INTRODUCTION

Mechanical characterization of articular cartilage, soft tissues and gels is important for biomechanical reference data and for functional assessment of biopsied or engineered tissue. Common techniques include dynamic mechanical spectroscopy [1] and stress relaxation [2], with the latter often favored for technical simplicity. While reliable, stress relaxation measurements of cartilage mechanical properties are still subject to significant uncertainty. Using [2], interpretation of the poroelastic governing equations, we have developed an approach which combines creep and stress relaxation to effectively double the amount of data available from soft tissue mechanical characterization experiments with only a small increase in the experimental time required.

METHODS

Adult bovine femurs were obtained fresh from a local butcher. Cylindrical osteochondral explants of 4.2 mm diameter were obtained from the femoral head using a trephine. Full-thickness cartilage explant disks were then separated from underlying bone with a razor blade. Only geometrically regular cylindrical specimens were tested. Prior to mechanical testing, explants were cultured for up to several days in supplemented DMEM (Cellgro) as previously described [3].

Cartilage explant disks were subjected to radially confined axial compression within a precision mechanical testing apparatus consisting of a load cell (Model 31, Honeywell) and displacement actuator (LTA-HL, Newport) mounted in an aluminum and stainless steel frame and interfaced to a PC using instrumentation software (LabVIEW). Explants were bathed in phosphate buffered saline (PBS), radially confined in a 4.2 mm diameter plexiglass well and compressed axially using a stainless steel plunger. Opposite the plunger, a rigid, porous ceramic filter permitted fluid flow from the cartilage disk in the axial direction. Starting from 0% bulk compressive strain, an alternating sequence of stress relaxation and creep transients was imposed. Stress relaxation was induced by an increment of ~5% bulk strain over a few seconds followed by constant explant thickness while stress relaxed to a steady-state value. Stress relaxation steps were performed up to ~30% total compressive strain. After each stress relaxation step, creep experiments were performed. Starting from the steady-state stress after each relaxation, a ~10% increase in stress was imposed using a control algorithm which increased sample compression when the measured stress fell below the desired stress. Therefore, during creep, explant thickness decreased while applied stress was relatively constant. After testing, explants were placed in PBS overnight, dried with a tissue, weighed, lyophilized, and weighed again to determine their free-swelling water content.

As previously described [2] steady-state stresses and strains were used to determine the bulk longitudinal (confined compression) elastic modulus \( H_c \). Stress relaxation transients were analyzed to determine the first-order exponential time constant governing relaxation just before equilibrium. This provided a means for measurement of hydraulic permeability \( k \) within a poroelastic description where compressive strain “diffuses” through explants during stress relaxation [2]. Creep transients were similarly interpreted. Interestingly, the distribution of compressive strain within explants during creep at relatively early times \( t \) following the change in applied stress was found to be analogous to descriptions of solute diffusion within semi-infinite media, with the result that explant thickness \( d \) during creep was expected to be a linear function of \( t^{0.5} \):

\[
d(t) = d_0 - \left( d_u - d_0 \right) \left( \frac{4H_c k t}{\pi \phi d_u d_s^2} \right)
\]

where \( d_0 \) and \( d_u \) represent explant thickness at the beginning and end of the creep transient, \( d_u \) is the unstrained thickness, and \( \phi \) is the fluid volume fraction at the early stages of creep.

RESULTS

Stress relaxation transients exhibited an exponential-like decay in the approach to equilibrium (Fig 1a). Since the apparatus functioned intrinsically in “displacement control”, there was little apparent noise in the thickness signal during stress relaxation (Fig 1b). Our algorithm for maintenance of constant stress during creep was reasonably successful, albeit with some noise in the applied constant stress (Fig 1a). Analysis of stress relaxation data provided values of \( H_c \) and \( k \) which were consistent with previous measures \([1,4]\); for the sample illustrated here, those values were \( H_c = 420 \) kPa and \( k = 1.5 \times 10^{-15} \) m^2/Pa s.

As anticipated, explant thickness was a linear function of \( t^{0.5} \) (Fig 2). Application of this theory for these data provided \( k = 3.4 \times 10^{-15} \) m^2/Pa s, which was consistent with the results of stress relaxation measurements.

DISCUSSION

The use of stress relaxation for measurement of equilibrium properties (e.g. \( H_c \)) of cartilage is logical, since displacement control hardware is easy to implement and stress relaxation is often faster than creep. It therefore makes sense to take advantage of the stress relaxation approach to equilibrium [2]. However, a drawback is that values of \( k \) measured in this way can be quite noisy. Complementary measures of this same parameter, using creep transients as illustrated here, could therefore provide more accurate determination of \( k \) and its variation with cartilage compression. Since this requires only the early stages of creep and not the entire transient, creep measurements can be efficiently combined with stress relaxations for more accurate characterization of soft tissue mechanical properties.

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REFERENCES