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OsteoArthritis and Cartilage (2006) **14,** 403–412
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doi:10.1016/j.joca.2005.10.011

Osteoarthritis and Cartilage

International Cartilage Repair Society

A review of the effects of insulin-like growth factor and platelet derived growth factor on *in vivo* cartilage healing and repair

M. B. Schmidt Ph.D.†*, E. H. Chen Ph.D.‡ and S. E. Lynch D.M.D., D.M.Sc.§||
† *Schmidt Technical Consulting, LLC, USA*
‡ *Chen Consulting, Inc., USA*
§ *BioMimetic Therapeutics, Inc., USA*
|| *Department of Surgery, Vanderbilt University School of Medicine, USA*

Summary

Growth factors may enhance current cartilage repair techniques via multiple mechanisms including recruitment of chondrogenic cells (chemotaxis), stimulation of chondrogenic cell proliferation (mitogenesis) and enhancement of cartilage matrix synthesis. Two growth factors that have been studied in cartilage repair are insulin-like growth factor (IGF) and platelet derived growth factor (PDGF). IGF plays a key role in cartilage homeostasis, balancing proteoglycan synthesis and breakdown. Incorporating IGF into a fibrin clot placed in an equine cartilage defect improved the quality and quantity of repair tissue and reduced synovial inflammation. PDGF is a potent mitogenic and chemotactic factor for all cells of mesenchymal origin, including chondrocytes and mesenchymal stem cells. Resting zone chondrocytes cultured with PDGF demonstrated increased cell proliferation and proteoglycan production, while maturation of these cells along the endochondral pathway was inhibited. Pretreating chondrocytes with PDGF promotes heterotopic cartilage formation in the absence of any mechanical stimulus. PDGF has also been shown to be a potent stimulator of meniscal cell proliferation and migration.

These studies and others suggest a potential role for these potent biological regulators of chondrocytes in cartilage repair. More work needs to be performed to define their appropriate dosing and the optimum delivery method. Combining tissue growth factors with a biological matrix can provide a physical scaffold for cell adhesion and growth as well as a means to control the release of these potent molecules. This could result in biological devices that enhance the predictability and quality of current cartilage repair techniques.
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Key words: Insulin-like growth factor, IGF, Platelet derived growth factor, PDGF, Cartilage, Meniscus, Repair.

Introduction

Traumatic cartilage and meniscus injuries have limited capacity for repair due to the avascular nature of these tissues and relatively low cell density. Left untreated, defects in the cartilage layer often do not heal at all. Biochemical and biomechanical tissue degradation ensues, leading to the irreversible changes that characterize osteoarthritis. A number of tissue engineering approaches have been developed which attempt to provide a foundation for cartilage repair. Scaffolds may be implanted to allow existing cells from the surrounding cartilage or synovium to migrate into the injury site and form repair tissue. A supplemental source of cells may be added prior to or at the time of scaffold implantation in an attempt to insure that sufficient repair tissue forms and that the characteristics of the tissue closely resemble native hyaline cartilage. These techniques have had variable success, however, because the neocartilage often fails to completely fill the defect, become integrated with the surrounding tissue and effectively bear loads[1].

The metabolism of mature articular cartilage is regulated by a number of growth factors that originate from cellular production within the cartilage, as well as from the synovial fluid and surrounding tissues. As the mechanisms of action for these growth factors are established through well-defined *in vitro* studies, it is becoming clear that growth factors may eventually serve to augment current cartilage repair techniques. Chemotactic growth factors may be used to encourage cell migration into an injury site. Cell numbers can be increased and matrix production upregulated by the release of appropriate local growth factors via scaffolds or other methods of intra-articular delivery. The ability of two specific cartilage growth factors, insulin-like growth factor (IGF) and platelet derived growth factor (PDGF), to improve *in vivo* cartilage and meniscus repair will be discussed in this review.

Effects of IGF on cartilage repair

IGF is a circulating cytokine that reaches articular cartilage through the synovial fluid. IGF is a single polypeptide with protein sequencing similar to insulin. Two distinct forms exist: IGF-1 with a molecular mass of 7.5 kDa and p*I* (isoelectric point) of 8.5, and IGF-2 with a molecular mass of 7.4 kDa and p*I* of 7.0[2]. IGF-1 is the most widely studied form with respect to cartilage injury and repair.

IGF-1 is the main anabolic growth factor for articular cartilage. It plays a key role in cartilage homeostasis, balancing proteoglycan synthesis and breakdown by the chondrocytes. *In vitro* studies demonstrate that IGF-1 stimulates proteoglycan production in a dose-dependent manner, as evidenced by increased [^{35}S]-sulfate incorporation[3]. Similarly, IGF-1 has been shown to slow proteoglycan catabolism in a dose-dependent fashion[4]. Collagen production and degradation do not appear to be regulated by IGF-1.

*Address correspondence and reprint requests to: Mary Beth Schmidt, Ph.D., Schmidt Technical Consulting, LLC, 7 Amberg Drive, Pomfret Center, CT 06259, USA. Tel: 1-860-428-3544; Fax: 1-860-963-1991; E-mail: SchmidtConsulting@earthlink.net
Received 20 October 2005; revision accepted 27 October 2005.

Studies of human articular cartilage indicate that serum IGF-1 levels and chondrocyte responsiveness to IGF-1 progressively diminish with age[5]. This suggests that a simultaneous decrease in the amount of IGF-1 available and a reduced ability for the cells to respond to the remaining IGF may make cartilage less capable of maintaining its structural and functional integrity. IGF nonresponsiveness has been further observed to exist in chondrocytes from arthritic cartilage or in the presence of inflammation[2]. The cellular response to IGF-1 is receptor mediated and IGF binding proteins in synovial fluid appear to regulate the amount of free IGF-1 that is available for receptor binding[5]. The age-related decline in the responsiveness of chondrocytes to IGF-1 appears to be due at least in part to overexpression of IGF binding proteins. Chondrocytes from patients with osteoarthritis have been observed to generate excessive levels of IGF-1 binding proteins[6,7]. It has also been suggested that a defect in IGF receptor binding or postreceptor signaling may contribute to IGF nonresponsiveness in aged and arthritic cartilage[2–4].

ACELLULAR REPAIR TECHNIQUES

In the absence of a supplemental source of chondrocytes or precursors, cartilage repair is dependent on cellular recruitment, retention and proliferation at the injury site. An equine model has been developed to examine the effectiveness of cartilage repair methods in large, clinically relevant defects under high intra-articular loading conditions. Critical size, full thickness defects, 15 mm in diameter and extending 1 mm or less into the subchondral bone, were arthroscopically created on the lateral trochlear ridge of the distal femur of adult horses[8]. The defects were filled with fibrin clots loaded with IGF-1. The treatment consisted of adding 25 μg of the growth factor to 1.0 mL calcium-activated thrombin during preparation of the fibrin clot. Control defects were filled with fibrin clots alone. Timed IGF-1 release from the fibrin clot was assumed to occur, although the release kinetics were not characterized.

Analysis of the synovial fluid and synovial membrane at various interim time points showed a mild transient inflammatory reaction to the surgery, but no apparent reaction to IGF-1. All defects were grossly observed to be filled with repair tissue at 6 months, with the IGF-1 treated defects being more completely filled and better attached to the subchondral bone than the control defects (Fig. 1). Histological analysis indicated that the control defects contained poorly organized collagen which was predominantly type I, with pockets of type II collagen. Fibroblasts were the primary cell type found in these defects. In contrast, IGF-1 treated defects contained mainly chondrocytes in an organized collagen matrix consisting predominantly of type II collagen. Overall, IGF-1 treatment was found to significantly improve quantity and quality of defect repair tissue in this model.

The chemotactic effect of IGF-1 on the repair of partial thickness defects created in healthy knee joint articular cartilage was also studied in skeletally mature rabbits and mini pigs[9]. In rabbits, aggressive, rectangular defects 1 mm wide, 0.2–0.25 mm deep and 4–6 mm long were created in the patellar groove and the medial femoral condyle. Similarly located defects in the mini pigs were 0.5 mm wide, 0.6 mm deep and 7–9 mm long. In an effort to render the defect surfaces more favorable to cell adhesion, the defects were briefly exposed to chondroitinase ABC or trypsin to remove surface proteoglycans. A single application of IGF-1 (50 ng/mL) was then either administered topically to the surface of the empty defect or added to a fibrin clot that was placed in the defect site. Based on defect volume, the total dose of IGF-1 was approximately 40–75 ng in the rabbits and 110–140 ng in the mini pigs. The effects of these treatments on the repair process were examined at intervals out to 48 weeks.

IGF-1 treatment resulted in an inconsistent but increased number of mesenchymal cells present in the defect site at 5

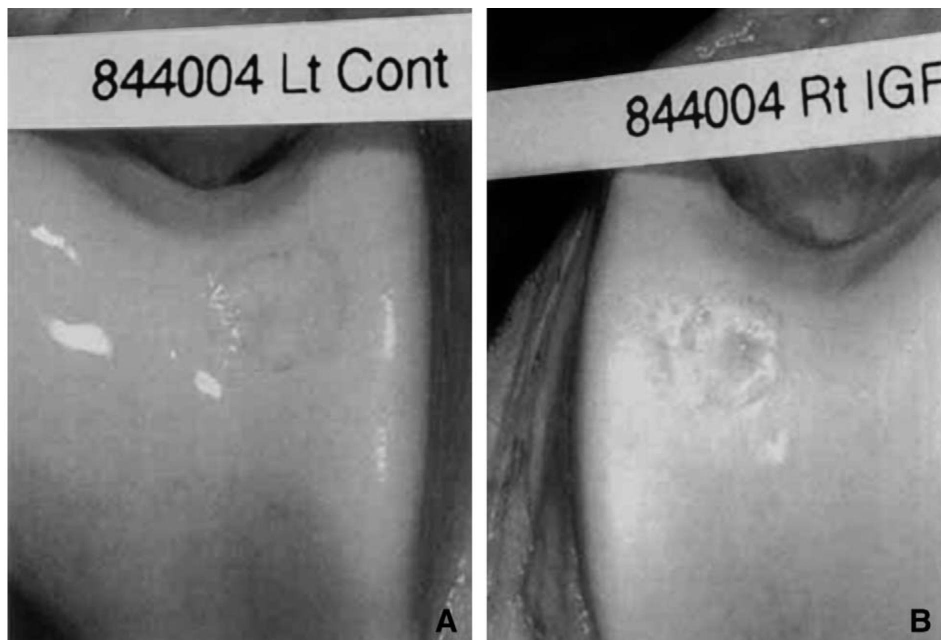

Fig. 1. Macroscopic appearance of control (A) and IGF-1 treated (B) cartilage defects in an equine model at 6 months post-op. Treated defects were filled with a fibrin clot supplemented with IGF-1 at the time of surgery. Control defects were filled with a fibrin clot alone. IGF treatment improves the quantity and quality of new cartilage filling the defect[8].

weeks. The source of these early repair cells was suggested to be the synovial membrane and its underlying tissues. The addition of the fibrin clot enhanced the cell density, although the repair cells eventually remodeled the fibrin matrix and replaced it with an avascular fibrous tissue. Good integration was seen at the defect margins in IGF-1 treated animals, with thin peripheral bands of differentiated chondrocytes and hyaline-like cartilage present. Few chondrocytes appeared to migrate into the defect site, however, despite the presence of the fibrin scaffold. At 48 weeks, predominantly fibrous connective tissue persisted in all defects treated with IGF-1 in the rabbits and the mini pigs. The reduced effectiveness of IGF-1 treatment in this study, as compared to the results obtained in the equine model, may be related to the lower total dose of IGF used in rabbits and mini pigs and the absence of a carrier scaffold.

CELL-BASED REPAIR TECHNIQUES

A second study using the full thickness defect model in horses, previously described, demonstrated that IGF-1 may also be used to enhance cell-based cartilage repair[10]. In this case, chondrocytes were isolated from the patellofemoral cartilage of immature horses and expanded in culture. The control and IGF-1 loaded (25 μg/defect) fibrin clots were prepared as before, with 20×10^6 cells added to all clots immediately before implantation into mature horses.

Synovial fluid analysis indicated that early white blood cell counts were reduced in the IGF-1 treated joints, suggesting that there may have been a decreased postoperative inflammatory response. The histological score for the synovial membrane was also significantly better for the IGF-1 treated joints as compared to the controls, further demonstrating that IGF-1 may have reduced the effects of inflammation. Quantitative gross assessment scores at 8 months were significantly better for the IGF-1 treated defects as compared to the control defects (Fig. 2). The addition of IGF-1 improved the overall continuity and consistency of the repair tissue. IGF-1 treated defects appeared to be more completely filled with repair tissue, better integrated with the surrounding host tissue than the controls and exhibited less surface fibrillation.

Although the mean cartilage histological scores for the control and IGF-1 treated defects were not statistically different, it appeared that the addition of IGF-1 to a cell-seeded fibrin scaffold improved the quality of the resulting repair tissue. The cellularity and pattern of cell distribution in repair tissue for IGF-1 treated defects appeared to be more organized and less like fibrous tissue than the control joints. Toluidine blue matrix staining for extracellular proteoglycan distribution was significantly improved for the IGF-1 treated defects. In addition, there was a trend toward greater type II collagen gene expression in the IGF-1 treated lesions. However, there were no statistically significant differences in DNA content, hydroxyproline content or measured proteoglycan content in the repair tissue for treated and control joints.

INTRA-ARTICULAR INJECTION

Since growth factors have short biological half-lives, it may be necessary to provide extended exposure in order to sufficiently stimulate cellular repair activities. The effect

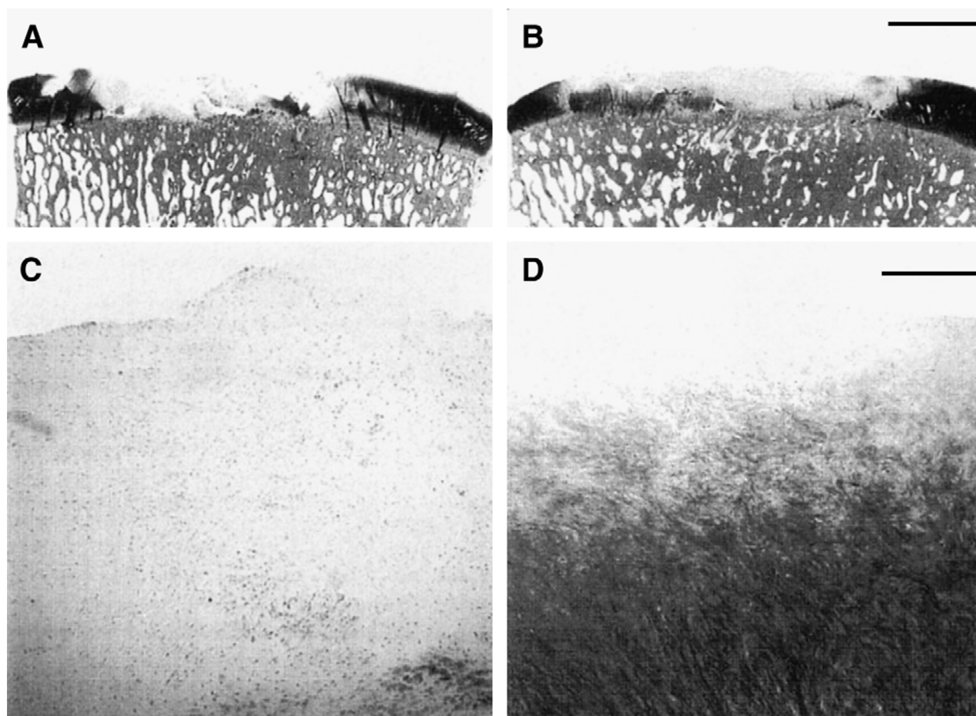

Fig. 2. Histological appearance of cell-seeded cartilage defects in an equine model at 8 months post-op. Treated defects were filled with a fibrin clot seeded with chondrocytes and supplemented with IGF-1. Control defects were filled with a similarly seeded clot without the growth factor. Hematoxylin and eosin staining of entire control defect (A) and IGF-1 treated defect (B) demonstrates slightly improved fill in the treated defects. Comparison of toluidine blue staining of control tissue (C) and treated (D) repair tissue shows a significant increase in staining intensity in IGF-1 supplemented repair tissue[10] (A, B bar = 5 mm; C, D bar = 80 μm).

of continuous intra-articular IGF-1 infusion on cartilage healing was studied in rabbits implanted with osmotic drug pumps[11]. Full thickness defects 1.2 mm wide and 8 mm long were created on the femoral condyles of adult rabbits, without disrupting the subchondral bone. No scaffold or cell supplementation was provided within the defect. IGF-1 or a control solution (saline) was delivered directly into the joint space for up to 8 weeks. The daily delivery volume was 62 μL, which corresponded to a daily dose of 30 ng of IGF-1. Growth factor treatment at this dose level produced no significant improvement in cell number or proteoglycan synthesis in the defect, or in the amount or quality of repair tissue that filled the defect.

The therapeutic value of periodic, low dose IGF-1, intra-articular injections into osteoarthritic knee and temporomandibular joints in mice was also found to be ineffective[12]. The effects of IGF-1 administration on the cartilage morphology, as well as the anabolic and mitogenic activity of the chondrocytes, were measured. Serial 60 μL intra-articular injections of IGF-1 (25 ng/60 μL) were made in 20-month-old mice every 3 days for a total of 9 days. IGF-1 treatment slightly increased DNA content and proteoglycan content, as compared to untreated controls. Acid phosphatase activity was also moderately increased, while alkaline phosphatase activity was significantly reduced. These results indicate that short-term exposure to the growth factor increased the metabolic activity of the chondrocytes. It is unknown whether this effect is sustained after the treatment is terminated.

Similarly, IGF-1 alone failed to slow or reverse ongoing cartilage degeneration in a canine anterior cruciate ligament (ACL) deficient model (Pond–Nuki model) of osteoarthritis[13]. Ligament transection was performed and the animals received no treatment for 3 weeks. Intra-articular injections of 1 μg of IGF-1 were then administered three times a week for the next 3 weeks. At the conclusion of the study, the damage to the condylar cartilage for the IGF-1 treatment group was extensive and indistinguishable from the osteoarthritic group that received no treatment. Tissue protease levels for IGF-1 treated cartilage were more similar to osteoarthritic cartilage than normal cartilage. The uronic acid and hydroxyproline content were somewhat elevated relative to arthritic cartilage, but less than normal levels. When intra-articular IGF-1 treatment was coupled with systemic doses of polysulfated polysaccharide (PPS), however, a significant decrease in cartilage degradation was seen. This suggests that PPS may inhibit synthesis of proteases that degrade IGF-1 or its receptor, or affect its binding protein. Therefore, more IGF-1 may be able to reach the chondrocytes and an enhanced repair response is achieved.

Effects of PDGF on cartilage repair

PDGF is a locally produced and locally acting growth factor. It is synthesized by smooth muscle cells, fibroblasts, endothelial cells and macrophages and stored primarily in platelets[14]. PDGF consists of two distinct disulfide linked peptide chains, A and B, which share a 60% protein sequence identity. The growth factor can be expressed as a homodimer (PDGF-AA or PDGF-BB) or as a heterodimer (PDGF-AB), with a molecular mass of approximately 30 kDa.

PDGF plays a fundamental role in the wound healing cascade. It is present in high concentrations in platelets and in the fluids generated during the early stage of wound healing[15]. PDGF is a potent mitogenic and chemotactic factor for cells of mesenchymal origin, including fibroblasts, osteoblasts and chondrocytes, and is thus believed to be capable of enhancing tissue regeneration and repair. PDGF receptors have been identified on a number of cell types including chondrocytes and the number of receptors is upregulated by the presence of inflammatory cytokines such as interleukin-1 (IL-1)[16].

Indirect evidence for the role of PDGF and other growth factors active in the wound healing process can be seen from the healing response in cartilage defects treated with microfracture. This procedure involves creating microperforations in the subchondral bone with an arthroscopic awl in and around a chondral lesion[17]. The mechanical integrity of the bone is maintained through careful placement of the holes. The awl is driven to a depth of 2–4 mm to insure that the marrow space is accessed and bleeding is observed. A clot forms in the defect, which is anchored to the bone by the increased surface roughness produced by the microperforations. Growth factors such as PDGF are released into the defect site, exerting chemotactic and mitogenic effects on cells in the surrounding cartilage and infiltrating mesenchymal stem cells. This provides an enriched environment for new tissue formation which may be augmented by placement of a scaffold seeded with autologous cells[18,19].

CHONDROCYTE PROLIFERATION AND MATURATION ASSAY

The effect of PDGF-BB on chondrocytes was investigated by exposing confluent cultures of rat costochondral resting zone chondrocytes to a range of doses of PDGF-BB and culture times[20]. At confluence, cells were incubated with 0, 2.3, 4.7, 9.5, 19, 37.5, 75, 150 and 300 ng/mL for 24 h. Time course studies were also performed, where confluent cultures were exposed to either 37.5 or 150 ng/mL for 1–10 days with media changed every second day. Cultures were assayed for cell proliferation via cell number and [^{3}H]-thymidine incorporation, alkaline phosphatase specific activity via *p*-nitrophenol release, proteoglycans synthesis via [^{35}S]-sulfate incorporation, matrix protein synthesis by [^{3}H]-proline incorporation, and resting zone chondrocyte differentiation by monitoring responsiveness to vitamin D metabolites.

Increased proliferation of chondrocytes was observed with concentrations of PDGF-BB from 4.7 to 300 ng/mL with a maximum number of cells at 75 ng/mL. [^{3}H]-Thymidine incorporation was increased at all concentrations examined as compared to controls. Longer-term exposure to PDGF-BB resulted in increased cell numbers but the effect decreased over time and at 10 days, PDGF-BB treated cells were not significantly different from untreated controls.

Long-term exposure to PDGF-BB inhibited alkaline phosphate specific activity, particularly at the 150 ng/mL dose, while short-term incubation at all concentrations of PDGF-BB had no effect[20]. PDGF-BB stimulated proteoglycan production 2.5–3.5 fold in a dose-dependent manner (Fig. 3). In addition, PDGF-BB had no effect on [^{3}H]-proline incorporation, indicating no change in collagen production.

Strikingly, PDGF-BB treatment inhibited the maturation of the resting zone chondrocytes[20]. Cells maintained their chondrocytic phenotype compared with controls. These results indicate that PDGF-BB has a direct effect on chondrocytes isolated from growth plate cartilage. Exposure to PDGF-BB in culture appears to increase cell proliferation and cartilage proteoglycan production, while preventing progression of cell maturation along the endochondral pathway. In order to fill a defect site with cartilage, it is important that the chondrocytes should be capable to some extent of

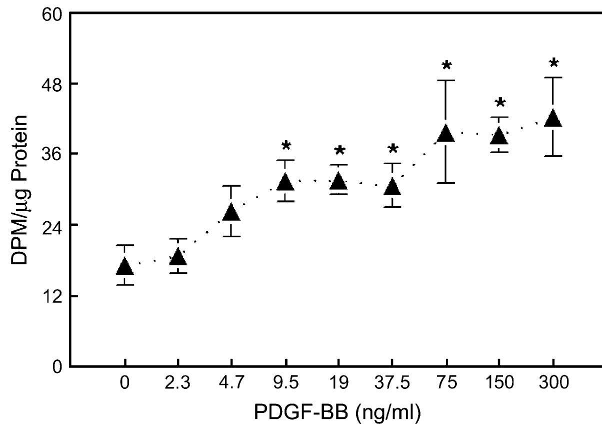

Fig. 3. [^{35}S]-Sulfate incorporation of confluent cultures of resting zone chondrocytes demonstrates a dose-dependent response, following treatment with PDGF-BB for 24 h. Values are the mean ± S.E.M. of six cultures, *$P < 0.05$, treatment vs control[20].

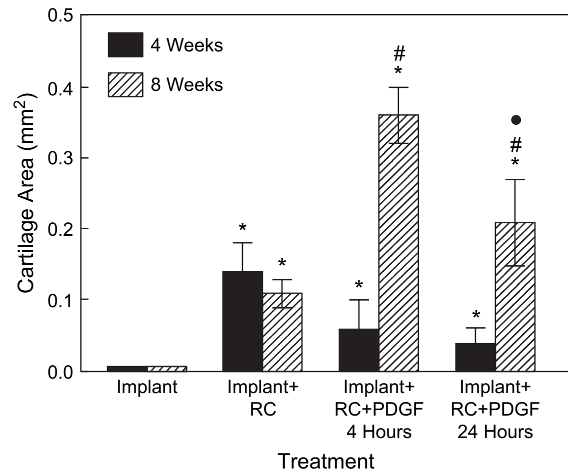

Fig. 4. The effect of PDGF-BB pretreatment of resting zone chondrocytes on heterotopic cartilage formation (*$P < 0.05$, all groups vs implant alone, #$P < 0.05$, 4 vs 8 weeks, ● $P < 0.05$ PDGF-BB treatment for 24 vs 4 h)[21].

both proliferation and differentiation. This is particularly critical at the interface of the cartilage and subchondral bone to prevent delamination of the repair tissue. Terminal chondrocyte differentiation, as evidenced by matrix mineralization, should be minimized for a chondrogenic implant in the joint space to prevent osteophyte formation.

HETEROTOPIC CARTILAGE FORMATION ASSAY

The effect of pretreatment with PDGF-BB on the ability of costochondral cells to form heterotopic cartilage was investigated to potentially develop a readily available source of chondrocytes for cartilage repair[21]. Cells were isolated from the rib tissue of adult rats and expanded in culture. Confluent cultures were then incubated in media containing 37.5 ng/mL PDGF-BB for 4 or 24 h. The cells were subsequently seeded onto scaffolds made from polylactic acid (PLA)/polyglycolic acid (PGA) copolymer foams. Controls consisted of scaffolds loaded with cells without growth factor pretreatment and scaffolds without cells. The scaffolds were implanted intramuscularly in nude mice and the chondrocyte response was evaluated at 4 and 8 weeks postoperatively.

Histological analysis revealed that cartilage was present in all scaffolds at 4 and 8 weeks. The amount of newly formed cartilage varied significantly with the pretreatment duration, as well as postoperative time. At 4 weeks, cells pretreated with PDGF-BB for 4 or 24 h produced less cartilage than untreated cells (Fig. 4). But by 8 weeks, the amount of cartilage produced by the pretreated cells was significantly increased as compared to the untreated control cells. The longer pretreatment with PDGF-BB was found to be detrimental, however. Cells pretreated for 24 h produced 42% less cartilage at 8 weeks than cells pretreated for 4 h. The results of this study indicate that short-term pretreatment with PDGF-BB can promote heterotopic formation of cartilage by costochondrocytes at 8 weeks in the absence of any mechanical stimulus. PDGF also caused increased proliferation and extracellular matrix sulfation without causing an increase in alkaline phosphatase, indicating that the cells remained in a less differentiated state. This effect may play a role in the reduced amount of cartilage seen at 4 weeks. By 8 weeks, chondrogenesis was increased, possibly due to the increased pool of chondrocytes with the resting zone phenotype[21].

INTRA-ARTICULAR INJECTION

The safety of repeated PDGF-BB injections on healthy knee joint cartilage and subchondral bone growth was investigated in skeletally immature rats[22]. Short-term effects were examined by giving the animals intra-articular injections of PDGF-BB (0.5 or 0.75 µg/injection) for 5 consecutive days. Histological samples were then collected at intervals from 7 to 35 days. Long-term effects were determined by administering injections of 0.5 µg PDGF-BB for 5 days, then 0.2 µg daily for 5 or 10 days. The injection series was then repeated on days 30, 45, 90 and 180.

No gross changes to the knees injected with PDGF-BB were noted for the short-term and long-term treatments. No changes in chondrocyte morphology were observed and there was no evidence of fibrosis or inflammatory cells. A minor transient increase in osteogenic activity was frequently noted in the subchondral bone following the series of PDGF-BB injections. PDGF-BB treatment also induced one incident of heterotopic cartilage formation in the patellar tendon and one incident of ossicle formation. Overall, PDGF-BB was found to be safe and well tolerated by young rat joint cartilage.

Growth factors and meniscus repair

The importance of the meniscus to normal knee joint function is well established. The meniscus provides load transmission across the joint, as well as shock absorption, stability and lubrication. Injuries to the meniscus are common and usually related to excessive torsional loading. Complete removal of the meniscus following injury results in accelerated cartilage degeneration due to the significant increase in contact stress. Even partial meniscectomy is acknowledged to be detrimental to joint cartilage and therefore methods to reconstruct and repair the meniscus continue to be developed.

The ability of the meniscus to heal is highly dependent on the location of the injury. The inner two-thirds of the meniscus has relatively low vascularity and cellularity, and thus does not heal well. Injuries to the peripheral, more highly vascularized region, have a more favorable prognosis. Growth factors and cytokines present in blood may in part be

responsible for the superior healing of meniscal tears in the peripheral region. Potentially, growth factors may be used therapeutically to stimulate proliferation of fibrochondrocytes in a meniscus injured in the avascular region, as well as migration of cells to the injury site. This concept has been explored to a limited extent *in vitro* with variable results.

RESPONSE VS LOCATION

The *in vitro* response of bovine meniscal fibrochondrocyte cells to PDGF-AB and IGF-1 has been compared for different regions of the meniscus[23]. Cells were isolated from the peripheral vascular region, the intermediate region and the inner avascular region. The effect on DNA synthesis was determined for growth factor concentrations of 0.1, 1.0, and 10 ng/mL. The chemotactic response was measured in cell migration studies using collagen coated chemotaxis chambers and growth factor concentrations of 1, 10, and 100 ng/mL. The upper chamber contained cells suspended in medium and no growth factor, while the lower chamber contained medium with growth factor. The number of cells that migrated through the separating membrane was then counted as a measure of the chemotactic effect of the growth factor.

PDGF-AB was found to stimulate DNA synthesis in cells from all three regions of the meniscus in a dose-dependent manner, with maximal stimulation at the highest concentration of 10 ng/mL. PDGF-AB also stimulated migration of meniscal cells from all regions with a maximum effect at 10 ng/mL. Migration appeared to be inhibited above this concentration. In contrast, IGF-1 had no significant effect on DNA synthesis at any of the concentrations studied. Significant IGF-induced chemotaxis was seen at 100 ng/mL only for cells isolated from the middle and inner zones. The results from this study indicate that PDGF-AB is a potent stimulator of meniscal cell proliferation and migration, while IGF-1 is not as effective.

A similar comparison of the mitogenic response to PDGF-AB in the peripheral and intermediate regions was performed using lateral and medial sheep menisci[15]. Menisci were sectioned vertically and explanted sections were cultured up to 4 days with 1–200 ng/mL PDGF-AB. Autoradiographic results showed increased DNA content for PDGF-AB treated tissue in both the peripheral region and the intermediate region. There were significantly more labeled cells present in the peripheral region, as compared to the intermediate region, for all growth factor concentrations. In this study, a dose of 100 ng/mL PDGF-AB produced maximal increase in DNA content in the peripheral region. No statistical differences in the response were observed between the lateral and medial menisci.

EFFECT OF MECHANICAL STIMULATION + GROWTH FACTORS

The effects of static compressive loading coupled with PDGF-AB or IGF-1 on matrix production were studied in bovine meniscal tissue explants[24]. Full thickness cores were obtained from the intermediate partially vascularized region of immature bovine menisci. These cores were sectioned and the sections were placed individually in culture. The effects of growth factor dose on newly synthesized proteins (primarily collagen) and proteoglycans were examined, as well as the temporal effects at a single concentration. The interaction between chemical and mechanical stimuli was explored by applying static compressive loads sufficient to achieve 25% and 50% deformation for 4 days.

PDGF-AB had a significant stimulatory effect on matrix production with maximal stimulation of matrix production at 100 ng/mL. IGF-1 was also found to stimulate matrix synthesis with maximally effective dose at 300 ng/mL. IGF-1 generally had a weaker stimulatory effect than PDGF-AB. Significant and sustained stimulation of proteoglycan synthesis was seen for both PDGF-AB and IGF-1 (Fig. 5), although in this study neither growth factor treatment significantly affected DNA content. Static compressive loading had an inhibitory effect on matrix production for both growth factors and at both levels of compression. This result suggests that PDGF especially enhances a beneficial effect on healing of meniscal injuries and the *in vivo* studies of growth factor enhanced meniscal repair may be affected by joint loading patterns and animal activity levels.

Animal models for cartilage repair

Evaluation of potential cartilage repair techniques requires the choice of an appropriate *in vivo* articular defect model. Unfortunately, due to the physiological and anatomical differences between human joints and those of experimental animals, there is no animal defect that perfectly models human cartilage injuries.

The size of the defect relative to the overall size of the joint must be carefully considered when planning a cartilage repair study. Creation of critical size defects is important, since variable and spontaneous healing in noncritical defects does not provide a proper control for comparison. But the effects of oversized defects must also be considered. For example, the defects created in the two rabbit studies summarized in this review were 1–1.2 mm wide and 6–8 mm long, representing a considerable proportion of the knee joint surface area for these animals[8,11]. The removal of an excessive number of native cells and destruction of the intrinsic mechanical integrity of the remaining cartilage may make tissue regeneration in these large defect sites extremely challenging.

The location of the defect on the joint surface is another important factor that influences cartilage repair. The stress distribution on the surface of the knee joint, for example, varies significantly with different animal species. In order to approximate cartilage injuries that often occur in high weight bearing areas on human knee condyles, for example, defects should be positioned at the appropriate location that corresponds to this condition for the species. An advantage of very large quadruped animal models, such as the previously described horse model, is that relatively large defects can be easily created in most locations on the knee surface.

The penetrating depth of the cartilage defect significantly influences healing and generation of repair tissue. Defects can be divided into three depth categories: (1) partial thickness defects, in which the defect is entirely contained in the cartilage layer; (2) full thickness defects, in which the defect extends down to, but not into, the subchondral bone; and (3) osteochondral defects which extend into the subchondral bone[25]. Cartilage repair is generally minimal in partial or full thickness defects that are wholly contained in the cartilage layer because there is no contribution from vascular elements[3]. Perforating the subchondral bone results in bleeding, influx of marrow cells and clot formation at the defect site. Circulating cytokines, a supplemental source of mesenchymal cells and a natural scaffold enrich the defect site and enhance the healing process.

Finally, most cartilage repair investigations utilize acute defects, which are surgically created and treated in the

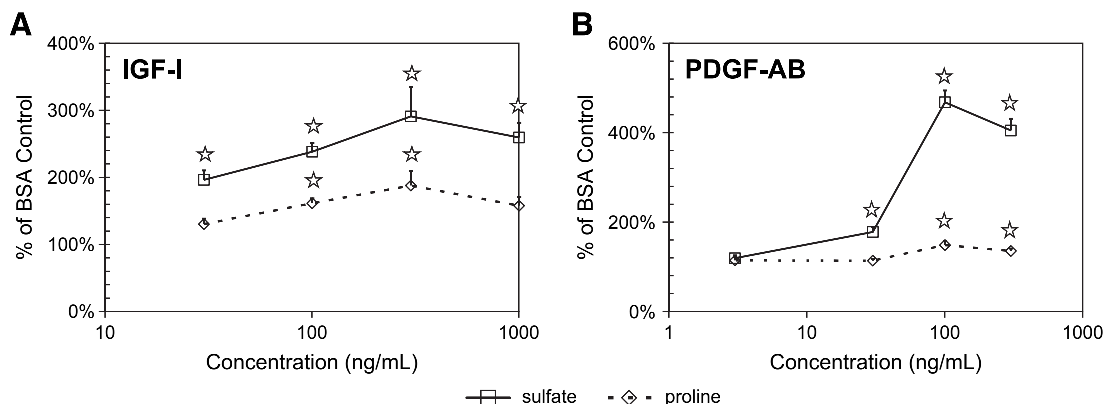

Fig. 5. Dose–response results for proteoglycan synthesis in meniscal explants exposed to IGF-1 (A) and PDGF-AB (B). Explants were subjected to static compressive loads for 4 days. Data are normalized to basal medium (BSA) control values. Star denotes that the value is significantly different from the BSA controls ($P < 0.05$). Both growth factors stimulated proteoglycan synthesis, with PDGF-AB exhibiting a stronger effect[24].

same procedure. But many patients seeking clinical treatment present with persistent joint pain caused by chronic cartilage injuries and degradation. Therefore, the age of the defect and the development of prior degenerative changes are important considerations for *in vivo* study design. Using multiple surgeries, a defect can be created and left untreated for a period of time before intervention, initiating changes in the joint environment that are analogous to chronic defects[25]. Alternatively, an established model of osteoarthritis, such as the Pond–Nuki, ACL-deficient canine model[26], may be utilized in conjunction with a cartilage defect. This combination may provide a better representation of the clinical condition.

Carrier materials for growth factor delivery

Growth factors are made up of soluble proteins of relatively small molecular mass that are rapidly absorbed or degraded. It is assumed that sustained release is required to make them available to cells in sufficient quantity and for sufficient duration to be of therapeutic value, although there is little data available on the effects of release kinetics. Furthermore, the optimum release profile is likely to differ depending on the specific action of the particular growth factor. For example, it may be advantageous to release chemotactic agents rapidly in the initial phase of the repair process to recruit the cells needed to lay down the repair tissue. Mitogenic agents, in contrast, may need to be released over a longer period to insure that the repair tissue adequately fills and matures within the defect.

Native or synthetic carrier materials may be used for cartilage repair, depending on the mechanical demands of the defect site and the rate of degradation required. Native materials offer the advantage of typically providing excellent biocompatibility and optimal surface properties for cell attachment and ingrowth. But the ability to control growth factor loading and release from native materials such as fibrin, collagen, hyaluronic acid (HA) and demineralized bone matrix may be limited by the sensitivity of these materials to processing conditions, as well as the nature and extent of the physical and chemical interactions which occur with the growth factors. In contrast, degradable, synthetic polymers, such as the PLA/PGA copolymer used for the heterotopic cartilage formation assay discussed previously[21], may offer tailorable growth factor release and carrier degradation profiles due to the wide variety of ways these polymers can be processed.

Discussion

The effects of IGF and PDGF on cartilage and meniscus repair are summarized in Figs. 6 and 7. These growth factors offer a promising way to augment existing cartilage regeneration techniques by stimulating cell migration to the wound site, cell proliferation and matrix production. The correct balance of these repair activities must be achieved in order for biologically and mechanically functional tissue to be successfully formed. The role of growth factors in regulating the metabolic balance is now beginning to be understood. Systematic *in vitro* studies have allowed the cellular responses to specific growth factors to be characterized without the complicated biological interactions that exist in the *in vivo* environment, the effects of mechanical stress, and the limited half-life of most growth factor peptides. However, a more comprehensive understanding of the effect of growth factors on the *in vivo* enhancement of the cartilage repair process is needed.

Variable results for growth factor treatments are still commonly reported for *in vivo* cartilage repair studies. For example, defects within the cartilage layer treated with fibrin clots loaded with IGF-1 were filled by predominantly fibrous tissue in rabbits and mini pigs, but well-organized tissue resembling hyaline cartilage was observed in the horse model. The more favorable outcome in the latter study may be due in part to the increased IGF-1 dose and concentration within the defect, as compared to smaller animal models (Table I). But the optimum amount of IGF-1 for successful cartilage repair may in fact be more dependent on factors such as load bearing and defect characteristics than on the size of the animal. The dose adjustments that are traditionally made relative to body weight for systemically administered substances may not be applicable when dealing with locally applied and locally acting growth factors. It is also possible that upregulating proteoglycan synthesis through IGF-1 treatment alone is insufficient to generate adequate repair tissue for some defect sizes and geometries.

Published data on the use of intra-articular injections of either IGF-1 or PDGF-BB alone are relatively scant. The three

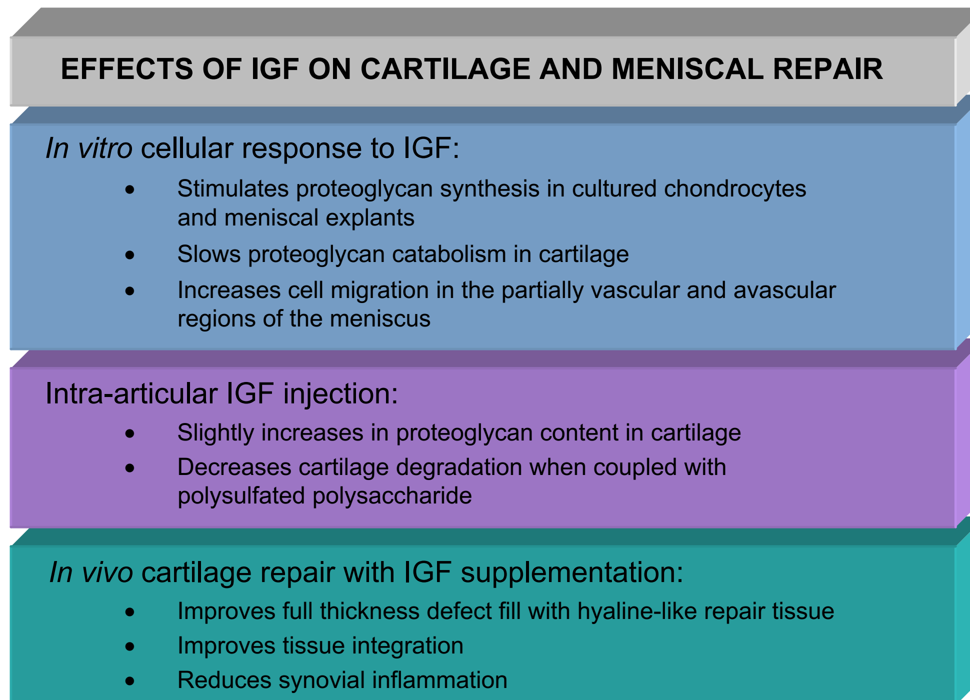

Fig. 6. Summary of the *in vitro* and *in vivo* effects of IGF on cartilage and meniscal repair.

studies of IGF-1 reviewed here demonstrated little therapeutic effect of these treatments for cartilage repair in osteoarthritic joints in mice[12] and dogs[13] and in a cartilage defect model in rabbits[11]. The data are limited to only these models and dosages. In a study utilizing normal, immature rat knees[22], repeated injections of PDGF-BB were demonstrated to be safe at the given dosing regimen. No gross changes to the chondrocytes occurred, no accumulation of fibroblasts or inflammatory cells was observed in the joint space and no effect on subchondral bone growth was detected. Although the effectiveness of PDGF injections on cartilage repair was not examined, PDGF-BB has been shown to stimulate resting zone chondrocytes *in vitro*, increasing proliferation and inhibiting maturation of these cells[22].

To date, no detailed studies optimizing the dose, injection regimen, or combinations of factors in cartilage repair

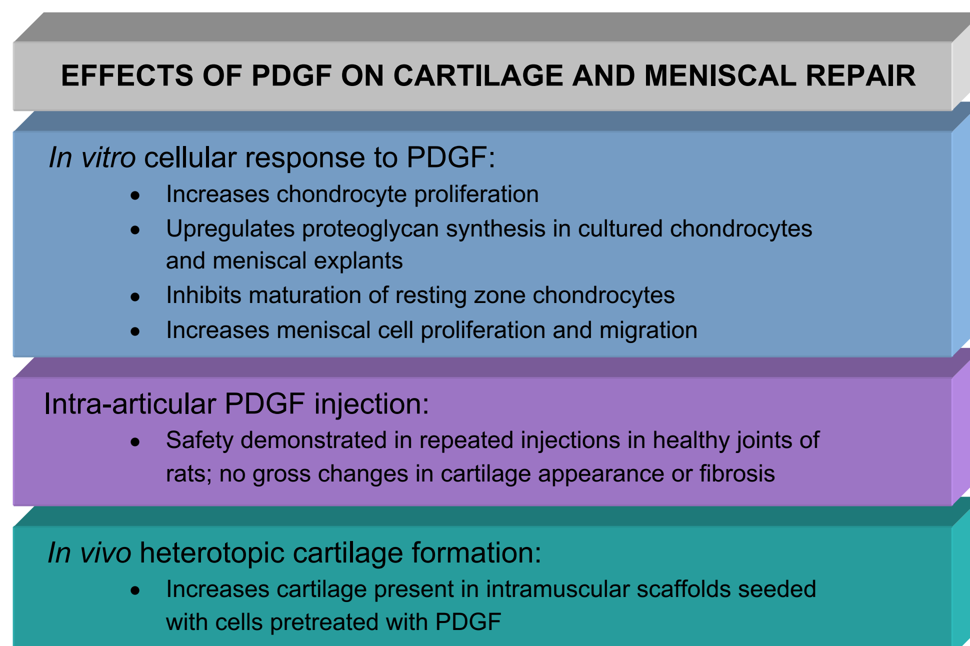

Fig. 7. Summary of the *in vitro* and *in vivo* effects of PDGF on cartilage and meniscal repair.

Table I
Comparison of IGF-1 doses

Investigator	Animal model	Concentration in defect (μg/mL)	Total dose (μg)
Hunziker and Rosenberg[9]	Rabbits	0.050	0.00004–0.000075*
	Mini pigs	0.050	0.00011–0.00014*
Sviri *et al.*[12]	Mice	N/A	0.025/day
Neidel[11]	Rabbits	2.97/day	0.030/day
Rogachefsky *et al.*[13]	Dogs	N/A	1/day
Nixon *et al.*[8]	Horses	47.12*	25
Fortier *et al.*[10]	Horses	47.12*	25

*Calculated.

models have been published. More extensive research is needed before the clinical benefits of injections of IGF-1 or PDGF-BB into the joint space can be clearly delineated. Injectable carriers may need to be developed to provide controlled release of growth factors into the joint space. It is well known that the biological half-lives of most growth factor peptides are very short and this may limit the effectiveness of the current intra-articular injection therapies. Combining a growth factor with HA, for example, allows for a more prolonged delivery of the growth factor, potentially enhancing this widely-used clinical treatment for cartilage degeneration and joint pain. HA gels have been prepared as injectable carrier vehicles for growth factors and bioactivity assays demonstrate that sustained release is achieved over periods as long as 10–15 days[27].

Loading growth factors onto a carrier material may also offer a better method to enhance the consistency and quality of current cell-based and acellular cartilage defect repair techniques. PDGF has been incorporated into polymerized HA scaffolds, showing uniform distribution and good retention. Increased cell proliferation on the growth factor-loaded matrix was observed, as compared to untreated controls[28,29]. Pretreating cells with growth factors prior to implantation into the wound site represents an alternative approach that can be used to jump-start the repair process. Once a thorough understanding of *in vivo* growth factor actions, dosing requirements and optimum exposure rates is achieved, growth factor treatment may provide a valuable tool to control and improve the cartilage repair and healing process.

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