Clinical trials in humans utilizing perichondrial grafts have also had mixed results. Engkvist and Johannson reported some cases with slightly improved motion and decreased pain, but an equal number with worsened complaints (52) Seradge has used perichondrial grafts in the metacarpophalangeal and interphalangeal joints in humans and found that the age of the patient seemed to influence the results, in that no patient over the age of 40 had good results (53).

Periosteum is more abundant and available than perichondrium and has produced some positive results as a graft material. The cambium layer of periosteal grafts can differentiate into hyaline-like tissue in full-thickness articular defects in rabbits (37,51). Autologous perichondrial grafts and periosteal grafts were tested in the tarsocrural joint of horses. Periosteal grafts resulted in neochondrogenesis in 83% of the animals; the repair rate was 17% with perichondrial autografts (36).

Periosteal grafts in combination with CPM have been examined in the rabbit (51,54-56). The gross appearance of these graft sites, with four weeks of post-operative CPM, showed restored articular surfaces; degeneration was noted in groups that were immobilized or permitted intermittent active motion. In the periosteum/CPM group, the resurfacing remained largely intact at one-year follow-up with slight degeneration at the edges of the adjacent cartilage (56). Again, healing of large defects (>3.5 mm diameter) was significantly inferior to that of small defects (<1mm diameter).

Tsai *et al.* noted enhancement of repair with the use of fibrin adhesive on periosteal grafts in rabbits (57). Fibrin was not as successful in larger animals (58,59). Few clinical trials of periosteal grafts with fibrin glue and joint motion have been performed. In patellar defects of 13 patients using the above combination, Hoikka *et al.* found mixed results at an average of four-years follow-up (60). Clinical concerns persist with the nature and longevity of the repair tissue. As with perichondrial grafts, young patients may be best suited for this procedure (4,42).

Some of the failures of this type of graft (and others) were deemed secondary to inadequate fixation and graft instability that led to loss of chondrocytes and subsequent graft degeneration (61-63). A variety of techniques and "adhesives" for stable fixation have been tested. Adhesives have a long history of use in orthopedic surgery, as they have been utilized in the past for fracture fixation. A good adhesive and delivery substance must be biocompatible, support the transplanted chondrocytes, and provide growth-promoting conditions while protecting the graft from the host immune response. Ossocol, a combination of collagen and fibrous tissue proteins, was one of the earliest adhesives employed. It, however, caused allergic reactions thereby negating its clinical use. Epoxy, acrylic resins, Ostamer (a polyurethane foam), cyanoacrylate, and Tisseel (a mixture of fibrinogen and factor XIII) have all been tried as adhesives with relatively poor results. Fibrin has been used successfully in repairing defects in meniscus. It mimics a fibrin clot and thus elicits an inflammatory response and stimulates the repair cascade (61,62). Fibrin has also been shown to provide selective permeability of nutritive liquids and trophic agents (64). Other substances utilized include mussel adhesive protein, collagen, chondronectin, and fibronectin. Other methods of graft fixation included use of a sutured periosteal flap, which is technically demanding and may cause micro-trauama to the adjacent tissue (61,62).

5.3.2. Osteochondral Transplantation

Osteochondral transplants have been extensively studied in both animal and human models. Seligman *et al.* evaluated whole knee fresh autogenous grafts and frozen allografts in dogs (65). The frozen allografts showed subchondral collapse (by radiography and histology) at one year. In contrast, the autogenous graft group maintained normal architecture for two years, although histologic evaluation showed survival of cells only in the superficial layer of the articular surface. No evidence of antibody response was found; the investigators thus suggested that the subchondral collapse altered the joint mechanics leading to cartilage destruction. This hypothesis was supported by other studies, which reported on fresh osteochondral grafts that resulted in subchondral bone collapse and creeping substitution of grafted bone in the failed grafts with subsequent altered joint mechanics and deterioration of transplanted cartilage (63,66). Rodrigo *et al.* found that 60% of dogs receiving allografts had cytotoxic antibodies present in their serum, although there was no evidence of graft rejection (67).

Due to long-term problems associated with prosthetic replacement in young patients, osteochondral allografts continue to be used for treatment of various osteochondral defects (especially when associated with

tumors) despite the results of animal studies. Clinical studies have shown mixed results. McDermott *et al* reported on 100 cases of fresh small-fragment osteochondral allografts in femoral osteochondral defects (68). At five-year follow-up, the cartilage showed fissuring, loss of matrix staining with safranin-O, and chondrocyte clumping and degeneration. Some specimens showed no evidence of hyaline cartilage; many had fibrocartilage, while others had denuded bone. There was no evidence of immune reaction; they hypothesized that weaking of the osseous portion of the graft during the revascularization phase, which takes from one to four years to complete, resulted in mechanical alterations leading to degeneration (mcdermott/langer). Kandel *et al.* reported necrosis of the subchondral bone and marrow with creeping substitution of host bone (69). The response seen at the articular surface varied; some specimens showed viable chondrocytes even after 7 years, while others showed degenerative changes ranging from fibrillation to erosion.

Garrett performed a series of fresh osteochondral allografts in 24 patients with defects in the femoral condyles who had failed treatment with abrasion arthroplasty (70,71). All defects were greater than 1.5 cm in diameter. At two-year follow-up, all patients had improved clinical symptoms with decreased pain, buckling, and swelling. Eleven patients had subsequent arthroscopies after grafting and only 2 patients exhibited significant deterioration of the graft. No problems with graft extrusion or rejection were encountered. Others have also reported good clinical results with osteochondral allografts. Gross *et al.* reviewed ninety-two of cases using fresh osteochondral allografts to treat post-traumatic osteochondral defects in the knee (72). They found good results at five (75%) and ten (64%) year follow-up.

These clinical studies have shown that use of osteochondral allografts can provide temporary functional and symptomatic improvement in selected patients. However, the reports of animal studies showing significant immune responses to the grafts with subsequent graft degeneration raises clinical concern. More long-term results are needed to determine whether those clinical cases with osteochondral allografts that are still functioning well can withstand normal joint function and loading and avoid the development of degenerative joint changes (41).

A recently introduced related technique is mosaicplasty, which uses multiple small osteochondral autograft cylinders obtained from non-weight-bearing areas (i.e., the femoral trochlear groove) to resurface defects. The surface of the transplanted plugs maintain their hyaline nature in 60-80% of cases, 73 with fibrocartilaginous tissue acting as filler between the plugs, integrating the transplants with the host tissue. Small grafts are necessary to minimize donor site morbidity; these sites refill with cancellous bone and fibrocartilage (74). Three-year follow-up data of 57 patients was reported by Hangody *et al* ., who found that patients who underwent condylar mosaicplasties had a more frequent repair result than those who had patellar mosaicplasties (73). Biopsies showed that the tissue remained predominantly hyaline in nature.

5.3.3. Chondrocyte Transplantation

For over thirty years, attempts have been made to stimulate repair by transplanting isolated chondrocytes. Several animal models have examined both allograft and and autogenous cells as transplant material. The possibility of host immune response as well as the possible transmission of viral and other diseases favor the use of autogenous tissues. Autologous transplants are being performed clinically; however, the history of allografts for cartilage repair and their immunogenicity merits review.

Cartilage immunogenicity. Cartilage was long considered an immunologically privileged tissue. However, studies have shown that cartilage, and specifically chondrocytes, do possess antigens and are capable of eliciting an immune reponse. In response to isolated chondrocyte allografts in full-thickness articular cartilage defects in rabbit knees, host lymphocytes accumulated around the allografts. Only 26% of the grafts contained viable cartilage cells after three weeks. Both a cell-mediated cytolytic reaction and a humoral immune response were present (75). Macrophages produced a factor which caused release of collagen and proteoglycan degrading enzymes; activated lymphocytes produced factors which inhibited the synthesis of glycosaminoglycans and collagen. Antibodies directed against the major histocompatibility complex of chondrocytes activated lysozymes which may have inhibited matrix synthesis or caused matrix degeneration by inducing release of proteolytic enzymes from chondrocytes (75). Other studies have shown that antibodies may also be directed against other antigenic sites on chondrocytes or against specific sites in the surrounding matrix (76). Many other reports have confirmed the antigenicity of isolated chondrocytes; however, the immune response does not cross the intact matrix (75,78-82). The matrix appears to prevent recognition of antigens on the chondrocytes, protecting them from the host immune response (70,77,81,83-85).

Fresh, frozen, and freeze-dried allografts have decreasing immunogenicity in that order (84). Freezing of allografts diminishes the induction of allo-antibodies, but allows ice crystals to form eventually destroying the tissue. Freeze-drying reduces the overall strength of the graft tissue (86). Liquid nitrogen has been used to provide a cryogenic range of -70 to -80 oC, at which most frozen grafts are now kept; this ensures cessation of all biochemical activity. Irradiation of grafts increases the solubility of collagen and glycosaminoglycans and causes destruction of the fibrillar network of the matrix (83). Techniques have been developed using cryoprotectants such as dimethylsulfoxide (DMSO) and glycerol to allow cells to survive the freezing process (85,87). Schachar *et al.* demonstrated that cryopreserved chondrocytes isolated from rabbits maintained their phenotype and function *in vitro* (88). Chondrocyte viability averaged 91% at 2 weeks.

Culture of isolated chondrocytes. Moskalewski noted that chondrocytes isolated from immature animals resulted in more regular arrangement of cells and matrix similar to that elicited by normal articular cartilage; cells

isolated from mature animals produced a more irregular arrangement (89). Other studies have also shown that isolated chondrocytes from immature animals had significantly better repair capacity than those isolated from mature donors (90,91).

Chondrocytes grown in collagen gels maintain normal morphology and evidence of GAG and type II collagen synthesis *in vitro* for five weeks (92). In monolayer cultures, isolated chondrocytes behave more like fibrous tissue and fibrocartilage, with production of a mixture of both type I and II collagen (93). Approximately 50% of the newly synthesized glycosaminoglycans are lost into the medium; an increased proportion of hyaluronic acid relative to the total GAG content reflected the dedifferentiation of the chondrocytes (92). In clonal or re-aggregated cultures, however, chondrocytes produce a repair tissue which contains type II collagen and whose GAG profile resembles that of articular cartilage (93). The chondrocytes remain differentiated and phenotypically stable and retain their ability to accumulate metachromatic matrix (92). Thus, it would appear that cultured chondrocytes in a clonal or re-aggregated pattern are more appropriate for transplantation. Although type II collagen is the predominant type found in normal hyaline cartilage, type II collagen gels do not function as well as type I collagen as a delivery substance for cultured chondrocytes (94).

In 1995, van Susante *et al.* compared the behavior of chondrocytes in collagen gels to that of alginate gels (a linear polysaccharide isolated from brown algae) *in vitro* (95). There were advantages and disadvantages noted with each type of gel. Whereas there appeard to be increased cellular proliferation in the collagen gel culture, the alginate gel allowed the chondrocytes to maintain their differentiated phenotype for longer periods of time. The collagen gels did not preserve chondrocyte morphology as well as did alginate, but the alginate gel resulted in a significantly lower total number of viable chondrocytes. The success of transplantation would seem to involve a combination of the positive factors of each gel; not only are sufficient numbers of viable chondrocytes required, but these chondrocytes must also maintain their ability to maintain their normal phenotype, migrate, and function *in vivo*.

5.3.4. Allografted Isolated Chondrocytes

With the use of a carrier material, attempts at repair of articular defects with allografted chondrocytes appeared more successful. Itay *et al.* in 1987 used cultured embryonal chick chondrocytes embedded in a biological resorbable immobilization vehicle (BRIV) composed of fibrin to repair 1.5 mm full-thickness articular cartilage defects in the tibial condyles of roosters.64 Defects filled with a smooth, shiny surface which was maintained at 6 months. No sign of rejection was observed.

Wakitani *et al.* reported on a series of allografts which were used to repair defects slightly larger than in the Itay study (96). Cryopreserved articular chondrocytes suspended in collagen gels were thawed and transplanted into 4mm diameter full-thickness articular cartilage defects in rabbit femurs. At one week, there was evidence of hyaline cartilage formation, which became more organized and was maintained up to twenty-four weeks. Chondrocytes in the repair site were determined to have been derived from the implanted chondrocytes, using autoradiographic analysis.. Immunologic response as determined by host direct and indirect blast formation reactions was insignificant (96).

Noguchi *et al.* compared the results of allografted and autografted chondrocytes in repair of osteochondral defects in the rat (97). Cells were embedded in a collagen gel and implanted into 1.5 mm full-thickness defects in femurs. The animals were sacrificed at up to 52 weeks. There was slightly more lymphocytic infiltration noted in the allograft group, although there was no evidence of graft destruction. At twelve weeks, the articular surfaces of both groups were completely covered by hyaline cartilage, and subchondral ossification was noted in 100% of the isograft and 50% of the allograft groups respectively. At 52 weeks, healing was maintained in 100% of the autograft group and 75% of the allograft group. The delay in subchondral ossification of the allograft group prompted the investigators to recommend delayed weight bearing in that group (97).

Small full-thickness defects heal more readily without intervention than large osteochondral defects. Hendrickson *et al.* (1994) reported the use of cryopreserved chondrocyte-fibrin allografts in 12mm diameter full-thickness articular cartilage defects in the trochlea of horses (98). The grafted areas were significantly improved over ungrafted defects at 4 and 8 months, with significantly increased levels of glycosaminoglycans and type II collagen. Sams and Nixon used allogeneic chondrocytes embedded in collagen scaffolds for resurfacing articular defects in an equine model (99). Cryopreserved chondrocytes were incorporated into a type I collagen scaffold, allowing the cells to secrete matrix, thus shielding their surface antigens and providing protection from the host immune response (99). At 4 and 8 month follow-up, the cells in the superficial zone were predominantly fibroblasts. The repair tissue did not fill the defects completely. GAG levels in the repair were significantly higher than that of controls, demonstrating chondrocyte survival and matrix synthesis. When compared to untreated control groups, the GAG levels in the surrounding cartilage were much better maintained, suggesting that the deleterious effects of the full-thickness defect on the surrounding cartilage were diminished by the chondrocyte grafts (29). The percentage of type II collagen in the repair tissue was reported as "high", although percentages did not approximate those seen in normal articular cartilage. There was also no histological evidence of lymphocytic infiltration or other immune response. Sams and Nixon (1995) concluded that only moderate improvement in the gross and histologic quality of the repair tissue in large osteochondral defects was observed at 4 and 8 months (97).

Not all studies, however, reported the incorporation of allogeneic chondrocytes without any evidence of host immune reaction. Kawabe and Yoshinao

(1991) performed isolated chondrocyte allografts in full-thickness articular cartilage defects in rabbit knees (75). They found good healing initially up to 15 weeks; however, at 24 weeks, they noted replacement of the regenerated tissue with fibrous tissue and subsequent complete degeneration of the cartilage graft, with evidence of both humoral and cell-mediated cytotoxicity. They postulated that even with the relative "immunoprivileged" status of chondrocytes, a host immune response is not completely precluded.

5.3.5. Autogenous Chondrocyte Transplantation

Grande *et al.* (1987) reported the use of autologous cultured chondrocytes in articular defects in immature rabbits (14). Cells were cultured and transplanted autogenously into 3mm diameter full-thickness defects in the patellae, using a sutured periosteal flap to hold the grafted cells in place. At six weeks the implanted area contained a hyaline-like regenerate (14,62). Brittberg *et al.* reported on the results of autogenous chondrocyte grafts in full-thickness defects in adult rabbit patellas (100). Chondrocytes which had been expanded *in vitro* were injected under a sutured periosteal flap, with the patch sealed with fibrin. At 52 weeks, the repair tissue appeared to be fully mature with a high degree of chondrocyte columnarization and organized extracellular matrix formation. Control groups with only a periosteal flap sutured over the defect showed fragmented, dense, and disorganized fibrous repair tissue with mild synovitis and osteophyte formation.

The results of the control periosteal group observed by Brittberg *et al.* (100) conflict with those of others who demonstrated the proliferative and reparative qualities of mesenchymal stem cells in periosteum (51,54-56). However, several differences exist between these laboratory studies. In Brittberg *et al* .'s study, mature animals were used; as noted above, their periosteal flaps appear to be limited in their repair capacity. O'Driscoll *et al.* (1986) used primarily immature animals, with a greater population of mesenchymal stem cells than older animals. Brittberg *et al*. (100) sutured periosteal flaps with the cambium layer (the source of the mesenchymal stem cells) facing the defect, whereas the O'Driscoll studies positioned the cambium layer facing into the joint. O'Driscoll *et al*. showed that orientation of the cambium layer plays a significant role in the subsequent repair response (56).

Experiments using autogenous chondrocyte transplantation were also performed in larger animal models . Autologous chondrocyte transplants were performed in chondral and osteochondral defects in dogs (101,102). Defects measuring 2-3 mm in diameter were created in the weight-bearing portions of femoral condyles of 29 dogs. Autologous chondrocytes grown in culture were transplanted into defects (secured with sutured periosteal flaps) and the results were compared with an ungrafted defects or a defects treated with fibrin glue (and no cells) secured with a periosteal flap. At six weeks, the defects with chondrocyte grafts showed a slight increase in the total number of chondrocyte-like cells with evidence of some type II collagen present. The periosteal flap/fibrin group defects and ungrafted defects filled with fibroblast-like cells and fibrous scar tissue. At one-year follow-up, degeneration of all the regenerated tissue was seen, with little difference between the periosteal flap/fibrin group and the chondrocyte transplantation group. The failure was attributed in part to the unmonitored activity of the dogs over the course of healing. It was postulated that the regenerated tissue seen at six months was probably not yet completely mature and mechanically sound; as a result, the unmonitored weight-bearing and loading of the repair tissue may have contributed to its degeneration.

Brittberg *et al.* (1994) utilized autogenous chondrocyte transplantation in human subjects (103). Twenty-three patients ranging from 14 to 48 years of age who had previously diagnosed full-thickness defects of the articular surface in either the femur or patella secondary to trauma or osteochondritis dissecans and who had failed initial treatment such as arthroscopic shaving and debridement were included in this study. Previously isolated and cultured chondrocytes were transplanted into defects ranging from 1.6 to 6.5 cm in diameter with sutured periosteal flaps. Initially all patients improved. Arthroscopic evaluation at 3 months showed repair tissue in the defects although the borders of the repair had not become fully incorporated. At 12 months grafts were firmer and more closely resembled the normal surrounding articular cartilage. Histologic evaluation of samples obtained at 12 months showed that 11 of 15 femoral grafts had a hyaline-like appearance, with positive immunostaining for type II collagen. The results in patellar transplants were poorer; only 1 of 7 grafts exhibited hyaline-like regeneration. Biopsies of the remaining femoral and patellar specimens revealed central areas of fibrous tissue surrounded by occassional areas of hyaline-like tissue. Percentages of chondrocyte viability and type II collagen composition were not provided, and more detailed examination of the underlying repair tissue was not performed.

Longer-term follow-up to an average of 24 months showed good results in 14 of 16 patients in the femoral group, and in 2 of 7 patients in the patellar group at 36 months. There was no evidence of immunologic reaction in any patient. Possible explanations for the discrepancy seen between the healing rates and tissue quality of the femoral and patellar groups include the fact that the underlying causes of the patellar lesions such as malalignment or lateral patellar subluxation were not treated at the time of grafting. There may also be greater contact stresses in the patellofemoral joint than in the tibiofemoral joint which could also account for the less successful results in the patellar group. It was recommended that concomitant correction of the underlying joint abnormality be performed with the transplant procedure to optimize the healing conditions (103).

There are several possible explanations for the repair observed by Brittberg *et al* (1994). The cultured chondrocytes may have proliferated and repopulated the

articular defect, subsequently modulating the repair process. Alternatively, the sutured periosteal flap used to maintain graft position may have been responsible for differentiation and subsequent synthesis of the repair tissue. Finally, the periosteum and transplanted chondrocytes may have acted synergistically to stimulate cells either in the grafts or the surrounding articular surface to regenerate the surrounding tissue.

Minas *et al.* (1996) have also reported preliminary data on a clinical series of autologously transplanted cultured chondrocytes in humans (102). Sixteen patients were treated for full-thickness chondral defects. With an average follow-up of six months, only one patient had a poor result requiring revision of the procedure. At three months, approximately 50% reported decreased pain and catching; by six months this percentage increased to 75-80%. However, autogenous chondrocyte transplantation is generally recommended for a limited group of patients. Menche *et al.* (1998) recommend the procedure only for patients younger than 50 years of age and only for grade IV chondromalacia or full-thickness chondral defects on the femoral side of the knee, but not for the patellar or tibial surfaces (104). Minas also advises against the procedure if there is concomitant grade III or greater chondromalacia on the tibial surface (102).

5.4. Tissue Engineering and Gene Therapy

5.4.1. Tissue Engineering

Tissue engineering concepts have been applied to a variety of bio-materials to design chondrocyte-seeded or cell-free implants for articular cartilage repair. Among the materials in these engineered devices are demineralized or enzymatically treated bone (105,106), polylactic acid (107), polyglycolic acid (108), hydroxyapatite/ Dacron composites (109), fibrin (98), collagen gels (62,96,110), and collagen fibers (111,112). Some of these materials allow formation of a repair tissue that resembles normal cartilage; however, the repair is often accompanied by substantial fibrocartilage formation. Limited availability of donor chondrocytes has led to the use of alternative cell sources and techniques. Synovial tissue has been used with limited success (113) Tissues rich in mesenchymal stem cells have also been tested as grafts. Wakitani *et al.* (1994) used osteochondral progenitor cells from either bone marrow or periosteal tissue, which they have termed "mesenchymal stem cells (MSCs)", to repair articular cartilage defects (110). Adherent cells from bone marrow and periosteum were isolated , cultured in a type I collagen gel, and transplanted into full-thickness defects in rabbit femoral condyles. Similar results were seen with both types of progenitor cells. By 2 weeks, the autologous MSCs had differentiated into chondrocytes. At 12 weeks, the subchondral bone was completely restored and the defects were filled with hyaline-like cartilage, although in some cases there was a gap between the repair tissue edges and the surrounding normal cartilage. By 24 weeks, however, the repair tissue showed thinning. The repaired surface in the periosteal group appeared split and fibrillated. The bone marrow group had a smoother surface, although the cartilage had thinned. Both groups had areas of incomplete integration of the repair and host cartilage. Mechanically, the repair tissue was more compliant than normal cartilage (110).

Peptide growth factors are important regulators of cartilage growth and cell behavior (i.e., differentiation, migration, division, or matrix synthesis or breakdown) (114). These factors are being investigated for their potential to induce host cartilage repair without transplantation of cells, and are being incorporated into engineered devices for implantation. A brief discussion of some of the better known factors will be reviewed.

Insulin-like growth factor (IGF-1) was one of the first chondrotrophic factors to be identified; it stimulates both matrix synthesis and cell proliferation in culture (115). In bovine cartilage explant culture, IGF-1 stimulates proteoglycan production in a dose-dependent manner, but probably does not have this effect on collagen synthesis (116). IGF-1 insufficiency may have an etiologic role in the development of osteoarthritis (117). Some studies have suggested that serum IGF-1 levels are lower in osteoarthritic patients than control groups, while other studies have found no difference. Nevertheless, in humans it has been shown that both serum IGF-1 levels and chondrocyte responsiveness to IGF-1 decrease with age (118). This simultaneous decrease in both the availability of IGF-1 and chondrocyte responsiveness may further upset cartilage homeostasis and lead to degeneration with advancing age.

IGF-1 may have a future role in the treatment or prevention of osteoarthritis. In a canine model of osteoarthritis (anterior cruciate ligament deficiency), investigators administered IGF-1 intraarticularly along with intramuscular sodium pentosan polysulfate (a chondrocyte catabolic activity inhibitor). The treated group had an improved histological appearance, and near-normal levels of degradative enzymes (neutral metalloproteinases and collagenase), tissue inhibitors of metalloproteinase (TIMP), and matrix collagen. These authors emphasized the need for modulation of both the anabolic and catabolic functions in order to improve the net reparative process (119).

Basic fibroblastic growth factor (bFGF) is synthesized by both chondrocytes and the pituitary; it affects articular chondrocytes by inducing replication (106). The effects of bFGF on matrix synthesis are less well understood. bFGF has been shown to either stimulate, inhibit, or have no effect on either proteoglycan or collagen synthesis by chondrocytes, although there do appear to be age-dependent variations in the response of chondrocytes to bFGF (120). In experiment using bovine cartilage explants, low doses of bFGF (3 ng/ml) in young tissues stimulated synthesis and inhibited breakdown of proteoglycan; higher doses (30-300 ng/ml) had the opposite effect of inhibited proteoglycan synthesis and increased degradation. The response of adult bovine cartilage to low doses of bFGF was accelerated degradation of proteoglycans; higher doses stimulated proteoglycan, protein, and collagen synthesis with no cell proliferation (120). bFGF also regulates cartilage homeostasis by inducing the autocrine release of

interleukin 1 (IL-1, a potent stimulator of catabolic behavior in cartilage) by chondrocytes, and enhances IL-1-mediated protease release (121). Due to its ability to stimulate mitosis *in vitro*, it may be useful as an accelerator of the proliferation of chondrocytes harvested for transplantation (117). *In vivo*, Cuevas *et al.* demonstrated that intraarticular infusion of b-FGF produced repair of partial-thickness chondral defects in rabbits at 3 weeks with hyaline-like cartilage containing clusters of viable chondrocytes surrounded by an apparently organized interterritorial matrix (122).

Bone morphogenetic protein (BMP) is a member of the large transforming growth factor beta (TGF-b) family of growth factors. *In vitro* and *in vivo* studies have shown that BMP is capable of inducing differentiation of mesenchymal cells into chondrocytes (123). Skeletal growth factor and cartilage-derived growth factor have been shown to be synergistic with BMP. BMP initiates mesenchymal cell differentiation in the presence of these growth factors and growth hormone; subsequent proliferation of these differentiated cells are stimulated by other factors (124). Recent studies using human recombinant BMP-2 in cell-free collagen sponges to heal articular cartilage defects in rabbits demonstrated repair tissue containing type II collagen at 24 weeks after implantation (125,126).

Transforming growth factor beta (TGF-b) is produced and found in bone matrix, cartilage, platelets, activated lymphocytes, and other tissues (117). The effects of TGF-beta in cartilage are variable; it has been reported to both stimulate and inhibit proteoglycan synthesis and articular chondrocyte proliferation. Many of these differences depend on the state of the target cell, dosage, and cell culture conditions (127-129). TGF-beta is a more potent stimulator of chondrocyte proliferation than other growth factors, including platelet-derived growth factor (PDGF), bFGF, and IGF-1 (127). As with IGF-1, there is a decrease in the proliferative response of human chondrocytes to TGF-beta with increasing age (127). TGF-beta also has the ability to down-regulate the effects of cytokines that stimulate chondrocyte catabolic responses (128). TGF-beta has been reported to have no effect on collagen synthesis, to stimulate proteoglycan production, and to decrease proteoglycan degradation in bovine tissue cultured chondrocytes (116).

In vivo TGF-beta can induce proliferation and differentiation of mesenchymal cells into chondrocytes. Hunziker and Rosenberg reported on the use of timed-release TGF-beta to elicit repair of partial-thickness articular defects using a rabbit model (130). Animals were treated with fibrin clot containing free TGF-beta or free TGF-beta plus liposome-encapsulated TGF-b. They hypothesized that the free TGF-beta would recruit mesenchymal cells to the defect site and that the liposome-encapsulated TGF-beta would subsequently result in chondrogenic cellular differentiation. In animals treated with free TGF-b, mesenchymal cells populated the defect without chondrogenic differentiation; the group treated with both free and liposome-encapsulated TGF-beta had defects containing differentiated chondrocytes with a hyaline-like repair tissue that was maintained through 12 months.

Because growth factors are soluble proteins of relatively small molecular mass that are rapidly absorbed and/or degraded, making them available to cells in sufficient quantity and for a sufficient duration is a challenge. It may be desirable to have different factors present at the repair site during different parts of the developmental cycle, and for varying lengths of time. Delivery vehicles should ideally be biocompatible, resorbable, have appropriate mechanical properties, and release no harmful degradation products; the same criteria can be applied to cell-carrier or tissue scaffold devices. Several natural and synthetic materials (including those used as cell carriers) have been tested as growth factor carriers. While each has several positive features, the optimal material has not been identified. To date, these methods have been hindered by the lack of knowledge of the appropriate dosage, duration of treatment, and joint clearance of growth factors that are administered by these routes.

5.4.2. Gene Therapy

The goal of cartilage gene therapy (a very recent technique) is to deliver a therapeutic gene to chondrocytes or synovial cells that would result in a local product advantageous to cartilage health and/or repair. Under the influence of specific cytokines, chondrocytes, synovium, and inflammatory cells release proteolytic enzymes that can mediate cartilage extracellular matrix degradation. Among these degradative factors are interleukin (IL)-1, IL-2, IL-6, interferon-gamma, and tumor necrosis factors (alpha or beta) (124,131). Introduction of factors that could inhibit matrix degradation and upregulation of the genes responsible for their synthesis are also being examined as therapeutic agents for repair. Both ex vivo and *in vivo* techniques have been used to deliver genes to the affected joint.

IL-I has catabolic effects on cartilage, including the generation of synovial inflammation and up-regulation of matrix metalloproteinases and prostaglandin expression (131-136). Soluble IL-1 receptor antagonist (IL-1ra) is a naturally occurring protein that can inhibit the effects of IL-1 by binding to and preventing IL-1 from interacting with chondrocytes, thereby lowering the effective concentration of IL-1. Much work has focused on delivering, by viral or nonviral vectors, the IL-1 receptor antagonist (IL-1ra) cDNA to synoviocytes or articular chondrocytes. Expression of the transferred genes has been limited, as cell lose their ability to synthesize that protein over a relatively short period of time (usually a few weeks). *In vivo* , gene transfer to chondrocytes by intraarticular injection of HVJ (Sendai virus)-liposome suspension has shown transfection of approximately 20-30% of cells in the superficial and middle zones of the cartilage (137). Results in animal have shown a reduction in the progression of experimentally-induced osteoarthritic and inflammatory arthritis models. With the advent of chondrocyte transplantation, isolated cells have also been

used as a target for gene delivery. The vectors that are currently being explored include the use of recombinant retroviruses and adenoviruses. Concerns over the use of retroviruses include the risk of insertional mutagenesis.

6. CONCLUSION

Injuries to articular cartilage continue to present challenges to orthopedic surgeons. A successful therapeutic method must restore the articular surface with a tissue that approximates the biological and mechanical properties of articular cartilage and withstands long-term joint function. No current method has demonstrated that it can generate a repair tissue that restores the articular surface to a normally functioning level over extended periods, especially in partial-thickness chondral defects. Chondrocyte transplantation has shown promising early results; however the technique remains controversial. Continued research into the role of growth factors in cartilage homeostasis and repair, as well as gene therapy and advances in biomaterial development may yield the optimal therapeutic modality for permanent restoration and prevention of deterioration of injured articular cartilage.

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