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Combined effects of growth factors and static mechanical compression on meniscus explant biosynthesis[1]

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Summary

Objective: To compare the actions of fibroblast growth factor-basic (bFGF), insulin-like growth factor-I (IGF-I), platelet derived growth factor-AB (PDGF-AB), and transforming growth factor-beta 1 (TGF-β1) on bovine meniscus tissue explants with and without static mechanical compression.

Design: Meniscus tissue explants were cultured in a serum-free environment supplemented with an individual growth factor (1) over a range of concentrations for 4 days, (2) at a single concentration for 2–14 days, and (3) at a single concentration for 4 days coupled with graded levels of static compression. Explants were analyzed for accumulation of newly synthesized proteoglycan and total protein as measured by ^{35}S-sulfate and ^{3}H-proline incorporation, respectively.

Results: Over the range of chosen concentrations, TGF-β1 was the most potent stimulator of both protein and proteoglycan production, whereas bFGF was the least effective stimulator. Over a 2-week period for all four growth factors, the stimulation of proteoglycan production was sustained while there was no stimulation of protein production during this period. The superposition of static mechanical compression inhibited matrix production in the presence of each anabolic factor, with comparable inhibition relative to uncompressed controls for all factors.

Conclusions: The growth factors chosen exhibited an anabolic effect on the meniscus tissue explants, encouraging matrix production and deposition. The addition of static mechanical compression produced comparable relative inhibition of matrix production for each growth factor, suggesting that static compression and growth factors may modulate meniscal fibrochondrocyte biosynthesis via distinct pathways.
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Key words: Meniscus, Fibrochondrocyte, Growth factor, Static compression.

Introduction

The menisci are fibrocartilaginous organs in the knee joint that are responsible for distributing roughly 50–70% of the load through the knee[1]. Other functions include the maintenance of joint stability and lubrication[2]. The tissue is highly hydrated (70–80%), and the main mechanically functional extracellular matrix components are collagen type I and proteoglycans[2]. These components make up roughly 70%[2–4] and 1–3%[5–8], respectively, of the dry weight of the matrix and are arranged in a highly ordered ultrastructure well suited for the tissue function. The outer region of the meniscus contains circumferentially oriented collagen fiber bundles capable of resisting tensile hoop stresses due to joint loading. The inner region is proteoglycan rich and is effective for resisting direct compressive loads[2,5]. This matrix is produced by meniscal fibrochondrocytes[9] which exhibit behaviors similar to both fibroblasts and chondrocytes.

The vascular supply of the mature meniscus is limited to the outer one-third of the organ (referred to as the "red-zone"). The remaining two-thirds (referred to as the "white-zone") is primarily avascular and has a small propensity for autonomous repair in response to injury[10–13]. Access to the vascular supply has been shown to be critical for promoting autonomous repair of the meniscus[11,14]. Specific growth and differentiation factors that may be contained within the vasculature have been identified in the healing and reparative mechanisms of meniscus tissue, specifically in stimulating fibrochondrocytes to produce necessary matrix components[15,16]. Common clinical repair techniques to increase the healing potential of a meniscus tear include debridement and the creation of vascular access channels[17], allowing a conduit to the vascular supply rich in these factors.

In vitro, growth factors have been noted to heavily influence the behavior of fibrochondrocytes in monolayer and explant culture[18–24]. Webber *et al.* found that the use of basic fibroblast growth factor (bFGF) at 10 ng/mL and ascorbate at 40 μg/mL caused fibrochondrocytes cultured in monolayer to proliferate, whereas using only ascorbate shifted the cells towards increases in matrix deposition, specifically increases in sulfated glycosaminoglycans (sGAG)[18]. Platelet derived growth factor-AB (PDGF-AB) has been shown to induce a mitogenic response in

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fibrochondrocytes in explant culture[21,24] and to stimulate migration amongst fibrochondrocytes in monolayer culture[22]. In addition, Bhargava *et al.* found that insulin-like growth factor-1 (IGF-I) stimulated migration of isolated fibrochondrocytes only from the "red-white zone"[22]. Collier and Ghosh found increased stimulation of proteoglycan production by fibrochondrocytes in monolayer and explant culture with the addition of TGF-β1 over serum-free media and media containing 10% fetal bovine serum, respectively[20].

In vivo, the majority of the fibrochondrocytes rely upon diffusion and mechanically driven convection through the extracellular matrix for nutrient delivery. The synovial environment is rich in nutrients and serves as a repository for cellular waste products. In addition, the paracrine effects of soluble factors produced by the fibrochondrocytes themselves are partially modulated by the transport phenomena within the matrix. Recognizing specific growth factors as major stimulators of cartilaginous extracellular matrix growth, it is important to characterize their actions and potency on fibrochondrocytes in their native tissue matrix. Therefore, this study explores the individual effects of four growth factors (bFGF, IGF-I, PDGF-AB, and TGF-β1) on meniscus tissue explants under serum-free culture conditions. The first aim of this study was to obtain the dose–responses of the meniscus explants to ranges of concentrations of each growth factor. Next, the temporal effects of these factors at a single concentration were explored for up to 2 weeks. Finally, the actions of the growth factors in the presence of graded levels of static mechanical compression were examined to explore the interactions, if any, between the two stimuli.

Methods

MATERIALS

Bovine stifle joints were from Research 87 (Marlborough, MA). High glucose Dulbecco's Modified Eagle's Medium (DMEM), penicillin, streptomycin, neomycin, kanamycin sulfate, Fungizone, gentamicin, phosphate buffered saline (PBS), HEPES buffer, non-essential amino acids (NEAA), and L-proline were from Invitrogen (Carlsbad, CA). Bovine serum albumin (BSA), ascorbic acid, sodium sulfate, ammonium acetate, and Hoechst 33258 dye were from Sigma (St. Louis, MO). 1,9-Dimethyl-methylene blue (DMMB) dye was from Aldrich Chemical Company (Milwaukee, WI). ^{35}S-sodium sulfate was from American Radiolabeled Chemicals (St. Louis, MO) or ICN Biomedicals, Inc. (Irvine, CA). L-5-^{3}H-proline was from American Radiolabeled Chemicals (St. Louis, MO) or Amersham Biosciences (UK). Recombinant human forms of bFGF (17.2 kDa), IGF-I (7.6 kDa), PDGF-AB (25.5 kDa), and TGF-β1 (25.0 kDa) were from PeproTech (Rocky Hill, NJ). Proteinase K was from FisherBiotech (Fair Lawn, NJ). Biopsy punches were from Miltex Instruments (Lake Success, NY).

TISSUE EXPLANTS

Full thickness meniscus cores were excised with 4 mm diameter biopsy punches from the lateral and medial menisci of immature (2–4 week) bovine stifle joints [Fig. 1]. In preliminary studies, we found that IGF-I supplementation and static compression induced comparable changes in ^{35}S-incorporation for explants from the inner and outer regions of both medial and lateral menisci, with no statistically significant differences based on location. Therefore, explants from the midsubstance of both menisci were randomly allocated across experimental groups. Using a custom designed slotted cutting block and sterile razor blades, the cores were sliced into either 1-mm (dose–response and time–course studies) or 2-mm-thick discs (static compression studies). Approximately 1 mm of the superficial most top and bottom layers of each core was discarded to remove the structurally distinct surface layers. The discs were first soaked in DMEM supplemented with 50 μg/mL gentamicin, 50 μg/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL neomycin, 100 μg/mL kanamycin sulfate, and 0.25 μg/mL Fungizone for 24 h in a 37°C, 5% CO_2 incubator. The explants were then precultured in basal serum-free culture medium (DMEM plus 0.1% BSA, 50 μg/mL gentamicin, 0.1 mM NEAA, 1.0 mM HEPES, 4 mM L-proline, and 50 μg/mL ascorbic acid) for 3 days to allow for equilibration of all samples.

STATIC COMPRESSION

Tissue explants were compressed within custom designed polycarbonate static compression chambers [Fig. 2(A)]. Each chamber base held up to 16 samples in individual wells (15 mm diameter × 10 mm), and samples were compressed between the well bottoms and 8 mm diameter impermeable platens attached to the lid [schematically shown in Fig. 2(B)]. Three stainless steel spacing blocks and a central annular spacer were used to limit the compression imparted by the platens upon the tissue explants. The chamber assembly was placed on an aluminum platform and a nut and washer assembly was used to slowly tighten the chamber lid to the platform/base assembly. After the initial tightening of the chamber lid, appropriate culture media were added to the chamber wells via the medium ports on the chamber lid. These ports allowed for the aspiration of old media and addition of fresh media while maintaining compression of the samples.

BIOSYNTHESIS MEASUREMENTS

During the final 21 h of each culture period, culture media were supplemented with 20 μCi/mL of L-5-^{3}H-proline and 10 μCi/mL of ^{35}S-sodium sulfate to measure the accumulation of newly synthesized proteins (primarily collagen) and proteoglycans, respectively. Explants were then removed and washed 4 times for 30 min each time in PBS supplemented with 0.8 mM sodium sulfate and 1 mM L-proline at 4°C in order to rinse out unincorporated isotopes. Samples were lyophilized and digested in 1 mg of proteinase K (0.2–0.4 mg/mL in 100 mM ammonium acetate) per 80 mg of tissue explant at 60°C overnight. Digests were assayed for radiolabel content with an LS5000TD liquid scintillation counter (Beckman, Fullerton, CA). Data for each sample were normalized by total DNA content as determined by the fluorescent Hoechst 33258 dye assay[25] read on a Spectra Max Gemini fluorescent plate reader (Molecular Devices, Sunnyvale, CA). Digests and spent media were also assayed for sGAG content as determined by the 1,9-DMMB dye assay[26] read on a Power Wave 340X-I plate reader (Bio-Tek, Winooski, VA).

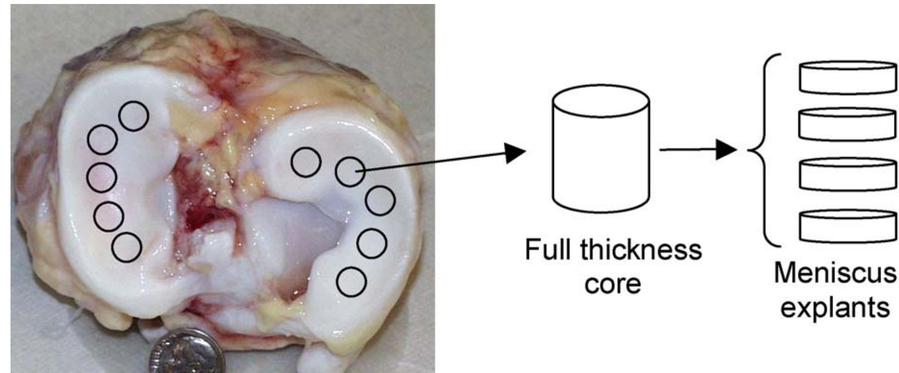

Fig. 1. Explant process showing meniscus explants from the lateral and medial menisci of immature bovine stifle joints. Meniscus cores were sliced to produce 1-mm or 2-mm-thick discs, discarding the inferior and superior superficial layers.

EXPERIMENTAL DESIGN

Study 1, dose–response

The first series of studies examined the response of meniscus explants to stimulation with a range of concentrations of each growth factor. Four concentrations of each growth factor spanning two orders of magnitude were chosen (Table I) based on previous studies in the literature involving cartilage tissue or isolated chondrocytes[27–33]. Four separate "sub-studies" were conducted, each of which utilized tissue from a different animal and included a control explant group cultured in basal medium alone (BSA controls). Study 1a included treatments with bFGF at 1–300 ng/mL (0.0581–17.4 nM), IGF-I at 30–1000 ng/mL (3.95–132 nM), or TGF-β1 at 0.05–5 ng/mL (0.002–0.2 nM). Study 1b included treatments with IGF-I at 30–1000 ng/mL or PDGF-AB at 3–300 ng/mL (0.118–11.8 nM). Study 1c included treatments with all four growth factors at the previously stated concentration ranges. Following the initial preculture in basal medium, the tissue explants (1 mm thick, $N = 6$ per condition in each study, total $N = 12$–18 per condition) were cultured for 4 days in 1.0 mL each of appropriately supplemented medium. A fourth study (study 1d, $N = 6$ per condition) was performed to explore a higher range of concentrations of TGF-β1 at 1–100 ng/mL (0.04–4.0 nM) based on the results of the first dose–response studies.

Study 2, time–course

The second study compared the duration of stimulation for up to 2 weeks by a single concentration (Table I) of each growth factor. After preculture, tissue explants from a single animal (1 mm thick, $N = 5$ per condition per time point) were cultured for 2, 4, 7, or 14 days in basal media plus bFGF at 100 ng/mL (5.81 nM), IGF-I at 200 ng/mL (26.3 nM), PDGF-AB at 100 ng/mL (3.92 nM), or TGF-β1 at 5 ng/mL (0.2 nM). Control explants were cultured in basal medium alone (BSA controls). In addition, spent media were assayed for sGAG release.

Study 3, static compression

The third study examined the effects on matrix synthesis of static compression combined with growth factor supplementation (Table I): bFGF at 100 ng/mL (5.81 nM), IGF-I at 100 ng/mL (13.2 nM), PDGF-AB at 100 ng/mL (3.92 nM), or TGF-β1 at 50 ng/mL (2.0 nM). After preculture, tissue explants (2 mm thick, $N = 6$ per condition) were cultured for 4 days in basal media with or without a single concentration of growth factor under static compression to 100%, 75%, or 50% of the original cut thickness (hereafter referred to as 0%, 25%, or 50% compression, respectively). These static compression levels were chosen both to span the range required to produce a consistent inhibitory

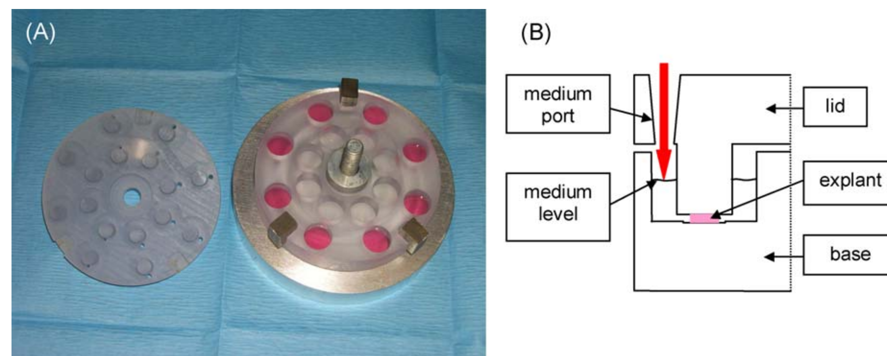

Fig. 2. Static compression chamber. (A) Individual explants were placed in isolated wells in the base and compressed between the base and platens on the lid. (B) Schematic showing the cross-section of a single well containing an explant and feed medium.

Table I
Growth factor concentrations for each study

Growth factor	Dose–response (ng/mL)		Time–course (ng/mL)	Static compression (ng/mL)
	Low	High		
bFGF	1	300	100	100
IGF-1	30	1000	200	100
PDGF-AB	3	300	100	100
TGF-β1	0.05	5	5	50
	1	100		

response and to allow comparison to results in the articular cartilage literature[34–36]. In addition, while the highest levels of compression appear to be non-physiologic, these levels could be seen *in vivo* due to prolonged exposure to loading. Two sub-studies were conducted using tissue from two different animals. During the preculture period, the explants swelled such that compression to the original cut thickness did impart a slight compressive deformation on the samples. Control free swell (FS) explants were cultured in a well of a culture dish.

STATISTICAL ANALYSIS

For all studies, the analyzed data were in the form of incorporation rates normalized to DNA content. Data are presented as values normalized to the average of a control group, but all statistical analyses were performed on non-normalized data. Dose–response data were analyzed separately for each individual growth factor, with the BSA control group included as a concentration of 0 ng/mL. Data were analyzed using a two factor (sub-study and growth factor level) General Linear Model (GLM) and Tukey's test for post hoc analysis using Minitab Release 12.23 (Minitab Inc., State College, PA). Time–course data were analyzed using a two factor (day and growth factor treatment) GLM and Tukey's test. Static compression data were analyzed using a three factor (sub-study, growth factor treatment, and compression level) GLM and Tukey's test. For the IGF-I dose–response and static compression studies, where each sub-study did not include identical treatments, sub-study was treated as a nested variable. Differences were deemed significant at $P<0.05$. All data are presented as mean+standard error of the mean (S.E.M.).

Results

DOSE-DEPENDENT STIMULATION OF TISSUE EXPLANTS

In general, the tissue explants responded to increasing levels of each growth factor with a dose-dependent stimulation of matrix production. Figure 3 illustrates both ^{35}S-sulfate and ^{3}H-proline incorporation rates for all growth factors normalized by the BSA controls.

For IGF-I and PDGF-AB [Fig. 3(A, B)], the dose–responses appeared to plateau within the chosen concentration ranges for both the ^{35}S and ^{3}H incorporation rates. IGF-I obtained maximal levels of stimulation by 300 ng/mL with ^{35}S and ^{3}H incorporation rates reaching 291% and 188% of BSA controls, respectively. The ^{35}S incorporation at 300 ng/mL IGF-I was significantly higher than incorporation at 0 ($P<0.0001$), 30 ($P<0.0001$), and 100 ng/mL ($P=0.0040$). The ^{3}H incorporation at 100 ng/mL IGF-I was significantly higher than at 0 ng/mL ($P=0.012$), and at 300 ng/mL IGF-I was significantly higher than at 0 ($P<0.0001$) and 30 ng/mL ($P=0.0008$). PDGF-AB obtained maximal levels of stimulation at 100 ng/mL with ^{35}S and ^{3}H incorporation rates reaching 468% and 148% of BSA controls, respectively. These maximal levels of stimulation were both significantly higher than incorporation rates at any of the lower concentrations (all $P<0.0002$).

In contrast to IGF-I and PDGF-AB, the initial concentration range chosen for TGF-β1 (0.05–5 ng/mL) did not appear to contain saturated incorporation of either ^{35}S or ^{3}H [Fig. 3(C), open markers]. The ^{35}S incorporation increased monotonically with TGF-β1 concentration and still appeared to be increasing at 5 ng/mL, the highest level in the initial dose–response study. At 5 ng/mL, the ^{35}S incorporation rate was 640% of the BSA control value and was significantly greater than incorporation at any lower concentration (all $P<0.0001$). In contrast, the ^{3}H incorporation rate at 5 ng/mL was 161% of the BSA control value and was only significantly higher than at 0 ($P<0.0001$) and 0.05 ng/mL ($P=0.0003$). When the concentration range was expanded to include concentrations of up to 100 ng/mL, maximal levels of ^{35}S and ^{3}H incorporation were seen at 100 ng/mL, reaching 1034% and 319% of BSA control values, respectively [Fig. 3(C), solid markers]. The ^{35}S incorporation at 100 ng/mL TGF-β1 was significantly higher than at 0 ($P<0.0001$), 1 ($P<0.0001$), 5 ($P=0.0070$), and 10 ng/mL ($P=0.0027$). The ^{3}H incorporation at 100 ng/mL TGF-β1 was only significantly higher than incorporation at 0 ($P<0.0001$) and 1 ng/mL ($P=0.0003$). However, the response to TGF-β1 still did not appear to be truly saturated by 100 ng/mL [Fig. 3(C)].

Finally, bFGF showed modest stimulation of ^{35}S incorporation and no stimulation of ^{3}H incorporation over the chosen concentration range [Fig. 3(D)]. The ^{35}S incorporation at 100 ng/mL bFGF was significantly higher than at 0 ng/mL ($P=0.0028$), and the ^{35}S incorporation at 300 ng/mL was significantly higher than at 0 ($P<0.0001$), 1 ($P=0.0072$), and 10 ng/mL ($P=0.017$). The greatest ^{35}S incorporation occurred at 300 ng/mL, reaching 169% of the BSA control value with no clear plateau in stimulation. At these concentrations, bFGF did not significantly affect ^{3}H incorporation ($P=0.19$).

Overall, the growth factors chosen were more potent stimulators of ^{35}S incorporation than of ^{3}H incorporation. There were differential levels of stimulation for the four factors chosen. TGF-β1 was the most potent stimulator of sulfate incorporation with an order of magnitude increase in incorporation rate at the highest concentration. bFGF was the least potent stimulator of both sulfate and proline incorporation over the ranges studied.

STIMULATION OF TISSUE EXPLANTS OVER A 2-WEEK CULTURE PERIOD

Based on the results of the initial dose–response study, single concentrations of each growth factor (Table I) were chosen to examine the effects of these factors over a 14-day period. A significant and sustained stimulation of ^{35}S incorporation over BSA controls was seen for TGF-β1, IGF-I, and PDGF-AB (all $P<0.0001$), but not for bFGF [$P=0.51$, Fig. 4(A)]. A significant dip in ^{35}S incorporation was seen at day 7 ($P=0.0031$ vs day 4 and $P=0.024$ vs day 14) for the TGF-β1 and PDGF-AB groups, but this was not sustained (incorporation at day 14 was not significantly different from that on days 2 or 4, $P>0.90$). In contrast, none of the growth factor groups exhibited ^{3}H incorporation

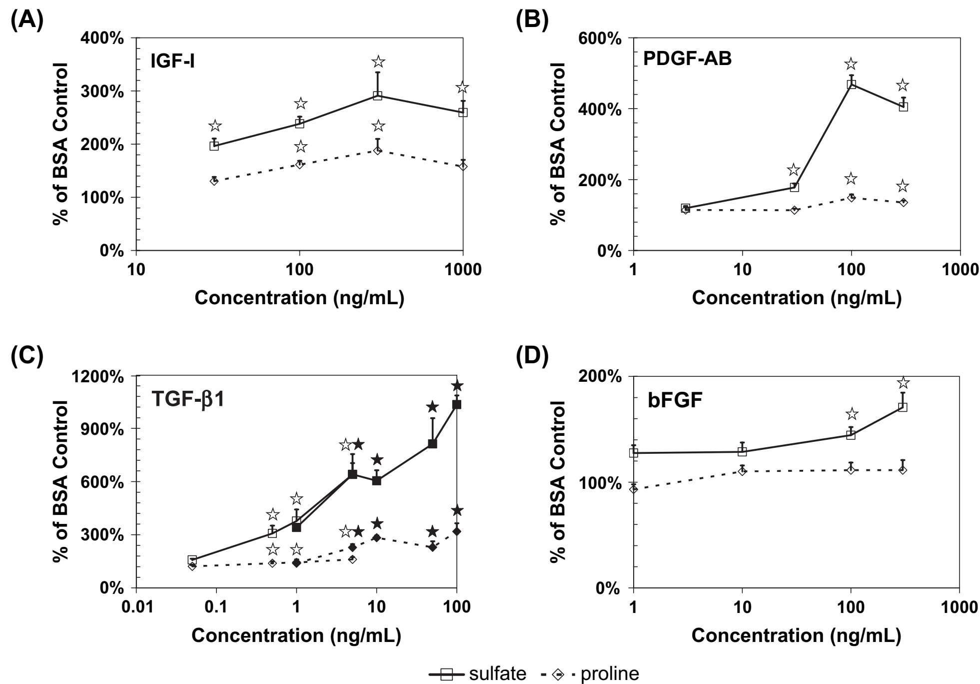

Fig. 3. Dose–response results for concentration ranges of (A) IGF-I (B) PDGF-AB, (C) TGF-β1, and (D) bFGF. For presentation purposes, data are normalized to basal medium (BSA) control values. In (C) solid markers and stars represent data from the second dose–response study for TGF-β1. Star denotes that the value is significantly different from BSA controls ($P<0.05$).

significantly different from the BSA controls [$P = 0.078$ to $P = 0.99$, Fig. 4(B)], although the ^{3}H incorporation for the PDGF-AB group was significantly less than that of the TGF-β1 ($P = 0.0063$) and IGF-I groups ($P = 0.0055$). The ^{3}H incorporation at day 7 was also significantly lower than at all other days (all $P<0.001$).

As only a fraction of the newly synthesized matrix molecules were incorporated into the tissue explants, we also quantified the total sGAG released to the media over the first 12 days [Fig. 5]. The cumulative sGAG release data for the TGF-β1 and PDGF-AB groups were generally consistent with the ^{35}S-sulfate incorporation data, with 12-day cumulative sGAG releases of 221% and 133% of BSA levels, respectively. TGF-β1 supplementation induced the greatest 12-day cumulative sGAG release ($P<0.012$ vs. all other groups). Interestingly, however, the cumulative sGAG released for the IGF-I and bFGF groups were lower than that of the BSA group (73.2% and 43.7%, respectively), despite the fact that both had higher ^{35}S-sulfate incorporations than the BSA controls. This indicates that the fraction of newly synthesized sGAG incorporated into the extracellular matrix varied considerably between treatment groups, suggesting that the growth factors differentially influence assembly and processing of proteoglycans as well as the overall synthesis rates.

INHIBITION OF MATRIX PRODUCTION WITH STATIC COMPRESSION

In the presence of any one growth factor, both the normalized ^{35}S and ^{3}H incorporation rates were significantly inhibited for all static compression levels as compared to the FS groups [$P<0.0001$, Fig. 6(A, B)]. Increasing

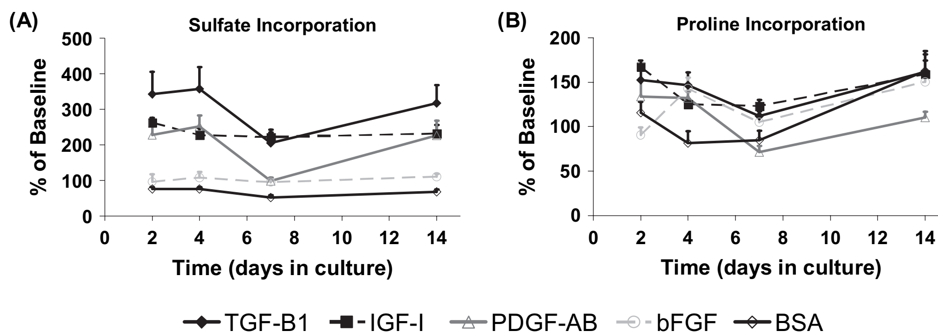

Fig. 4. Time–course results for (A) sulfate and (B) proline incorporation rates over the 2-week culture period. For presentation purposes, data are normalized to baseline BSA control values at day 0 (following preculture but before adding growth factors).

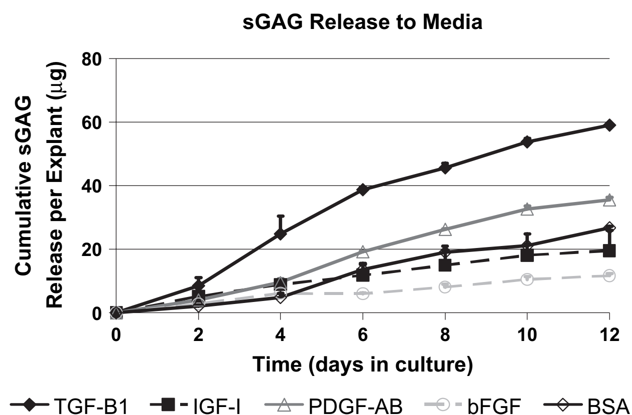

Fig. 5. Cumulative release of sulfated glycosaminoglycans (sGAG) into the media over the 2-week culture period. The data are presented on a per explant basis.

compression levels induced a significant dose-dependent inhibition of ^{3}H incorporation ($P<0.0001$), but not of ^{35}S incorporation ($P > 0.46$). Interestingly, although the overall levels of ^{35}S incorporation varied substantially among media conditions, the dose-dependent inhibition of ^{3}H incorporation relative to FS incorporation levels did not vary significantly among media conditions ($P = 0.086$). Since the samples swelled during the preculture period,

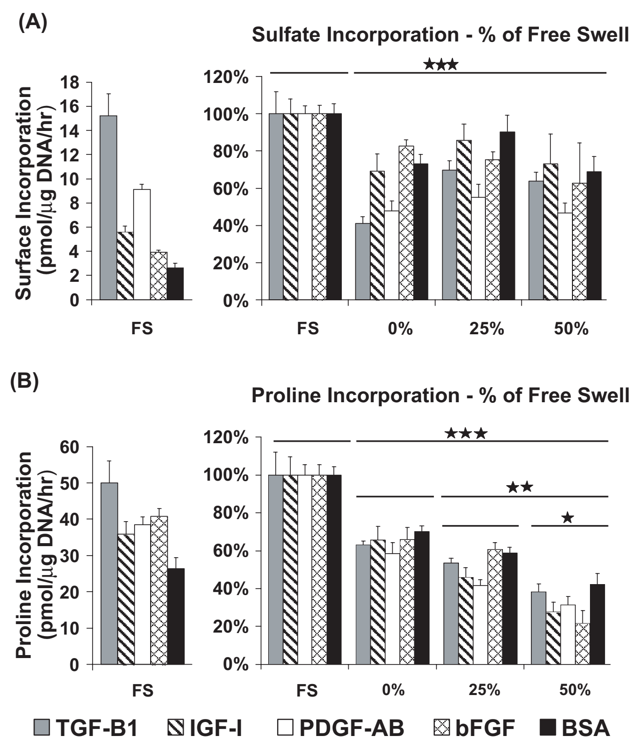

Fig. 6. Effects of static compression on (A) sulfate and (B) proline incorporation rates for the growth factor supplemented media conditions. For presentation purposes, results for compression groups are normalized to the average of the corresponding FS group (shown to the left). Star denotes that the value is significantly different from FS ($P<0.05$).

explants in the 0% compression group were actually compressed somewhat, resulting in significant inhibitions of ^{35}S and ^{3}H incorporation rates relative to FS controls for all media conditions ($P<0.0001$). Although the swelling was not quantified, this phenomenon is consistent with previous observations in our laboratory[37].

Discussion

The results of these studies illustrate the different effects of four growth factors on matrix production by bovine meniscus tissue explants. TGF-β1 was found to be the most potent stimulator of both protein and proteoglycan matrix accumulation, whereas bFGF was found to be the least effective stimulator. For all factors, the stimulation of proteoglycan accumulation was sustained over a 2-week period, while protein accumulation was modestly stimulated in the dose–response study and not significantly stimulated in the time–course study. The superposition of static mechanical compression inhibited matrix accumulation in the presence of each anabolic factor.

The concentration ranges chosen for the present studies were based on published studies on articular cartilage explants and chondrocytes in alginate bead and monolayer cultures[27–33]. Although many studies have characterized levels of these factors within the articular cartilage extracellular matrix and synovial fluid, little similar information for the meniscus (or fibrocartilage, in general) is currently available. Luyten *et al.* estimated 50 ng of IGF-I per gram (wet weight) of bovine articular cartilage[28]. This value is on the same order of magnitude as the concentrations shown to cause a plateau in matrix stimulation of articular chondrocytes[29,38,39]. Schneiderman *et al.* found a lower concentration of 10 ng total IGF-I per gram of adult human articular cartilage[40]. bFGF has been found in articular cartilage at concentrations of 1–50 ng per gram of tissue[29]. PDGF has been identified as a locally produced factor, with no traces of the growth factor in the circulation[41]. However, in a study exploring the use of meniscal rasping as a technique to promote healing within the avascular zone, Ochi *et al.* detected the induction of the expression of both TGF-β1 and PDGF using immunohistochemical staining[42]. These factors were thought to play an important role in the recruitment of fibrochondrocytes and synoviocytes to commence the repair process.

It should be noted that the choice of control conditions (specifically, serum supplemented vs. defined media) may confound comparisons between studies. The presence of serum increases the baseline synthesis rates and introduces the possibility of interactive effects with other cytokines and growth factors in the serum. For example, the stimulation of meniscus proteoglycan synthesis by TGF-β1 in the current study was substantially greater than that previously reported by Collier and Ghosh for mature ovine meniscus tissue, who found a twofold increase over 10% fetal bovine serum control levels with the addition of 4 ng/mL TGF-β1[20]. This is in contrast to a sixfold to sevenfold increase at 5 ng/mL TGF-β1 for bovine meniscus explants in the current study and a threefold increase at 3 ng/mL for porcine meniscus explants reported by Lietman *et al.* (both over BSA controls)[24].

Interestingly, the relative stimulation of meniscus ^{35}S-sulfate incorporation by TGF-β1 was even more robust than that previously reported for articular cartilage. Under culture conditions similar to ours, Morales and Roberts found that stimulation of immature bovine articular cartilage

proteoglycan synthesis reached a plateau by 10 ng/mL of TGF-β1 with a sevenfold to eightfold increase over BSA controls[43]. In contrast, the current dose−response study on meniscus explants indicated a monotonic increase in ^{35}S-sulfate incorporation for doses up to 100 ng/mL of TGF-β1, reaching a tenfold increase over BSA controls (to approximately the level of cartilage explants in basal medium) with no indication of a plateau in the response. This, along with previous evidence that TGF-β1 stimulation of fibrochondrocytes preferentially stimulates the production of large, aggregating proteoglycans[20], suggests that high doses of TGF-β1 induce the production of a more cartilaginous matrix.

Focusing on the mitogenic response, Spindler *et al.* reported that PDGF-AB induced a tenfold increase in proliferation by adult ovine explants from the outer one-third but no significant change in proliferation by explants from the inner two-thirds, even with PDGF-AB supplementation at 200 ng/mL[21]. They suggested that the fibrochondrocytes of the central region may not have the α-PDGF or β-PDGF receptors necessary to interact with the exogenous PDGF-AB. In the current study, neither PDGF-AB nor any other factor significantly increased explant DNA content (data not shown). However, PDGF-AB did modulate the biosynthesis of tissue explants from the midsubstance of bovine menisci, suggesting that immature bovine fibrochondrocytes do have functional PDGF receptors. This is consistent with previous findings that PDGF stimulation of both immature and mature bovine articular cartilage had no influence on DNA synthesis but induced a dose-dependent increase in proteoglycan biosynthesis[44]. It is also consistent with the dose−response studies of Lietman *et al.* showing a dose-dependent increase in proteoglycan biosynthesis of porcine meniscus explants[24].

The actions of bFGF on articular chondrocytes have been identified as both mitogenic[39,45] and biosynthetic[29,39]. Osborn *et al.* found saturation in proteoglycan synthesis at 100−1000 ng/mL for adult bovine articular chondrocytes[39], with [^{3}H]-thymidine incorporation also significantly stimulated at the highest concentrations of bFGF. Sah *et al.* found consistent results for adult bovine articular cartilage with bFGF supplementation[29], but found that matrix production by immature bovine cartilage was stimulated only at a concentration of 3 ng/mL of bFGF. Concentrations of 30−300 ng/mL of bFGF caused an inhibition of both matrix production and mitogenic activity and induced catabolism with increases in proteoglycan release from the tissue matrix. In contrast, we observed a significant stimulation of proteoglycan accumulation by immature meniscus explants with up to 300 ng/mL of bFGF, indicating that cells in the two tissues may respond in different manners to specific stimuli.

Studies on articular cartilage explants have found plateaus in IGF-I stimulation of proteoglycan synthesis from 20 to 200 ng/mL[28,29,38,39]. This wide range of concentrations has been attributed to differences in immature vs. mature tissue[29,39], where mature tissue requires lower concentrations of IGF-I for maximal responses in proteoglycan production. Our results for immature fibrocartilage are on the same order of magnitude of the upper end of that concentration range. While observing stimulation of proteoglycan production in response to IGF-I, Luyten *et al.* also found no change in DNA content over a 5-week-culture period with IGF-I supplementation of immature (newborn to 10-month olds) articular cartilage explants[28]. Our results were similar, with no significant change in DNA content normalized to explant dry weight over the 2-week-culture period (data not shown).

In addition to biochemical factors, biomechanical stimuli aid in directing the development and maintenance of the tissues within the knee joint. Bonassar *et al.* examined the combined effects of mechanical compression and IGF-I treatment on immature bovine articular cartilage explants. IGF-I stimulation of both protein and proteoglycan synthesis was reduced by static compression, and the time required to reach steady-state stimulation was increased[36]. Overall, however, the kinetics of IGF-I stimulation did not vary substantially with compression level. Oscillatory compression substantially reduced the time to steady-state stimulation by IGF-I, but this response was still substantially slower than the biosynthetic response to oscillatory compression alone[46]. Taken together, these studies suggest that stimulation of articular cartilage by IGF-I and mechanical compression occur through separate cellular mechanisms, with the primary interaction being inhibition (static) or enhancement (oscillatory) of IGF-I transport through the matrix.

Our results with meniscus tissue explants are consistent with an analogous hypothesis for meniscal tissue. For all four growth factors we observed an inhibition of matrix accumulation relative to FS controls with the addition of static compression at levels of 0%, 25%, and 50%. Despite the wide range in stimulatory potentials, the relative inhibition by static compression was comparable for all four growth factors. This may indicate that, regardless of biochemical stimulation, the inhibition caused by the biomechanical stimulus has a greater and more potent effect on the protein production of the cells. Our results appear to be consistent with the notion that fibrochondrocytes respond to biochemical and biomechanical stimuli via separate cellular pathways. Future short term studies examining the initial kinetics of stimulation with and without mechanical compression will further clarify this issue.

These studies demonstrate the effects of several growth factors, previously identified as having anabolic effects on cartilage tissue explants, on the biosynthesis of meniscal fibrochondrocytes in meniscus tissue explants. Several factors were identified as potent stimulators of meniscus protein and proteoglycan accumulation, and the addition of a biomechanical stimulus reduced the levels of biochemical stimulation. While the results were generally similar to those previously reported for articular cartilage, we observed several differences in the responses. As the two tissues occupy a common synovial environment in the knee joint, care must be taken in employing growth factor-based repair strategies for one tissue to ensure that a beneficial treatment for one tissue does not have detrimental effects on the other. An improved understanding of environmental factors capable of modulating meniscus metabolism will be crucial in the development of improved biological strategies for meniscus repair or regeneration.

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References

1. Shrive NG, O'Connor JJ, Goodfellow JW. Load-bearing in the knee joint. Clin Orthop 1978;131:279−87.

2. Fithian DC, Kelly MA, Mow VC. Material properties and structure–function relationships in the menisci. Clin Orthop 1990;252:19–31.
3. Bullough PG, Munuera L, Murphy J, Weinstein AM. The strength of the menisci of the knee as it relates to their fine structure. J Bone Joint Surg Br 1970;52:564–7.
4. Eyre DR, Muir H. The distribution of different molecular species of collagen in fibrous, elastic and hyaline cartilages of the pig. Biochem J 1975;151:595–602.
5. Ghosh P, Taylor TKF. The knee joint meniscus: a fibrocartilage of some distinction. Clin Orthop 1987;224:52–63.
6. Proctor CS, Schmidt MB, Whipple RR, Kelly MA, Mow VC. Material properties of the normal medial bovine meniscus. J Orthop Res 1989;7:771–82.
7. Nakano T, Dodd CM, Scott PG. Glycosaminoglycans and proteoglycans from different zones of the porcine knee meniscus. J Orthop Res 1997;15:213–20.
8. Ishima M, Wada Y, Sonoda M, Harada Y, Katsumi A, Moriya H. Effects of hyaluronan on the healing of rabbit meniscus injured in the peripheral region. J Orthop Sci 2000;5:579–84.
9. Ghadially FN, Thomas I, Yong N, Lalonde JM. Ultrastructure of rabbit semilunar cartilages. J Anat 1978;125:499–517.
10. Arnoczky SP, Warren RF. The microvasculature of the meniscus and its response to injury. An experimental study in the dog. Am J Sports Med 1983;11:131–41.
11. Hough Jr AJ, Webber RJ. Pathology of the meniscus. Clin Orthop 1990;252:32–40.
12. Gray JC. Neural and vascular anatomy of the menisci of the human knee. J Orthop Sports Phys Ther 1999;29:23–30.
13. King D. The healing of semilunar cartilages. Clin Orthop 1936;1990:4–7.
14. Cabaud HE, Rodkey WG, Fitzwater JE. Medical meniscus repairs. An experimental and morphologic study. Am J Sports Med 1981;9:129–34.
15. Kollias SL, Fox JM. Meniscal repair. Where do we go from here? Clin Sports Med 1996;15:621–30.
16. Koski JA, Ibarra C, Rodeo SA, Warren RF. Meniscal injury and repair: clinical status. Orthop Clin North Am 2000;31:419–36.
17. McAndrews PT, Arnoczky SP. Meniscal repair enhancement techniques. Clin Sports Med 1996;15:499–510.
18. Webber RJ, Harris MG, Hough Jr AJ. Cell culture of rabbit meniscal fibrochondrocytes: proliferative and synthetic response to growth factors and ascorbate. J Orthop Res 1985;3:36–42.
19. Webber RJ. In vitro culture of meniscal tissue. Clin Orthop 1990;252:114–20.
20. Collier S, Ghosh P. Effects of transforming growth factor beta on proteoglycan synthesis by cell and explant cultures derived from the knee joint meniscus. Osteoarthritis Cartilage 1995;3:127–38.
21. Spindler KP, Mayes CE, Miller RR, Imro AK, Davidson JM. Regional mitogenic response of the meniscus to platelet-derived growth factor (PDGF-AB). J Orthop Res 1995;13:201–7.
22. Bhargava MM, Attia ET, Murrell GA, Dolan MM, Warren RF, Hannafin JA. The effect of cytokines on the proliferation and migration of bovine meniscal cells. Am J Sports Med 1999;27:636–43.
23. Zaleskas JM, Kinner B, Freyman TM, Yannas IV, Gibson LJ, Spector M. Growth factor regulation of smooth muscle actin expression and contraction of human articular chondrocytes and meniscal cells in a collagen-GAG matrix. Exp Cell Res 2001;270:21–31.
24. Lietman SA, Hobbs W, Inoue N, Reddi AH. Effects of selected growth factors on porcine meniscus in chemically defined medium. Orthopedics 2003;26:799–803.
25. Kim YJ, Sah RL, Doong JY, Grodzinsky AJ. Fluorometric assay of DNA in cartilage explants using Hoechst 33258. Anal Biochem 1988;174:168–76.
26. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986;883:173–7.
27. Inoue H, Kato Y, Iwamoto M, Hiraki Y, Sakuda M, Suzuki F. Stimulation of cartilage-matrix proteoglycan synthesis by morphologically transformed chondrocytes grown in the presence of fibroblast growth factor and transforming growth factor-beta. J Cell Physiol 1989;138:329–37.
28. Luyten FP, Hascall VC, Nissley SP, Morales TI, Reddi AH. Insulin-like growth factors maintain steady-state metabolism of proteoglycans in bovine articular cartilage explants. Arch Biochem Biophys 1988;267:416–25.
29. Sah RL, Chen AC, Grodzinsky AJ, Trippel SB. Differential effects of bFGF and IGF-I on matrix metabolism in calf and adult bovine cartilage explants. Arch Biochem Biophys 1994;308:137–47.
30. Smith RL, Palathumpat MV, Ku CW, Hintz RL. Growth hormone stimulates insulin-like growth factor I actions on adult articular chondrocytes. J Orthop Res 1989;7:198–207.
31. Tyler JA. Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem J 1989;260:543–8.
32. van Osch GJ, van den Berg WB, Hunziker EB, Hauselmann HJ. Differential effects of IGF-1 and TGF beta-2 on the assembly of proteoglycans in pericellular and territorial matrix by cultured bovine articular chondrocytes. Osteoarthritis Cartilage 1998;6:187–95.
33. Yaeger PC, Masi TL, de Ortiz JL, Binette F, Tubo R, McPherson JM. Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. Exp Cell Res 1997;237:318–25.
34. Sah RLY, Kim Y-J, Doong J-YH, Grodzinsky AJ, Plaas AHK, Sandy JD. Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 1989;7:619–36.
35. Sah RLY, Grodzinsky AJ, Plaas AHK, Sandy JD. Effects of static and dynamic compression on matrix metabolism in cartilage explants. In: Kuettner K, Ed. Articular Cartilage and Osteoarthritis. New York: Raven Press, Ltd. 1992;373–92.
36. Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB. Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. Arch Biochem Biophys 2000;379:57–63.
37. Imler SM, Vanderploeg EJ, Hunter CJ, Levenston ME. Static and oscillatory compression modulate protein and proteoglycan synthesis by meniscal fibrochondrocytes. Trans Orthop Res Soc 2001;26:552.

38. Barone-Varelas J, Schnitzer TJ, Meng Q, Otten L, Thonar EJ. Age-related differences in the metabolism of proteoglycans in bovine articular cartilage explants maintained in the presence of insulin-like growth factor I. Connect Tissue Res 1991;26:101–20.
39. Osborn KD, Trippel SB, Mankin HJ. Growth factor stimulation of adult articular cartilage. J Orthop Res 1989;7:35–42.
40. Schneiderman R, Rosenberg N, Hiss J, Lee P, Liu F, Hintz RL, *et al*. Concentration and size distribution of insulin-like growth factor-I in human normal and osteoarthritic synovial fluid and cartilage. Arch Biochem Biophys 1995;324:173–88.
41. Nimni ME. Polypeptide growth factors: targeted delivery systems. Biomaterials 1997;18:1201–25.
42. Ochi M, Uchio Y, Okuda K, Shu N, Yamaguchi H, Sakai Y. Expression of cytokines after meniscal rasping to promote meniscal healing. Arthroscopy 2001;17:724–31.
43. Morales TI, Roberts AB. Transforming growth factor beta regulates the metabolism of proteoglycans in bovine cartilage organ cultures. J Biol Chem 1988; 263:12828–31.
44. Schafer SJ, Luyten FP, Yanagishita M, Reddi AH. Proteoglycan metabolism is age related and modulated by isoforms of platelet-derived growth factor in bovine articular cartilage explant cultures. Arch Biochem Biophys 1993;302:431–8.
45. Trippel SB. Growth factor actions on articular cartilage. J Rheumatol 1995;43:129–32.
46. Bonassar LJ, Grodzinsky AJ, Frank EH, Davila SG, Bhaktav NR, Trippel SB. The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I. J Orthop Res 2001;19: 11–7.