PRESTRAIN AFFECTS THE RESPONSE OF ARTICULAR CARTILAGE TO INJURIOUS COMPRESSION

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Introduction:
Nonphysiological mechanical loading is a contributing factor to the initiation and progression of degenerative diseases of articular cartilage [1]. Previous in vitro studies of the tissue response to injurious compression have shown that macroscopic superficial cracks and cell injury depend upon applied stresses and rates of strain [2,3], with the "gel diffusion" rate representing an apparent threshold separating different biomechanical regimes of tissue injury [4]. These studies highlight the importance of cell-scale micromechanical factors in determining the spatial distribution of injury within compressed cartilage. In the context of surgery or even normal locomotion, mechanical constraints on articular cartilage may be altered and thereby change its mechanical properties [5] and the local susceptibility to injury. We hypothesized that the loading history of cartilage may have important influences on its response to injurious compression. Therefore, different levels of prestrain were applied to cartilage explants before injurious compression, and tissue injury was assessed in terms of superficial cracks, cell death, and fluid loss for comparison with mechanical loading parameters.

Materials and Methods:
Osteochondral cores of 4 mm diameter were drilled from adult bovine humeral heads. The full-thickness cartilage layer was trimmed to 2.7 mm diameter and osteochondral explants were cultured in supplemented DMEM with daily media changes for 10 days. After 6 days, cartilage thickness was measured under a dissection microscope and used as the reference for strain during compression which was applied the same day. Compression was applied in three steps (Figure 1a): a prestrain of 0, 5, 10, 25 or 50% applied at 7x10^-3 s^-1 (which was previously found to induce no injury [4]), a 15 minute relaxation at the fixed prestrain, and a single ramp "injurious" compression characterized by a strain rate of either 0.07s^-1 or 0.007s^-1 and a maximal stress of either 3.5 or 14 MPa. Before compression, explants were randomly assigned to a group characterized by the level of prestrain, and the strain rate and peak stress of injurious compression. Control explants were subjected to prestrain and relaxation only. The loading apparatus continuously recorded stress and strain versus time. Three parameters were deduced from the stress-strain curve (Figure 1b): the maximal strain attained, the equilibrium modulus (E_eq) defined as the mean slope of the curve between 2.5 and 3.5 MPa, and 11 and 14 MPa respectively. Explants were weighed just before and after the compression sequence to determine fluid loss. On day 10 explants were weighed again, and inspected with the naked eye for the presence of superficial cracks, cell death, and fluid loss for comparison with mechanical loading parameters.

Results:
Mechanics of Loading: E_m was approximately constant around 0.25 MPa for 5, 10 and 25% prestrain but significantly increased to 0.66±0.059 MPa (n=20) at 50% prestrain (Figure 2a). For all prestrain conditions, E_m increased with stress during injurious compression (Figure 2b); however, the proportional increase was least dramatic for 50% prestrain. Among the different levels of prestrain between 0 and 25%, E_m was not significantly affected by prestrain nor by strain rate.

However, E_m was significantly increased by 50% prestrain compared to lesser prestrains. Prestrain had no influence on the maximum axial strain attained at the end of the compression sequence. Normalized fluid loss (the volume of exuded fluid divided by explant volume) during prestrain increased from 0.05±0.02 (n=10) at 5% to 0.34±0.09 (n=6) at 50%. Significantly less fluid was lost during injurious compression at 0.07s^-1 for 50% prestrain compared to all other conditions.

Discussion:
Prestrain modified the response of our cartilage explants to injurious compression. With increasing prestrain from 0 to 10%, the formation of surface cracks and the induction of cell death due to injurious loading decreased. These levels of prestrain correspond approximately to the extent which cartilage explants swell upon removal from a joint surface [6], suggesting that dissection itself may render cartilage more susceptible to injury due to swelling. The mechanics of loading indicate an important role for radial fluid outflow and matrix tensile loading in mediating injury of our cylindrical explants. With increasing prestrain the matrix fluid volume fraction decreased; at 50% prestrain E_m increased indicating stiffening of the solid part of the matrix. However, after 50% prestrain a relatively small amount of fluid was exuded during injurious compression and the stress-strain relationship became more linear. This tendency toward more elastic (as opposed to poroelastic) behavior with increasing prestrain was accompanied by a decrease in superficial cracking and cell death. Results therefore imply that matrix swelling, which may occur due to surgical slicing, could render cartilage more vulnerable to subsequent mechanical injury in vivo. Furthermore, since prestrains in the range of 10-50% reduced the injurious effects of subsequent loading, results suggest that quasistatic loading can reduce the susceptibility of cartilage to mechanical injury. These findings emphasize the importance of cell-scale physical factors resulting from the tissue loading history in the cartilage response to mechanical injury; results may be relevant to injury modelling, prevention, and treatment, as well as in surgical and tissue engineering applications.


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