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Dynamic Shear Stimulation of Bovine Cartilage Biosynthesis of Proteoglycan 4

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Objective. **The boundary lubrication function of articular cartilage is mediated in part by proteoglycan 4 (PRG4) molecules at the articular surface and in synovial fluid. The objective of this study was to determine the effects of dynamic shear stimulation on PRG4 biosynthesis by bovine cartilage explants.**

Methods. **Cartilage disks with intact articular surfaces were harvested from immature bovines. Some disks were subjected to 24 hours (day 1) of loading, consisting of a step load of 20% static compression either alone or with superimposed dynamic shear (3% amplitude and 0.1 Hz), while other disks were cultured free-swelling as controls. After the 24-hour loading period, disks were terminated or were further incubated for up to 72 hours (days 2–4) in free-swelling culture to assess chondrocyte responses to, and following, unloading. PRG4 products secreted into culture medium were quantified by enzyme-linked immunosorbent assay and characterized by Western blotting. Chondrocytes expressing PRG4 were localized by immunohistochemistry, and depth-associated variations in chondrocyte PRG4 expression were quantified by image analysis.**

Results. **Dynamic shear stimulation increased PRG4 secretion to 3–4 times that of unloaded controls and statically compressed samples. Sheared cartilage secreted more PRG4 of 345 kd relative to smaller molecular weight species, as compared with unloaded controls. Immunohistochemistry revealed that shear stimulation also increased the total number of cells expressing PRG4 by inducing expression by cells at a depth of 200–400 μm.**

Conclusion. **The paradigm that certain mechanical stimuli up-regulate biosynthesis in cartilage appears operative not only for load-bearing matrix constituents, but also for PRG4 molecules that mediate lubrication.**

Articular cartilage functions to provide a low-friction, load-bearing surface that allows the bones of diarthrodial joints to slide smoothly against each other while transmitting load. Cartilage tissue has classically been divided into 3 zones (superficial, middle, and deep) with distinct biochemical content and organization that impart specific functions to each zone. Chondrocytes in the middle and deep zones produce large amounts of aggrecan and collagen, which provide the load-bearing function of these zones. In addition to producing aggrecan and collagen, chondrocytes of the superficial zone also secrete specialized molecules, one of which is encoded by the proteoglycan 4 (PRG4) gene (GenBank accession no. AF056218 for bovine partial sequence and U70136 for complete human sequence) (1–4).

PRG4 molecules mediate, at least in part, the lubrication function of the articular cartilage surface layer (5–10). Mutations in the PRG4 gene can cause camptodactyly-arthropathy–coxa vara–pericarditis syndrome in humans (11), which results in early-onset noninflammatory joint failure (12) that is due in part to articular surface alterations and subsequent cartilage deterioration (13), demonstrating the functional importance of the gene in vivo. Lubricin, a 227-kd product of the PRG4 gene originally purified from synovial fluid (6,14), is highly expressed by cells of the synovial lining and functions to reduce friction in latex-on-glass (7–9) and cartilage-on-glass (5,6) friction assays. Superficial zone protein (SZP), a 345-kd product of the PRG4 gene, was first isolated from conditioned medium derived

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from cultured explants of cartilage from the superficial zone, but not the deep zone (1). SZP reduces friction in cartilage-on-cartilage sliding (10). These proteoglycan products of the PRG4 gene (4) will also be referred to below as PRG4.

Biomechanical regulation of chondrocyte metabolism of cartilage matrix molecules, such as aggrecan and collagen, is well documented both in vivo and in vitro (for review, see refs. 15 and 16). In general, static compression inhibits matrix biosynthesis, whereas dynamic compression and dynamic shear at certain frequencies and amplitudes can stimulate matrix metabolism, as demonstrated for cultured cartilage explants (17–20). While mechanical regulation of the metabolism of lubricant molecules is not as widely studied, recent evidence suggests that mechanical stimuli might also regulate PRG4 expression in the synovial joint. During embryonic development of the mouse elbow joint, PRG4 messenger RNA (mRNA) expression begins at the onset of joint cavitation (13), suggesting that PRG4 expression might be induced by the initiation of relative motion between the articular surfaces. A similar pattern is seen during postnatal growth, where fetal bovine cartilage exhibits inconsistent PRG4 expression by chondrocytes near the articular surface; in contrast, adult bovine tissue has abundant PRG4-expressing cells near the surface (21). In both cases (in utero and in vivo), increased chondrocyte expression of PRG4 coincides with increased joint motion.

While these studies implicate a role of mechanical stimuli in regulating chondrocyte expression of PRG4, a variety of other factors present in vivo may also affect PRG4 expression and metabolism. For example, certain chemical factors, including those present at elevated concentrations in the synovial fluid in osteoarthritis, markedly regulate chondrocyte PRG4 metabolism in cartilage explants that include the superficial zone (22,23). In explant culture, the exogenous chemical stimuli present in vivo can be eliminated or controlled to more precisely determine the effects of mechanical stimuli. Cartilage explant cultures also allow for application of well-defined mechanical stimuli to phenotypically stable chondrocytes, since they are still embedded within their native extracellular matrix.

The effects of mechanical stimulation on PRG4 protein expression by chondrocytes within their native extracellular matrix, however, remain unknown. Therefore, the objectives of this study were to determine the effects of dynamic tissue shear stimulation on cartilage metabolism of PRG4, as assessed by the quantity and structure of PRG4 products secreted into medium by chondrocytes in extracellular matrix of their native superficial and middle zones, and to determine chondrocyte expression of PRG4 and cartilage depth–associated variations in cells expressing PRG4.

MATERIALS AND METHODS

Cartilage explants. Cartilage disks were obtained as described previously (24). Briefly, stifle joints from 3 immature bovines (1–3 weeks old) were obtained from an abattoir. Under sterile conditions and with irrigation using phosphate buffered saline (PBS) supplemented with antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B), osteochondral cores (9 mm in diameter) were harvested from the patellofemoral groove using an Osteochondral Autograft Transfer System (Arthrex, Naples, FL). These cores were cut in a sledge microtome (Microm, Waldorf, Germany) to obtain slices (1 mm in thickness) that consisted of the intact articular surface (superficial zone; 0–1 mm from the articular surface) or the middle and deep zones (middle zone; 1–2 mm from the articular surface). From these slices, smaller disks (3 mm in diameter) were punched using a stainless steel dermal punch (Miltex, Tuttlingen, Germany). Finally, day 0 thickness was determined prior to the beginning of culture using a contact-sensing micrometer.

Culture and mechanical stimulation. For the first day, all disks were incubated in medium (low-glucose Dulbecco's modified Eagle's medium, 10 m*M* HEPES buffer, 0.1 m*M* nonessential amino acids, 0.4 m*M* L-proline, 2 m*M* L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B) supplemented with 10% fetal bovine serum and 50 μg/ml of ascorbate. Incubations were performed in a custom biaxial motion bioreactor designed by the authors. The bioreactor (similar to that described by Frank et al [19]) consisted of 2 polysulfone parts attached to a stand-alone mechanical tester (model V500cs; BioSyntech, Laval, Quebec, Canada). The bottom part had 12 independent culture wells (1 disk per well), and the top part had loading rods aligned with 8 wells, such that the other 4 wells allowed simultaneous free-swelling culture. The bioreactor also provided an enclosed, sterile atmosphere containing 5% CO_2 at a temperature of 37°C.

During the first day of culture, disks were subjected to 24 hours (day 1) of 1 of 3 mechanical stimulation conditions: free-swelling (Figure 1A), 20% static compression relative to swollen thickness (Figure 1B), or 20% static compression with superimposed dynamic sinusoidal simple shear of 0.1 Hz and 3% amplitude, centered about the axis of the disk (Figure 1C). In each experiment, 2 experimental groups were subjected to stimulation conditions: groups 1 and 2 or groups 1 and 3. Some disks were terminated immediately upon unloading (lifting the top part of the bioreactor off the samples), at which time day 1 thickness was measured, and metabolic analyses were performed (see below). Other disks were then placed into free-swelling culture in a tissue culture plate and put in a standard incubator for a subsequent 72-hour recovery period (days 2, 3, and 4) to assess chondrocyte metabolic responses to, and following, unloading and were terminated on day 2 or day 4. Disk thickness was also measured at the end of culture (day 4).

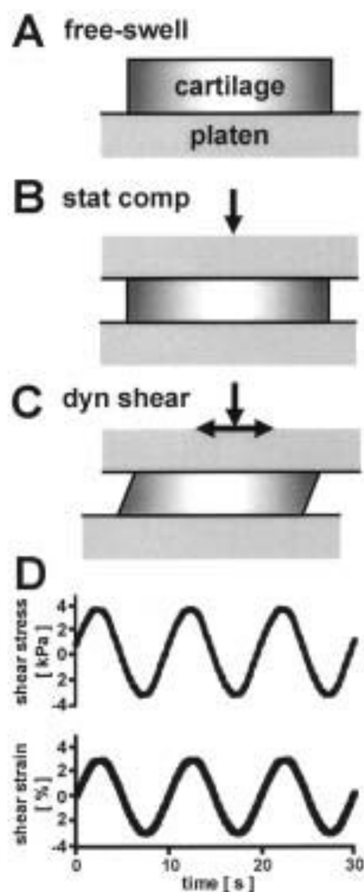

Figure 1. Mechanical stimulation of bovine articular cartilage. Cartilage was cultured under one of the following conditions: **A,** free swelling (free-swell), **B,** with static compression (stat comp), and **C,** with dynamic shear stimulation (dyn shear) superimposed on static compression. Arrows indicate the direction of applied force. **D,** Shear strain and shear stress waveforms were computed from data recorded during shear stimulation.

Compression and shear were applied via movement of the vertical and horizontal actuators, respectively, of the mechanical tester operating in the displacement mode of control. The amplitude of the horizontal load waveform recorded during mechanical stimulation was stable (within 4%) between 1 and 24 hours of loading. For this reason, load and displacement data sets of 1 minute duration (partial set shown in Figure 1D) were recorded (using Mach-1 software; BioSyntech) after 1 hour of stimulation as well as 1 hour prior to the end of stimulation (i.e., n = 4 data sets total for 2 runs of the bioreactor). Cartilage shear modulus (G) was computed from the load and displacement waveforms as $G = \tau/\gamma$, where τ is the amplitude of the horizontal force recorded during stimulation and normalized to the cartilage surface area and γ is the amplitude of the applied horizontal motion divided by the thickness of the tissue (25). The total harmonic distortion (calculated as 100% times the ratio of a, the square root of the sum of the squares of the powers of all higher harmonic frequencies, to b, the power of the fundamental frequency) of the load waveforms was determined as a measure of the quality of the sinusoidal shear stress imparted to the cartilage.

Metabolic analyses. *Enzyme-linked immunosorbent assay (ELISA) for quantification of secreted PRG4.* Medium samples that had been collected from cartilage cultures and replaced after each 24-hour period were quantitatively analyzed for PRG4 by indirect ELISA, as previously described (26), using monoclonal antibody (mAb) 3-A-4 (a generous gift from Dr. Bruce Caterson, University of Wales, Cardiff, UK) (21). Briefly, samples were diluted serially, adsorbed, and then reacted with mAb 3-A-4, horseradish peroxidase–conjugated secondary antibody, and ABTS substrate, with 3 washes of PBS plus 0.1% Tween between each step. A standard curve was generated from samples containing known amounts of PRG4 purified from conditioned medium derived from explants of the superficial zone of bovine calf cartilage, as previously described (1). The protein-equivalent amount of PRG4 in each sample was calculated from the linear region of the standard curve (between 0.078 μg/ml and 5 μg/ml of PRG4), as described elsewhere (26,27). The amount of PRG4 was normalized to the cartilage articular surface area.

Control studies indicated that all cartilage disks contained PRG4 at low levels (~1 $\mu g/cm^2$; quantified as previously described for meniscus [27]) relative to the amount secreted into the medium. Thus, the secreted quantities were representative of biosynthetic levels. Unlike superficial zone disks, cartilage middle zone disks subjected to the same loading protocols did not secrete PRG4 at levels above the detection limits of the assay (~0.1 $\mu g/cm^2$/day) for any of the loading conditions and were therefore not analyzed further.

Western blotting for characterization of secreted PRG4. Equal portions of medium samples collected from all 4 days of all free-swelling cultures were pooled, as were samples from all dynamically sheared cultures. PRG4 was purified from pooled samples by anion-exchange chromatography with DEAE-Sepharose gel, collecting the 0.3–0.6*M* NaCl eluate, and then concentrated with a Centricon Plus filter with a 100-kd molecular size cutoff. These samples were then reduced with 10 m*M* dithiothreitol for 30 minutes at 37°C and alkylated with 50 m*M* iodoacetamide for 30 minutes at 37°C. Samples (0.5 μg of total PRG4 per lane, as determined above by ELISA with mAb 3-A-4) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 4–20%-gradient polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and probed with mAb 6-A-1 (also a gift from Dr. Caterson), with the ECL Plus detection system (Amersham, Arlington Heights, IL). For visualization, the membrane was exposed to film for 30 seconds.

Immunohistochemistry for chondrocytes expressing PRG4. For free-swelling and dynamically sheared cartilage, the presence of PRG4 within chondrocytes was determined qualitatively in disks from cultures terminated at 24, 48, and 96 hours (i.e., at the end of loading and at 24 and 72 hours after unloading). For the 4 hours just prior to termination, these disks were incubated with medium supplemented with 1 μM

monensin. Upon termination, the disks were frozen in TissueTek OCT and sectioned (5-μm slices) perpendicular to the articular surface. Immunohistochemistry was performed as described previously (26). The sections were reacted with mAb 3-A-4, which was detected with a peroxidase-based system. The stained samples were examined to identify immunoreactive cells, indicating synthesis of PRG4. Sections probed with a nonspecific mouse IgG antibody served as negative controls. Qualitative results were documented by photomicroscopy.

From these photomicrographs, the depth-associated variation in chondrocyte PRG4 expression was determined. Using a custom program written in Matlab 6.5 software (MathWorks, Natick, MA), we analyzed a representative region of each section measuring 300 μm wide × 400 μm deep. Chondrocytes expressing PRG4 were identified manually. Then, the total number of PRG4+ cells and the number of PRG4+ cells as a function of depth from the articular surface were counted. Results are expressed as the number of PRG4+ cells per area in each successive 50-μm bin below the articular surface. Since the thickness of free-swelling cartilage increased during culture and the thicknesses of all loaded tissues did not (neither during loading nor after unloading), bin sizes were adjusted for loaded tissues based on thickness data collected here as well as axial strain profiles determined previously (28) for axially compressed immature bovine cartilage explants including the articular surface. Thus, for the compressed tissues, bins near the articular surface were 35 μm thick and increased with depth from the articular surface to 49 μm thick at a total depth of 320 μm (equivalent to the 400-μm depth on unloaded tissues that swelled 20%).

Statistical analysis. Data are expressed as the mean ± SEM. The effects of loading condition on cartilage thickness and PRG4 secretion were assessed using analysis of variance with repeated measures for day of culture. The effects of loading on the total number of PRG4+ cells in the area analyzed, as well as the number of PRG4+ cells in the area at particular depths, were analyzed by unpaired *t*-test. Tukey's post hoc test was performed to determine the effects of load within a given day. Statistical analysis was implemented with SyStat 10.2 (SyStat, Evanston, IL).

RESULTS

Biomechanical characterization of the load and displacement data showed that tissue shear deformation was applied in a reproducible manner. The total harmonic distortion of the load waveforms was 8.1 ± 0.2% (mean ± SEM), and both displacement and load waveforms were smooth (no visible distortion) (Figure 1D). Cartilage shear modulus was 0.11 ± 0.01 MPa (mean ± SEM).

Dynamic shear and static compression inhibited the increase in cartilage thickness that occurred during free-swelling culture (load/day interaction $P < 0.05$) (Figure 2). Immediately following harvest (day 0), cartilage thickness was similar ($P = 0.55$) for all explants (mean ± SEM 1.02 ± 0.02 mm for each loading group).

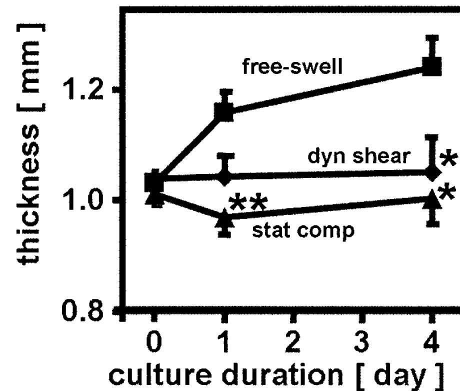

Figure 2. Thickness of free-swelling (free-swell), statically compressed (stat comp), and dynamically sheared (dyn shear) cartilage before culture (day 0), upon termination of loading (day 1), and upon termination of 72 hours of unloaded culture (day 4). Values are the mean and SEM of 4–8 samples. * = $P < 0.05$; ** = $P < 0.01$ versus free-swelling cartilage, by Tukey's post hoc test.

However, while the thickness of compressed and sheared cartilage remained essentially unchanged (within ±3%) during the 24 hours of stimulation, free-swelling cartilage thickness increased 14% during that same 24 hours. By the end of culture period (day 4), the thickness of free-swelling tissue had increased by a total of 22% over the initial thickness, while compressed and sheared cartilage remained within 3% of the initial thickness.

PRG4 secretion into culture media was markedly up-regulated by dynamic shear stimulation. Sheared cartilage superficial zone disks secreted 3–4 times the amount of PRG4 secreted by controls (Figure 3). Not only did shear stimulation up-regulate PRG4 secretion during the 24 hours of continuous loading, but this effect was maintained for the next 3 days following unloading ($P < 0.05$ for each day). In contrast, statically compressed controls secreted PRG4 at the same level as free-swelling controls ($P > 0.62$ for each day) and were therefore not analyzed further. Cartilage middle zone disks subjected to the same loading protocols did not secrete PRG4 at levels above the detection limit of the assay (~0.1 $\mu g/cm^2$/day) for any of the loading conditions (data not shown) and, thus, were also not analyzed further.

Western blot analysis (Figure 4) showed that medium from both free-swelling cartilage and dynamically sheared cartilage contained PRG4, predominantly of ~345-kd. Some smaller molecular weight immunore-

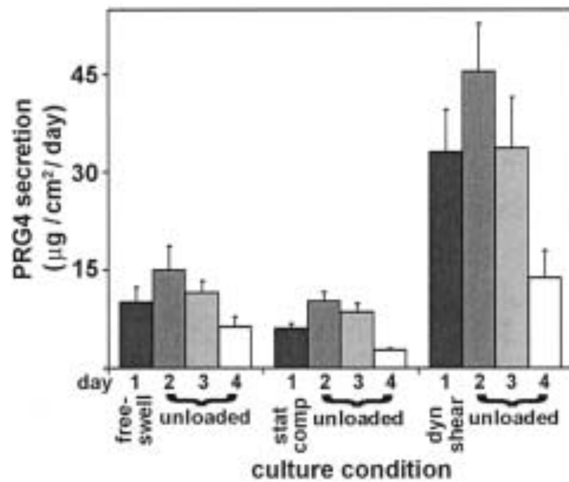

Figure 3. Secretion of proteoglycan 4 (PRG4) by free-swelling (free-swell), statically compressed (stat comp), or dynamically sheared (dyn shear) cartilage during 24 hours of continuous loading (day 1), followed by 72 hours (days 2–4) of unloaded culture. Values are the mean and SEM of 8–12 samples.

active species (∼220, ∼97, and ∼20 kd) were detected in medium from both free-swelling and dynamically sheared cultures, although medium from dynamically sheared tissue appeared to contain relatively more PRG4 of ∼345 kd than the smaller species, as compared with medium from free-swelling cartilage.

The total number of PRG4+ cells and the depth-associated variation in chondrocyte expression of PRG4 were also modulated by dynamic shear stimulation ($P <$ 0.001 and $P < 0.05$, respectively). During loading, cartilage subjected to dynamic shear had a mean ± SEM of 90 ± 10 total PRG4+ cells across the 0.3-mm width of the immunohistochemistry sample counted, while free-swelling tissue had 70 ± 3 PRG4+ cells. On days 2 and 4, sheared tissues had 120 ± 9 and 88 ± 9 PRG4+ cells, respectively, while free-swelling tissues had only 55 ± 4 PRG4+ cells on day 2 and 62 ± 6 PRG4+ cells on day 4. In free-swelling tissue, many cells in the top 0 to 100–200 μm were PRG4+ (Figures 5B and 6A–C), with very few cells deeper than 200 μm expressing PRG4. This pattern was consistent throughout the duration of culture (Figure 6). In contrast, sheared tissues exhibited more ($P < 0.05$) PRG4+ cells in certain bins deeper than 200 μm, both during the 24-hour loading period and after 24 or 72 hours following unloading (Figures 5C and 6A–C). Chondrocytes deeper than 400 μm from the articular surface did not express PRG4 under any conditions tested.

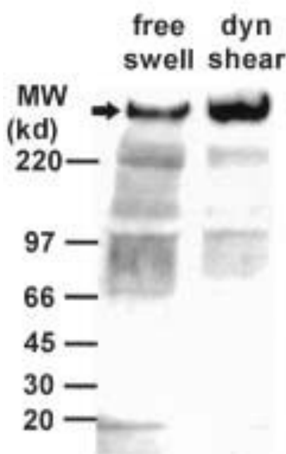

Figure 4. Characterization of proteoglycan 4 (PRG4) secreted into cultures of free-swelling and dynamically sheared cartilage. Western blot, showing that PRG4 secreted under free-swelling (free swell) and dynamically sheared (dyn shear) conditions (0.5 μg of total PRG4 per lane, as determined by enzyme-linked immunosorbent assay with monoclonal antibody 3-A-4) was predominantly of ∼345-kd molecular weight (arrow).

DISCUSSION

The results of our study demonstrate that dynamic shear stimulation markedly up-regulates cartilage biosynthesis of PRG4. The quantity of total PRG4 secreted was higher for cartilage subjected to dynamic shear stimulation than for unloaded and statically compressed cartilage (Figure 3). The number of cells expressing PRG4 was also higher in dynamically sheared cartilage than in unloaded cartilage (Figures 5 and 6). In addition, dynamic shear induced PRG4 expression by cells at depths of 200–400 μm from the articular surface, a region which did not express PRG4 under control conditions. Furthermore, shear stimulation may have affected the forms (different molecular weights) of PRG4 secreted and metabolized during culture (Figure 4). Static compression and dynamic shear stimulation protocols both prevented the increase in thickness typical of cartilage explants cultured under free-swelling conditions (Figure 2). These results suggest a possible feedback mechanism for mechanical regulation of the production of lubricant PRG4 molecules in vivo, that is, the tissue strains imparted to cartilage during motions

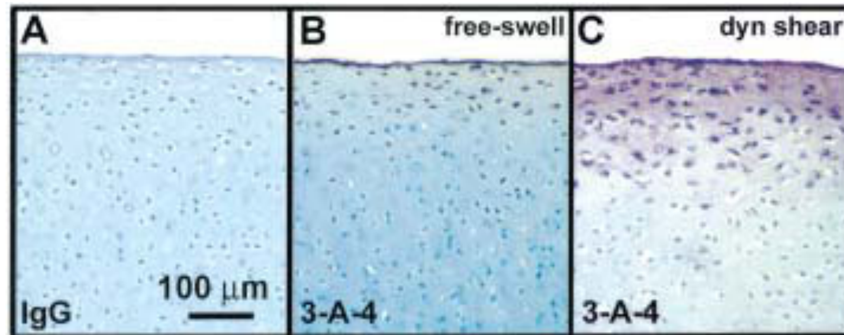

Figure 5. Immunohistochemistry for the presence of proteoglycan 4 (PRG4) within chondrocytes at different depths of cartilage. Shown are representative images of **A,** cartilage probed with a nonspecific mouse IgG antibody (representative control staining for both free-swelling and dynamically sheared tissues), **B,** free-swelling (free-swell) cartilage probed with monoclonal antibody 3-A-4, and **C,** dynamically sheared (dyn shear) cartilage probed with monoclonal antibody 3-A-4.

utilizing, and thereby depleting, boundary lubricant molecules at the articular surface simultaneously signal the nearby chondrocytes to secrete more PRG4.

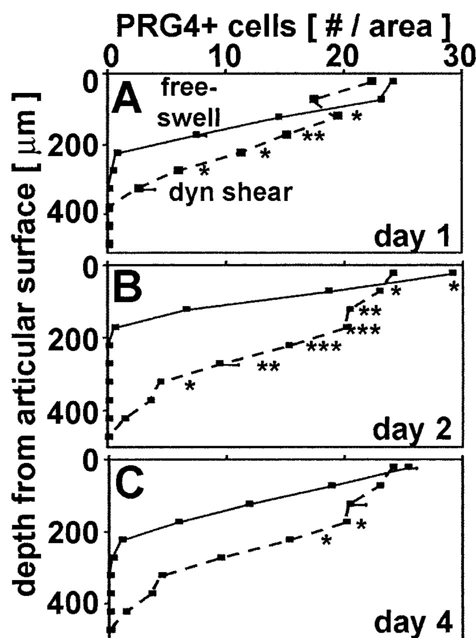

Figure 6. Depth-associated variation in chondrocyte expression of proteoglycan 4 (PRG4) in free-swelling (free-swell) and dynamically sheared (dyn shear) cartilage. Data are presented as the number of cells per area as determined on **A,** day 1, **B,** day 2, and **C,** day 4. Values are the mean of 8–12 samples; horizontal bars show the representative SEM. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ for the effect of loading, by unpaired *t*-test.

The bioreactor we used was developed based on a previous design used to study the effects of shear stimulation on cartilage protein and proteoglycan synthesis (19), with additional adaptations to provide tissue culture conditions without the use of an incubator. It was designed to impart dynamic shear frequencies and amplitudes that up-regulate chondrocyte metabolism of extracellular matrix molecules such as aggrecan and collagen (19,20) in cartilage explants. In addition, the compression we applied was in the range of cartilage deformation that has been shown experimentally to occur during motion in vivo (29). While the exact nature of the cartilage deformation during shearing was not quantified, the low total harmonic distortion, along with the smooth (no visible distortion) shape of the load waveforms (Figure 1D), indicated that the platens did not slip relative to the articular surface. In addition, the computed cartilage shear modulus was consistent with previously reported values (19,30,31), suggesting that the cartilage was indeed subjected to tissue-shear deformation (as opposed to surface motion, where the platen slides across the cartilage surface). The shear modulus reported herein is at the low end of the range (0.18–2.5 MPa) previously reported for middle zone immature bovine cartilage tested under simple shear (19) or pure shear (30), as well as for full-thickness adult bovine cartilage under simple shear (31), analogous to the lower compressive modulus in the superficial zone of cartilage compared with that of deeper zone and full-thickness cartilage (32).

PRG4 secretion and expression were analyzed to give a quantitative measure of PRG4 production as well as qualitative information to identify chondrocyte pop-

ulations that were expressing PRG4 under the various loading conditions. Since the initial PRG4 content of cartilage disks is an order of magnitude lower than that detected in culture medium, the PRG4 detected in medium samples provided an index of that synthesized during culture. Such secretion levels could result in an underestimation of the total synthesis, depending on the partition coefficients of PRG4 in cartilage under various conditions (33), which remain unknown. It would be of interest to determine how the level of PRG4 secretion relates to the amount of PRG4 that is bound and functional as a lubricant at the articular surface.

These results support the hypothesis that chondrocyte metabolism depends on the local microenvironment, which is determined by a complex combination of chemical and mechanical stimuli. Previous studies have shown that PRG4 synthesis and expression by chondrocytes in culture are markedly regulated by chemical factors. Inclusion of fetal bovine serum and ascorbate in culture media up-regulates PRG4 secretion levels (23). Certain growth factors and cytokines that are present at high levels in injury and arthritis also affect PRG4 metabolism. For example, transforming growth factor β1 increases PRG4 mRNA synthesis by chondrocytes cultured in monolayer (34,35), as well as protein secretion by chondrocytes in explants (22) and 3-dimensional constructs (2). Conversely, interleukin-1α has inhibitory effects on PRG4 mRNA expression (2) and protein synthesis (22).

The microenvironmental mechanisms by which mechanical stimuli regulate chondrocyte biosynthesis, however, remain to be established. In contrast to pure shear, where no fluid flow is expected, the tissue shear configuration used in our studies results in a small amount of fluid flow (20), which appears to be partly responsible for the stimulation of matrix biosynthesis by dynamic compression (17,36). Dynamic compression applied for the same duration did not result in the marked up-regulation of PRG4 secretion seen with dynamic shear (37). Alternatively, cell matrix deformation itself may cause changes in metabolism. Using a cell-tracking methodology (32), a preliminary study (Bae W: personal communication) demonstrated that application of ~4% shear strain to full-thickness human cartilage resulted in a level of shear strain that is high near the articular surface and decreases with depth, with ~8–13% shear strain in the superficial zone (top 400 μm) and ~3% shear strain in the middle zone (30–60% of the cartilage depth). The region of high shear strain, the superficial zone of the tissue, is the same region in which the percentage of chondrocytes expressing PRG4 is high, suggesting that chondrocyte responsiveness may be due, at least in part, to the local shear strain to which the chondrocytes are exposed.

The regulatory effect of dynamic shear stimulation reported herein is also consistent with recent evidence that certain mechanical stimuli, including cyclical tension (38) and articular surface motion (39), can up-regulate chondrocyte PRG4 mRNA expression by chondrocyte-seeded cartilaginous constructs, and our findings extend the current knowledge to include effects on secretion of PRG4 protein products by chondrocytes within their native extracellular matrix. The extent to which PRG4 synthesis was up-regulated by dynamic shear (300–400% over unloaded controls), was similar in magnitude to the 300–700% up-regulation of PRG4 mRNA expression due to surface motion reported by Grad et al (39). In contrast, shear stimulation of cartilage explants and engineered tissue up-regulates collagen and matrix proteoglycan synthesis by only 18–50% and 19–35%, respectively, over unloaded controls (19,20,40,41). In general, mechanical loading of cartilage regulates the expression of a large number of genes, including those sometimes considered to be constitutively expressed, such as GAPDH (42). The overall regulatory effects of shear loading on cartilage metabolism may be further clarified by simultaneous analysis of the expression of a broad range of genes.

The possibility that dynamic shear influences the structure of PRG4 products synthesized would supplement recent findings that joint motion applied to chondrocytes in polyurethane constructs differentially regulated mRNA expression of different PRG4 isoforms (43). Alternatively, the lower molecular weight bands could represent posttranslational cleavage of PRG4 products that were secreted as full-length molecules (44). Disruption of disulfide bonds could also have allowed the separation of several species of various molecular weights under reducing conditions that are normally held together under nonreducing conditions. Taken together, these results suggest that mechanical stimuli may affect the transcription, translation, or secretion of PRG4, and additional studies are needed to elucidate the molecular details of these regulatory effects.

The molecular mechanism of joint lubrication remains a somewhat controversial subject (see, for example, refs. 7, 45, and 46). However, if PRG4 molecules accumulate in synovial fluid as a lubricant for the articular cartilage surface in a manner that is dependent upon secretion rates by the surrounding tissues, the contribution by cartilage itself could significantly impact

the low-friction properties of the surface during joint motion. Understanding the role of mechanical stimulation in maintaining PRG4 levels in the synovial fluid could therefore lead to possible treatments to prevent the loss of the low-friction function of articular cartilage. In addition, the ability of dynamic shear stimulation to induce PRG4 expression in chondrocytes at a depth of 200–400 μm from the articular surface, but not those below 400 μm, may also prove useful for creating stratified tissue-engineered cartilaginous constructs from isolated chondrocyte subpopulations that have functional, lubricating articular surfaces.

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