Effect of tissue maturity on cell viability in load-injured articular cartilage explants

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Summary

Objective: During joint maturation, articular cartilage undergoes compositional, structural, and biomechanical changes, which could affect how the chondrocytes within the cartilage matrix respond to load-induced injury. The objective of this study was to determine the effects of tissue maturity on chondrocyte viability when explanted cartilage was subjected to load-induced injury.

Design: Cartilage explants from immature (4–8-week-old) and mature (1.5–2-year-old) bovine humeral heads were cyclically loaded at 0.5 hertz in confined compression with a stress of 1 or 5 megapascals for 0.5, 1, 3, 6 and 16 h. Cell death was assessed at 0, 24 and 48 h after load removal using cell viability dyes and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay. The organization of pericellular matrix (PCM), biochemical composition and biomechanical properties of the cartilage were also determined.

Results: For the immature and mature cartilage, cell death began at the articular surface and increased in depth with loading time up to 6 h. No increase of cell death was found after load removal for up to 48 h. In both groups, cell death increased at a faster rate with the increase of stress level. The depth of cell death in the immature cartilage was greater than the mature cartilage, despite the immature cartilage having a higher bulk aggregate modulus. A less organized PCM in immature cartilage was found as indicated by the weak staining of type VI collagen.

Conclusion: Cells in the mature cartilage are less vulnerable to load-induced injury than those in immature cartilage.

Key words: Cell death, Tissue maturity, Load-induced injury, Pericellular matrix.

Introduction

Load-induced injury is a known risk factor for the development of secondary osteoarthritis (OA). Several studies have shown that joint trauma in adolescents and adults leads to a higher incidence of OA. However, in a young child, joint trauma does not usually result in secondary OA. The pathogenesis leading to such a low incidence in children is, at large, unclear. The objective of this study was to determine the effect of tissue maturity on chondrocyte death in cartilage explants subjected to a defined load-induced injury.

From birth to skeletal maturity, articular cartilage undergoes endochondral ossification, forms a subchondral bone plate, and progressively decreases in thickness. In doing so, the cartilage alters its structural, biochemical and biomechanical properties to optimize its ability to resist and redistribute joint loads. The percentage of the solid component of the tissue matrix, mostly collagen, continues to increase after birth, gradually leveling off during maturation. On the other hand, there is a decrease in proteoglycan (PG) and link protein (LP) syntheses and in PG size, which accompanies the transition during the maturation process. Maturation also involves changes in the collagen fiber orientation within the matrix, and in the size, shape, and organization of the pericellular matrix (PCM). These compositional changes also lead to alterations in the biomechanical properties of the matrix, including decreases in tensile and compressive moduli.

Recently, we have shown that excessive cyclic mechanical loads (high stress level and long loading duration) applied to healthy, mature articular cartilage explants can cause matrix damage and cell death similar to that observed in the early stages of OA. In vitro, cell death has been shown to increase with load duration, load magnitude, strain rate, and post-load incubation time, and to negatively correlate with stress rate. In vivo, cell death (apoptosis and necrosis) increases with aging and arthritic severity. However, it is unknown how tissue maturity affects chondrocyte viability when cartilage is subjected to load-induced injury. Due to the significant changes occurring in the cartilage matrix during the maturation process, the ability of chondrocytes to survive load-induced injury, such as joint trauma that occurs with single and repeated excessive loads, could be affected by these changes. The objective of the present study was to determine the effect of tissue maturity on chondrocyte viability when the cartilage was exposed to load-induced injury. We hypothesized that cells in immature cartilage would be less vulnerable to mechanical injury than the cells in mature cartilage.

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Received 29 November 2003; revision accepted 16 January 2005.
Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, MO). Culture medium and supplies were purchased from Gibco BRL (Grand Island, NY).

CARTILAGE EXPLANTS

Immature (calf: 4–8 weeks old) and mature (adult: 18–24 months old) bovine shoulder joints were obtained from a local abattoir within 4 h post-mortem. Full-thickness cartilage explants were excised from the central region of the humeral head, 1 cm away from the periphery, using a 7-mm diameter circular biopsy punch to core the cartilage, and then removing the core from the subchondral bone using a surgical blade. Immature and mature explants were taken from eight joints and four animals each, pooled by age, and randomly distributed into the various loading protocols. Each cartilage explant was then sliced through its deep zone (D2), using a custom-fabricated cutting template and double-edged razor, to produce a uniform thickness of 2.5% (2.0 ± 0.05 mm with an intact articular surface. After washing with Dulbecco’s Modified Eagle Medium (DMEM, low glucose) supplemented with 10% antibiotic/antimycotic (3× for 30 min each), the explants were transferred to 24-well plates containing serum-free culture media (DMEM, 1% antibiotic/antimycotic, 10 mM HEPES buffer, and 50 μg/mL ascorbic acid), and maintained in an incubator at 100% humidity, 5% CO2 and 95% air at 37°C for 48 h before loading. The culture media with fresh ascorbic acid was changed three times per week.

BIOMECHANICAL AND BIOCHEMICAL PROPERTIES

To determine the biomechanical and biochemical properties of the immature and mature cartilage, additional explants were used. Explants were frozen at −20°C in phosphate-buffered saline (PBS) containing protease inhibitors until biomechanical testing was performed. Biomechanical relaxation tests were performed in confined compression using a custom designed computer-automated soft tissue test apparatus as previously described28. Prior to testing, the explant was cut to a diameter of 5 mm in order to fit into the loading chamber, and trimmed to a uniform thickness of 1 mm using a sledge microtome to remove matrix from the deep layers, leaving the articular surface intact. The bulk mechanical properties of the explants were measured as described previously28,30.

Briefly, a 5-mm diameter porous load platen was slowly lowered until it just contacted the surface; a 25 μm (2.5%) pre-displacement was then applied and held for 30 s to ensure uniform initial contact between the load platen and the articular surface. The cartilage sample was loaded to a maximum strain of 15% at a rate of 50 μm/s (5%/s). The instantaneous dynamic modulus was calculated by fitting a straight line to the linear region (last 30%) of the stress-strain response, using least-squares linear regression analysis. The cartilage sample was allowed to recover from the deformation for 30 min after removal of the load. A five-step stress-relaxation test was then performed to determine the bulk aggregate modulus and hydraulic permeability. Five steps of 40 μm (4%) were applied to the explant, allowing an equilibrium stress to be reached before applying the next step. The displacements and loads were continuously recorded, and the strains calculated after correcting for system stiffness. The bulk aggregate modulus was calculated by curve-fitting a straight line to the equilibrium stresses and strains (steps 1–5, 4–20%), using least-squares linear regression. The hydraulic permeability at 4% strain was calculated by fitting the data to a linear isotropic biphasic model29,30.

After the biomechanical tests, the explant was placed in PBS (0.1 M sodium-phosphate, 0.15 M NaCl, pH = 7.2) for 1 h to allow the matrix to regain its initial thickness (free-swelling). The explant was then gently blotted with a paper tissue (Kimwipe) and a wet weight recorded using an ultrasonic (±1 μg) microbalance (Cahn 25, Ventron, Cerritos, CA). The dry weight of the cartilage explant was determined after overnight lyophilization in a freeze-dryer (Labconco, Kansas, MO). The tissue solid content was calculated from the wet and dry weights. The explant was then digested in a 1 mg/ml papain solution containing 50 mM phosphate buffer (pH 6.5), 2 mM N-acetyl cysteine, and 2 mM ethylenediaminetetraacetic acid, with agitation at 65°C for 4 h. Aliquots from the digestate were then used to determine the PG content using the 1,9-dimethylmethylen blue dye-binding method31.

MECHANICAL LOADING PROTOCOL

Mechanical load was applied to the cartilage using a mechanical explant test system (METS) as previously described28. Briefly, each explant was placed within the loading chamber of the METS with the articular surface facing a porous load platen and immersed in DMEM culture media. The explants were cycled loaded (sinusoidal waveform) in confined compression with a stress of 1 or 5 megapascals (MPa) at a frequency of 0.5 hertz (Hz) for 0.5, 1, 3, 6 or 16 h. For each loading condition, a total of 10 (N = 10) explants were loaded in the METS, five (n = 5) immature and five (n = 5) mature explants; an additional 10 explants (five immature and five mature) served as non-loaded controls by placing them in the loading chambers but without the load platens (n = 5, N = 10). In all loading conditions, the METS was housed in an incubator at 37°C and 100% humidity environment with 95% air and 5% CO2.

ANALYSES OF CELL DEATH

Cell viability within control and loaded explants was evaluated immediately after loading. Cell viability was evaluated using a combination of two fluorescent probes: propidium iodide (PI), a cell membrane-impermeable dye which indicates cell death (stains dead cells red), and fluorescein diacetate (FDA), a cell metabolic dye which indicates cell viability (stains viable cells green)17. After loading, the explants were allowed to swell in PBS for 20 min. A thick slice (cross-section around 200 μm) was cut transversely through the explant and stained with 60 μM PI (Sigma) and 10 μM FDA (Molecular Probe, Portland, OR) in PBS for 5 min. The section was washed in PBS for 5 min to remove residual dye from the matrix, and viewed using a fluorescent microscope (excitation at 475–490 and 545–570 nm; NIKON Optiphot-2, Melville, NY) with a dual green and red barrier filter (wavelength ranges of 505–535 and 580–620 nm, respectively). The fluorescent images were captured using a cooled-color CCD camera (Optronics, Goleta, CA). Cell death was always found to start at the articular surface and to be uniform across the explant’s width. Thus, we measured the depth of cell death from the articular surface using SigmaScan Pro imaging software (SPSS Science, Chicago, IL), as previously described17.
To determine whether there was a delayed cell death after load removal, the remaining explants were incubated unloaded for an additional 24 and 48 h (three from each group). One section from each explant was taken to evaluate cell viability before being frozen for TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay and staining of type VI collagen. The frozen samples were sectioned to 6 μm thickness using a microtome cryostat, and analyzed for the presence of nicked ends of fragmented DNA in dead chondrocytes, using the TUNEL assay (Roche, Indianapolis, IN), as previously described.18

ANALYSIS OF PCM

In order to determine the structure of the PCM in immature and mature cartilage, sections from the non-loaded control and cartilage loaded with 5 MPa for 1 h were used to determine the organization of PCM by staining type VI collagen as previously described.13 Prior to staining, slides were thawed and fixed in 4% paraformaldehyde for 10 min and washed three times in PBS. Slides were then incubated with 1 mg/mL hyaluronidase (pH = 5.5) for 15 min, and with 1 mg/mL pronase (Calbiochem, San Diego, California) (pH = 7.4) for 30 min at 37°C for antigen retrieval. Non-specific binding was blocked with 10% goat serum (Chemicon, Temecula, CA) and 1% bovine serum albumin (BSA) for 10 min at room temperature. The slides were then incubated with type VI collagen antibody (Chemicon) at 37°C for 1 h. After washing with PBS and 0.1% BSA, sections were then incubated with a fluorescein isothiocyanate-conjugated goat anti-rabbit IgG secondary antibody in the dark at 37°C for 1 h, counter-stained with 0.5 μM PI for cell nuclei, and embedded in mounting medium with an anti-fade agent. Sections were viewed using a fluorescent microscope as described above.

STATISTICAL ANALYSES

The effects of tissue maturation on the biomechanical and biochemical properties of the cartilage were analyzed using the unpaired Student’s (two-tailed) t tests using Systat (SPSS, Chicago, IL) or Excel (Microsoft, Redmond, WA) software. The effect of tissue maturation on cell viability was analyzed using analysis of variance (ANOVA). Tukey/Kramer post hoc tests were used to determine statistical differences between treatment groups. The rate of cell death (depth vs loading time) was determined using a least-squares linear regression model (Systat). The number of explants within each loading group is designated by "n," while the total number of explants analyzed is designated by "N." All data are expressed as mean ± standard error of the mean (s.e.m.). Statistical significance level (α) was set at 0.05.

Results

BIOCHEMICAL AND BIOMECHANICAL PROPERTIES

Tissue maturity was a significant factor affecting the biomechanical and biochemical properties of the articular cartilage (n = 4 for each age group). Tissue solid content was significantly lower in the immature cartilage as compared to the mature cartilage (Table I). PG content [s-GAG (sulfated glycosaminoglycan) content per dry weight] was significantly higher in the immature cartilage as compared to the mature cartilage (Table I). The instantaneous dynamic and bulk aggregate moduli in the immature cartilage were statistically greater than those of the mature cartilage (Table I). The hydraulic permeability at 4% strain was not statistically different (P = 0.14, Table I).

CELL DEATH IMMEDIATELY AFTER 1 MPa LOADING

After mechanically loading the mature cartilage with 1 MPa, dead cells were found only in the superficial zone (SZ) of the matrix, consistent with our previous findings.17 Similar to the mature explants, cell death in the loaded immature explants was localized in the SZ [Fig. 1(C)]. No dead cells were seen in the middle and DZs of any explant as well as in the non-loaded controls, except along the cut edges [Fig. 1(A–D)]. These dead cells death along the cut edges was thought to be caused by a cutting artifact, and not related to load-induced injury, because it was consistent in the non-loaded controls and loaded cartilage.

Quantitative analyses showed progressively increased cell death, in the loaded immature and mature cartilage as a function of loading time (n = 5 for each group). In the mature cartilage, the depth of cell death increased linearly with the duration of loading from 11.3 ± 3.5 μm at 1 h to 54.3 ± 10.7 μm at 6 h. This was significantly higher than the non-loaded controls (5.9 ± 1.0 μm). There was no further increase in the depth of cell death between 6 h and 16 h of loading (61.8 ± 14.4 μm). A similar trend was seen in the immature explants, in which cell death increased from 45.9 ± 6.1 μm at 1 h to 124.8 ± 27.0 μm at 6 h, with no change after 6 h of loading.

The depth of cell death due to load-induced injury in the immature cartilage was significantly greater than that in the loaded mature explants (P < 0.001), despite a slightly greater depth of cell death in the immature controls as compared to the mature controls (17.1 ± 3.5 vs 5.9 ± 1.0 μm, respectively) [Fig. 2(A)]. Tissue maturation and load duration were significant factors for chondrocyte

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<th>Table I</th>
<th>Biochemical and biomechanical properties of immature and mature bovine cartilage (n = 4, in each group). * indicates p &lt; 0.05</th>
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<td>Instantaneous dynamic modulus</td>
<td>Bulk aggregate modulus (Ha)</td>
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<tr>
<td>Immature</td>
<td>6.99 ± 0.60 MPa</td>
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<tr>
<td>Mature</td>
<td>3.54 ± 0.36 MPa</td>
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<tr>
<td>P-values</td>
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Instantaneous dynamic modulus (strain rate = 5%/s), bulk aggregate modulus, and PG (s-GAG) content per tissue dry weight in immature cartilage were greater than those of mature cartilage. Tissue solid content increased with tissue maturity. No significant difference in hydraulic permeability (Kp, at 4% strain) was found between the two groups. Data are presented as means ± S.E.M.
death, as determined by two-way ANOVA ($P < 0.001$). Using a least-squares linear regression model fitting the data from the samples loaded for up to 6 h, the depth of cell death in the loaded cartilage increased with loading duration at a rate of $18.6 \pm 2.7 \, \mu m/h$ ($R^2 = 0.57$, $P < 0.001$) in immature cartilage and $8.4 \pm 1.3 \, \mu m/h$ ($R^2 = 0.52$, $P < 0.001$) in the mature cartilage, both of which were significantly higher than the non-loaded controls.

**CELL DEATH IMMEDIATELY AFTER 5 MPa LOADING**

In explants loaded with 5 MPa, cell death was again initiated at the articular surface. The depth of cell death at 5 MPa was significantly greater at each time point, compared to the response at 1 MPa [Fig. 2(B)]. Similar to the response at 1 MPa, the immature explants had a significantly greater depth of cell death, as compared to the mature explants ($P < 0.01$). A small increase was seen in the depth of cell death between 0.5 and 3 h of loading in both age groups, while dramatic increases were seen in the depths of cell death from 3 to 6 h [Fig. 2(B)].

In immature and mature explants loaded with 5 MPa for 6 h, the depth of cell death was 4.5-fold and 5.4-fold higher than that loaded with 1 MPa ($677 \pm 50$ vs $125 \pm 27 \mu m$ and $242 \pm 38$ vs $54 \pm 11 \mu m$, respectively). When loaded for 16 h, the mature explants had a further increase in the depth of cell death, as compared to that at 6 h, which was also found in the immature explants. After 16 h of loading, cell death extended into the middle zone of the immature cartilage, but only into the upper regions of the middle zone of the mature cartilage. The depth of cell death in the immature loaded cartilage was increased with loading duration at a rate of $65.3 \pm 15.7 \, \mu m/h$ ($R^2 = 0.51$, $P < 0.001$) and significantly higher than the non-loaded controls. However, the depth of cell death in the mature loaded cartilage was not increased with loading duration (rate $= 16.8 \pm 7.9 \, \mu m/h$, $R^2 = 0.18$, $P = 0.07$), consistent with our previous finding for the mature cartilage. In immature cartilage loaded for 6 h or longer, dead cells were evident in the middle zone, randomly distributed with viable cells. However, in mature loaded explants, dead cells were clearly separated from viable cells. The region with a mixture of viable and dead cells was not included for the quantitative calculation, which could result in a slight underestimation of the total amount of cell death under those loading conditions.

**DELAYED CELL DEATH DURING POST-LOADING**

Cell viability was also evaluated for intervals of 24 and 48 h after load removal. The amount (depth) of cell death after 24 and 48 h post-loading did not change for either age
The presence of PCM was determined using immunofluorescent staining against the type VI collagen (abundant in the PCM). Staining of type VI collagen in the PCM was less intense in the immature cartilage [Fig. 6(A, B)] than that in the mature tissue [Fig. 6(C, D)]. Flattened PCMs were consistently seen in the SZ of loaded immature cartilage as compared to unloaded controls, suggesting unrecoverable changes and possible damage to the PCM.

**Discussion**

The objective of this study was to determine the effect of tissue maturity on chondrocyte viability when cartilage explant was exposed to load-induced injury. The viability of the chondrocytes within immature and mature bovine articular cartilage was determined after the application of a continuous, cyclic stress of 1 or 5 MPa for time intervals ranging from 0.5 to 16 h. Our results showed that under the same loading conditions of stress magnitude and duration, there was a greater depth and faster rate of cell death in the immature cartilage explants than in the mature cartilage explants. We also found that the location of the cell death in the immature cartilage was the same as in mature cartilage loaded with 1 MPa, which was located only in the SZ. However, at 5 MPa cell death progressively spread to the SZ and middle zone with increasing duration of loading.

We believe that this pattern of cell death was due primarily to the heterogeneity in the material properties (organization and content) of the cartilage matrix, which, to a large part, determines the biomechanical response of the matrix to mechanical loads. Several studies have shown that the compressive modulus of the SZ is much less than those in the DZ and of the average (bulk) modulus. In immature cartilage, the apparent equilibrium modulus of the DZ is 10-fold higher than that in the SZ. This is compared to 6.1-fold difference in the mature cartilage. Thus, when the tissue is loaded the SZ will deform more (greater strain) than the zones below it. In the normal loading of a joint, the SZ probably functions to cushion the middle and DZs by deforming more to absorb the mechanical energy. However, under excessive loading conditions, such as those applied to the explants in this study (high stress and long duration), the deformation of extracellular matrix in the SZ could be the most (estimated to be greater than 60%) and rupture the cell membrane beyond the capability for a chondrocyte to repair. Thus, cell death would then become unavoidable.

It should be noted, however, that cell death in normal immature and mature cartilage does not usually occur under physiological joint loads. Although the stress magnitudes used in this study (1 and 5 MPa) are at physiological levels, the loading times used were much longer than the normal. In our study, the explants were loaded for the entire cycle, and loading continued for up to 16 h. In normal walking or running, the cartilage would only be loaded for a continuous, cyclic stress of 1 or 5 MPa for time intervals ranging from 0.5 to 16 h. In normal immature and mature cartilage does not usually occur under excessive loading conditions, such as those applied to the explants in this study (high stress and long duration), the deformation of extracellular matrix in the SZ could be the most (estimated to be greater than 60%) and rupture the cell membrane beyond the capability for a chondrocyte to repair. Thus, cell death would then become unavoidable.

A surprising finding was that the greater depth of cell death found in the mechanically stiffer immature cartilage than in the mature cartilage, which was contrary to our hypothesis. Decreased cell death with tissue maturity was also reported in a recent study by Shah et al. using static unconfined indentation. The finding of the decrease of aggregate modulus in our study is consistent with that in rabbit cartilage. Therefore, the question remains as to why the mechanical properties did not correlate with the amount of cell death, that is, stiffer — less strain — less cell death. One possible reason may be the differences in the organization of the PCM. The PCM, while almost 10 times weaker (more compliant) than the rest of the extracellular matrix, is believed to have a greater ability to bear stress and may have a different role in matrix mechanics.
matrix, can protect the chondrocyte from high compressive deformation. In our study, a thicker and more intense staining for type VI collagen was found in the mature than immature cartilage, which is consistent with previous findings. This suggests that the increased organization of the PCM in the mature cartilage might provide better protection from load-induced injury, in spite of weaker overall (bulk) matrix compressive properties.

Two other factors contributing to the lower cell death within mature cartilage need to be addressed as well. The first factor is the greater collagen content and fiber organization in the matrix of mature cartilage. Collagen content can double during the maturation process. The organization of the collagen fibers progresses from a somewhat random collagen meshwork in immature cartilage to a more organized arrangement with maturation.

Fig. 3. Low-power fluorescent photomicrographs of cartilage loaded with 5 MPa for 6 h. Live cells were stained green by FDA, whereas dead cells were stained red by PI: mature cartilage (A) immediately after loading and (B) 48 h after unloading; immature cartilage (C) immediately after loading and (D) 48 h after unloading. Images (A) and (B) were taken from the same cartilage explant, so were images (C) and (D). (Bar = 300 μm.)

Fig. 4. Depth of cell death in immature and mature cartilage explants loaded with 5 MPa for 0.5, 1, 3, 6, and 16 h. Explants were analyzed either immediately after loading or 48 h after unloading. Data are presented as means ± S.E.M.; N = 100.
Fig. 5. Low-power fluorescent photomicrographs of TUNEL-stained cartilage sections loaded with 5 MPa for 6 hours. TUNEL-positive (green fluorescent) cells were seen in (A) immature and (B) mature cartilage 48 h after unloading as compared to positive-control (C) which was treated with DNase. (Bar = 200 μm.)

Fig. 6. Low-power and high-power (inset) micrographs of type VI collagen in immature and mature cartilage using immunofluorescent assay: (A) immature non-loaded controls; (B) immature cartilage loaded with 5 MPa for 1 h; (C) mature non-loaded control; and (D) mature cartilage loaded with 5 MPa for 1 h. The staining of the type VI collagen in the PCM was more intense in the mature (C, D) than in the immature tissue (A, B). The high-power images of the articular surface highlight the pericellular matrices of the SZ chondrocytes. Flattening of PCMs, as indicated by arrows, was found in the SZ of the loaded immature cartilage, suggesting load-induced damage. (Bar = 150 μm.)
In mature cartilage, the reinforcing collagen fibers are oriented in relation to tensile stresses experienced by the tissue. The collagen fiber orientation in the SZ, as observed using polarized light microscopy or after silver impregnation, is aligned parallel to the articular surface. The organization of the collagen in the SZ is optimized to resist tensile forces, and thus reduces the in situ tensile deformation experienced by the cells in the SZ. The second factor is that the thickness of the SZ in immature cartilage is greater than that in the mature cartilage. Since the apparent compressive modulus in the SZ is much less than those in middle and DZs, as described above, the greater thickness of the SZ in the immature cartilage can result in a greater depth of cell death.

The greater depth of cell death in the immature cartilage, as compared to the mature cartilage, remained consistent for all loading times and even after an unloading interval of up to 48 h. The later post-loading incubation period was used to determine whether there was delayed cell death due to apoptosis, which can take several hours before becoming observable. Our findings showed that there was no increase in cell death in either age group after 24 and 48 h of unloading, and that the TUNEL-positive cells were primarily necrotic cells. It is interesting to note, however, that the immature explants, while having no increase in cell death after load removal, did exhibit many TUNEL-positive cells. This is consistent with the study by Tew et al. who showed a greater percentage of TUNEL-positive (delayed) cell death in immature than mature bovine cartilage after wounded using a trephine. It is unclear why the necrotic chondrocytes in immature cartilage were more likely to undergo DNA fragmentation than in mature cartilage.

The lower GAG content and bulk aggregate modulus of the mature cartilage as compared to the immature cartilage, is a consequence of protein turnover and assembly. Our result is consistent with previous findings of decreases in cellularity, total protein production, LP synthesis and aggrecan production with tissue maturity. Together, these changes in protein turnover and assembly can help to explain the variation in biomechanical properties during the maturation process. Consistent with previous studies reporting age-related differences in tensile strength and compressive stiffness, our biomechanical testing showed a significant decrease in the bulk aggregate modulus as the cartilage matures. It should be noted that the bulk aggregate modulus measured in our study was comparable to the studies with the inclusion of the SZ, but lower than other findings where the SZ was removed.

Our finding that the load caused an initiation of cell death in the SZ may help clarify the factors responsible for cartilage degeneration and the onset of OA. It is known that mechanical damage to the cartilage matrix leads to secondary OA and that the disease process is often correlated with advanced age. Collagen degradation and loss of cellularity in the SZ of the cartilage matrix are pathological features of load-injured cartilage and the early stages of OA. However, our finding that cell death in the immature cartilage was greater than that in the mature cartilage was quite unexpected, since OA is not a disease process found in immature individuals. Therefore, the reasons for a low incidence in younger individuals could be from other factors, which include: (1) less injury due to the lower body weight (impact energy) and softer subchondral bone; (2) the presence of unique growth or repair mechanisms, such as a greater chondrogenic potential, higher mitotic activity, or the induction of chondrocyte differentiation; (3) fewer mechanical insults occurring over a lifetime, which could be additive to cause the degree of cell death and matrix damage needed for the onset of OA; (4) the greater thickness of immature cartilage resulting in a smaller percentage of overall injury as compared to mature cartilage; or any combination of the above factors. As a result, immature cartilage may indeed be more susceptible to load-induced injury, but less likely to accumulate enough degradation/injury to initiate the development of OA.

Acknowledgments

This study was supported by grants from the Arthritis Foundation – New York Chapter (ASL), HSS MacArthur Cartilage Fund (CTC), and National Institutes of Health AR45748 (PAT).

References


