Introduction: Nonphysiologic mechanical loading of articular cartilage, as can arise in obesity or traumatic injury, may be a contributing factor in the development of degenerative diseases of cartilage. Previous investigations [1] have suggested that relatively rapid (strain rate ~0.5 s⁻¹) and very slow (strain rate ~0.00005 s⁻¹) ramp loads applied to osteochondral explants can give rise to differing patterns of cartilage matrix and cell injury. Our goals were to extend these studies to include strain rates closer to those characteristic of the “gel diffusion” rate (~0.001 s⁻¹) [2], in order to further characterize relationships between matrix mechanics and injury, and identify micromechanical factors which can cause cell injury in cartilage and may lead to subsequent degeneration.

Materials and Methods: Osteochondral cores of 4 mm diameter were drilled from adult bovine humeral heads. The full-thickness cartilage layer was subsequently trimmed to 2.7 mm diameter and the osteochondral explants were incubated in supplemented DMEM [1] with daily media changes for a total of 10 days. Spent media was stored at 4°C. On day 6 cartilage thickness was measured under a dissection microscope and a single uniaxial radially unconfined ramp compression was applied individually to each explant. Compression was characterized by a defined strain rate (0.7, 0.07, 0.007, 0.0007, or 0.00007 s⁻¹) and maximum stress (3.5, 7, or 14 MPa). Wet weights were measured immediately before and after compression by removing surface water from explants and weighing on an analytical balance. On day 10 explants were removed from culture, inspected with the naked eye for the presence of surface cracks, and weighed again. To assess chondrocyte viability, a vertical slice of ~1 mm thickness was cut manually with a razor blade, from the middle of the explant and perpendicular to the articular surface. Slices were incubated for 5 minutes in a solution of calcein AM and ethidium homodimer-1 and examined under a confocal fluorescence microscope to visualize spatial distributions of live and dead cells [3]. The cross-section of cartilage was divided into superficial, intermediate and deep zones in the axial direction (10%, 50%, and 40% of tissue thickness from the cross-section of cartilage) as well as three equally-sized zones in the radial direction (from the center of explants to the radial edge). A human user identified areas of images containing cartilage (regardless of cell viability) and containing viable cells with a normal appearance. The proportion of tissue containing viable chondrocytes was thereby estimated within each defined tissue zone. After viability measurements explants were reconstituted, digested in 125 µg/ml papain overnight, and assayed for proteoglycan (PG) content along with media aliquots by the DMMB method [4]. Differences between compressed explants and uncompressed controls were identified by two-tailed T-tests. Data are reported as mean±sem (n).

Results: The likelihood of the appearance of superficial cracks increased with strain rate and peak stress, with a complete lack of visible cracks for strain rates ~0.00005 s⁻¹. As expected, the maximal uniaxial strain behaved similarly to the changes in wet weight (Figure 2). For example, explants compressed at 0.07 s⁻¹ to 3.5 MPa exhibited a peak compressive strain of 0.39±0.01 and a weight loss of 0.88±0.16 mg while 14 MPa peak stress resulted in a maximal strain of 0.64±0.02 and a weight loss of 1.02±0.15 mg. For strain rates from 0.7 s⁻¹ to 0.0007 s⁻¹ cell death occurred mainly in the superficial zone and near superficial cracks (Figure 3a). A dramatic decrease of the volume fraction of tissue containing viable cells (v; Figure 3b,c,d) emerged at 0.00007 s⁻¹ strain rate; the zone of dead cells extended to the full tissue depth including more of the full radial extent of the explant (Figure 3c). Consistent with previous findings [1], PG release was elevated during the three days following dissection and then stabilized. It increased relative to uncompressed controls for 2 days following compression for strain rates higher than 0.007 s⁻¹, but did not change for any others.

Discussion: At the extremes of strain rates tested (0.7 and 0.00007 s⁻¹), matrix injury as evidenced by superficial cracks and PG release to culture media was consistent with previous findings [1]. The present study included additional intermediate strain rates (0.07, 0.007, and 0.0007 s⁻¹) and demonstrated a lack of matrix injury for strain rates similar to or slower than the characteristic rates associated with cartilage “gel diffusion” (~0.001 s⁻¹) [2]. This suggests an important role for interstitial fluid pressurization as an important criterion for matrix mechanical failure. Compression was also associated with changes in the spatial distributions of viable cells in the matrix (Figure 3). Cell death appeared to be associated with superficial cracks at relatively high strain rates but also occurred in the absence of cracks, suggesting different sets of contributing micromechanical factors. In contrast with previous findings [1], very slow (0.00007 s⁻¹) compression to 14 MPa caused extensive cell death which was least severe near the center of explants. This difference may have been due to the slightly different explant geometry used (in the present study the cartilage could not “escape” compression by “bulging” over the edge of the bone in osteochondral explants) and appears to argue in favor of dehydration (Figure 2) and solid deformations as factors contributing to chondrocyte death at very low strain rates.