Supporting information (Zareian et al)

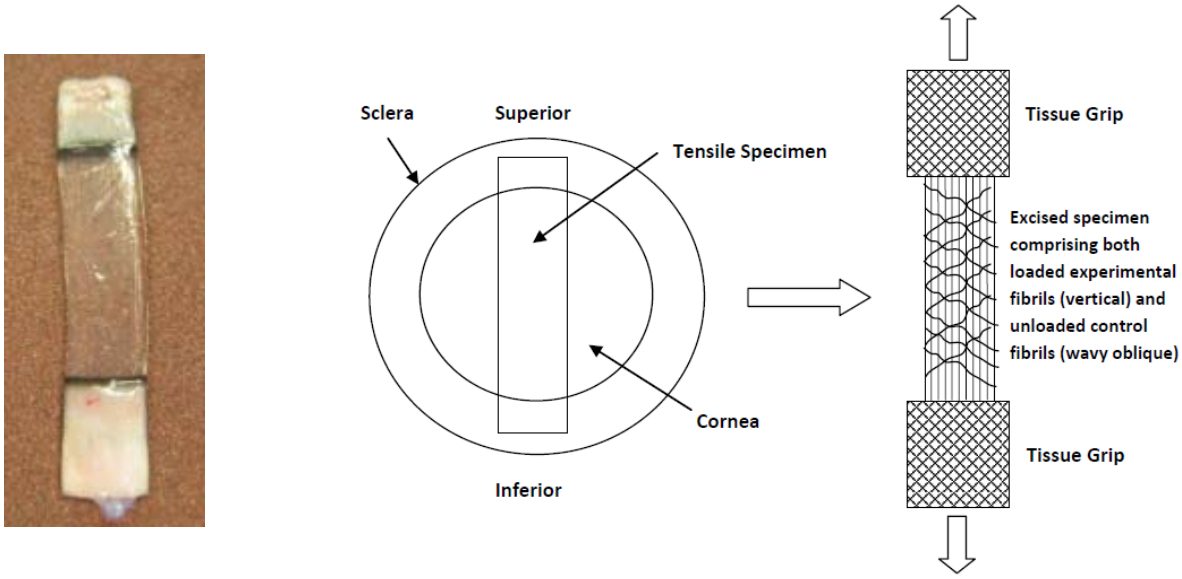


Fig. S1. Specimen geometry, fibril orientation and loading. A tissue strip was excised from bovine ocular globe (left/center) in the superior/inferior direction leaving small scleral extensions which were clamped into the tissue grips (right). The unique architecture of the cornea provides an internal control set of collagen fibrils with composition and diameter (oblique wavy lines) identical to the experimental fibril set (straight vertical lines). The control fibrils are mechanically uncoupled from the loaded experimental fibrils in the system and will experience a markedly lower tensile strain.

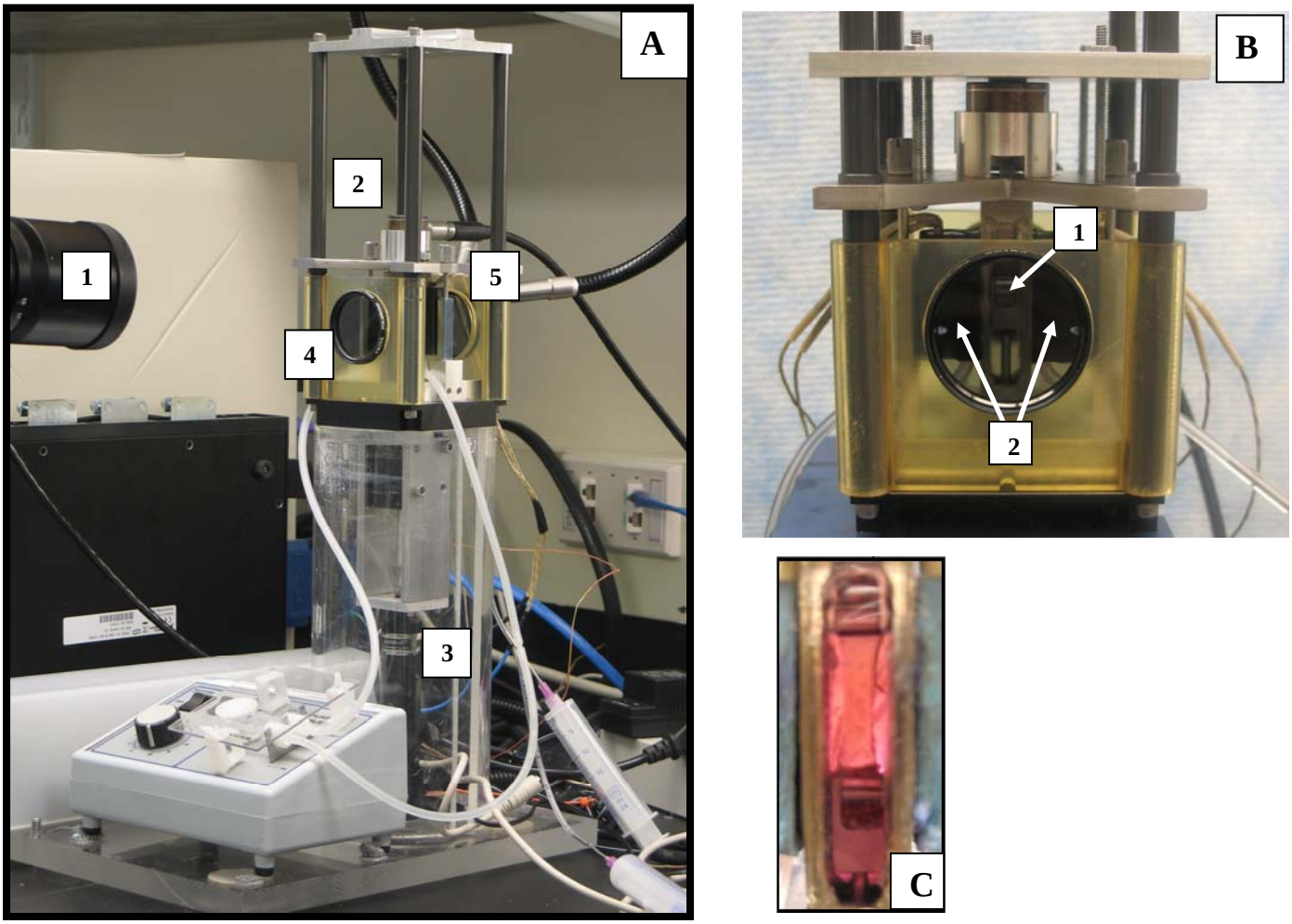
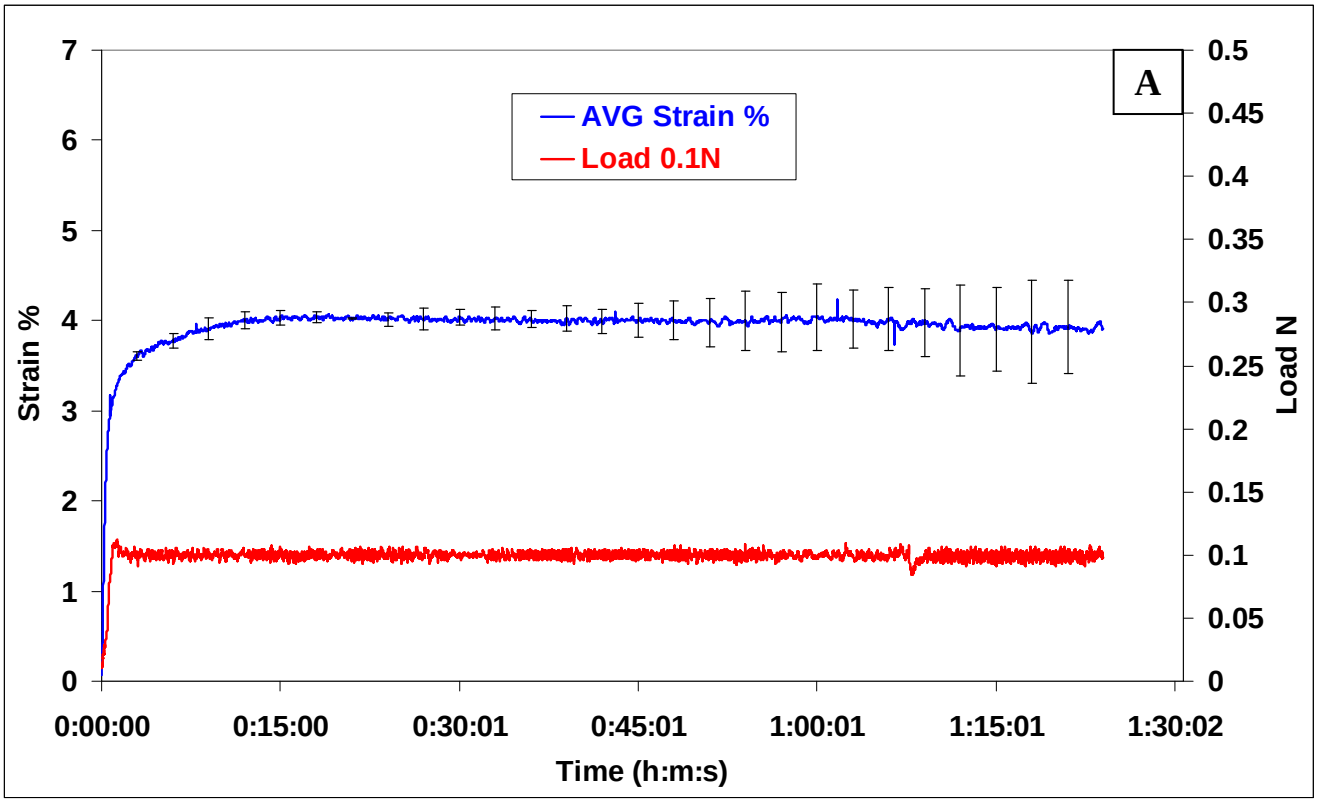


Figure s2. Miniature incubating, uniaxial loading bioreactor. A) The bioreactor, features include 1- digital camera (Prosilica, Model CV640, Black and white, Frame rate 120 fps, Resolution 9.9x9.9 mm per pixel, Newburyport, MA), 2- load cell (Honeywell Sensotec, Model 31; Max - 5 lb; Resolution 0.0002 lb, Columbus, OH) 3- uniaxial motor (Zaber Technologies T-LA60; Resolution: 16 mm; Max Speed: 4 mm/s, Speed resolution 0.001 mm/s, Vancouver, BC, Canada). 4- polarizing lens and 5- custom-made heated specimen chamber. B) Closer view of chamber with copper block heaters in situ. 1- custom cam grip on moveable arm; 2- custom copper heater blocks for maintenance of temperature. C) Tissue strip in the main chamber during degradation test in the presence of BC. The initial prototype for this device was produced via Northeastern University's Capstone Design Course [Group members: Kelli Church, Brad Jaworski and Ryan Cahill]. The bioreactor is fully described in the Master's Thesis of K. P. Church - Effect of tensile mechanical load on the degradation of native collagenous matrices – May 2007



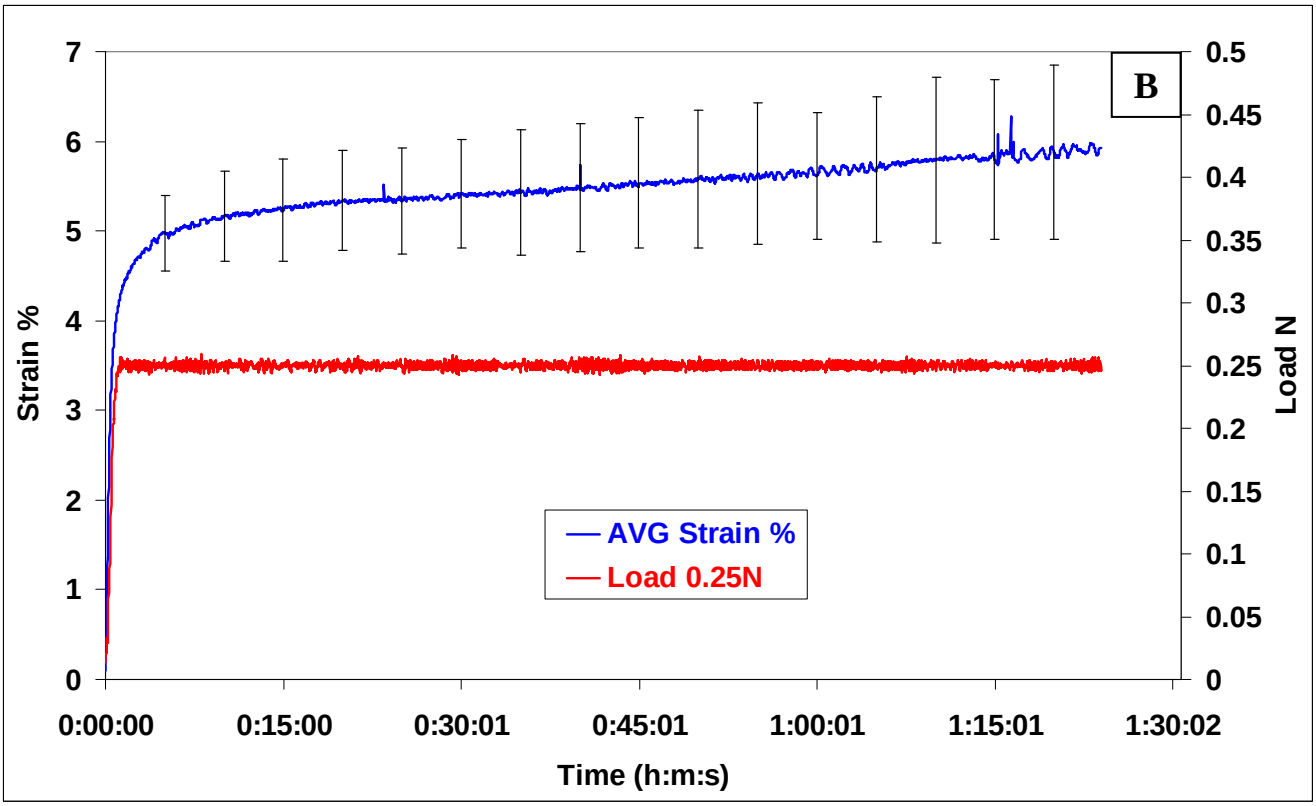


Fig. s3. Control experiment creep response. Strain and load vs. time for control test with 0.1N (A) and 0.25N (B) loads. Strain data is average for 3 experiments (bars are standard deviations). Load was the controlled variable and the load data is representative of one experiment.

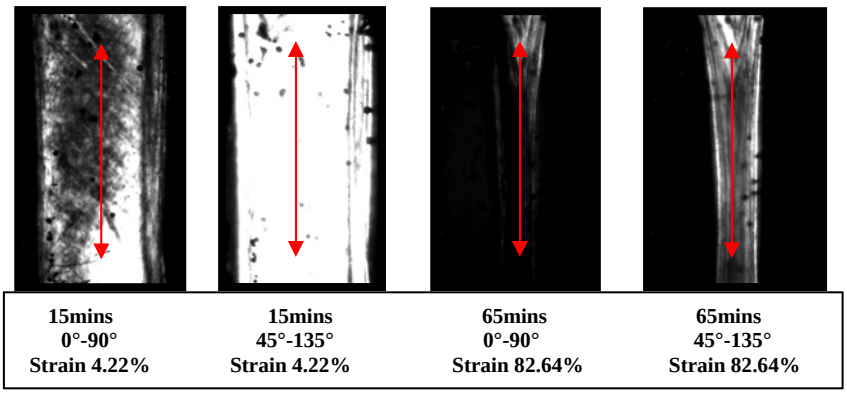


Fig. s4. Polarization imaging of tissue strips before collagen exposure and after sample failure. Polarization axes were oriented at either 0°-90° or 45°-135° (see first frames of figure 2A in main paper for polarization axis orientation). This series depicts digestion Test with 0.1N load.

Supplementary movie. Representative experiments for low and high-load degradation tests. The movie is provided to demonstrate the correlation between the actuator reported "grip" strain and the observed "image" strain which is extracted directly from the movie image sequence. The left two frames are the raw polarized images captured from the sample at 0.1 N load (top) and 0.25 N load (bottom). To extract the image strain information, two features in the center of each specimen were tracked (marked with dots red or green). The features were chosen by taking advantage of the natural heterogeneity of the tissue under the polarized light. Features had to be both optically stable and locally static relative to immediate surrounding structures. The right panels are concurrent plots of the "image" strain and the "grip" strain for the high and low load experiments. The "grip" strain is just a conversion of the motor displacement data to strain. The plots show that the two strains match each other well and also confirm that the sample under lower load is straining more quickly during the first few minutes of the experiment (our most critical measurement in this investigation).

Collagenase Activity Assay

The enzymatic degradation of collagen by *Clostridium histolyticum* collagenase (BC) has been shown to follow two-step Michaelis-Menten kinetics with Michaelis-Menten constant, K_M, and catalysis rate, k_{cat} for BC on bovine type I collagen reported at 3.5μM and $0.277s^{-1}$, respectively[1]. For concentrations of substrate equal to or greater than K_M, the equations governing product formation in Michaelis-Menten kinetics depend linearly on initial enzyme concentration and k_{cat}. To work within this regime, we chose reaction concentrations based on K_M reported by Mallya *et al.*[1] BC (C0130, Sigma-Aldrich, St. Louis, MO), kept at -80°C until use to retain activity, was activated by dissolving in Dulbecco's Modified Eagle's Medium (DMEM: 10-017-CM, Mediatech, Manassas, VA) and incubating for 30 min. at 37°C. Bovine type I collagen (# 5005-B, Advanced Biomatrix, San Diego, California) standards were made by diluting collagen (3mg/mL in 0.01M HCl) with 0.01M HCl. Samples and standards, as well as 0.01M HCl (control), were pre-warmed 15 min. at 37°C. BC (in DMEM for calcium activation of BC, as well as neutralization of collagen) was added to collagen samples, 20nM final [BC], 5uM final [collagen]. Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) (E5134, Sigma-Aldrich, St. Louis, MO), final concentration 10mM, was added to each sample to stop enzymatic degradation. Degradation times ran 0-30 min. in 2 min. intervals. EDTA and DMEM without BC were added to collagen standards, while EDTA and DMEM + BC were added to controls. All samples experienced the same time (45 min.) in the 37°C water bath.

Once all degradation samples had been stopped, all samples were transferred to a 35°C water bath for 30 min to permit the digested collagen molecular fragments to thermally denature [2]. All samples were then diluted in 0.5M Acetic Acid and stored at 4°C for 3 hours to allow aggregated molecules to re-dissolve and to bring the collagen concentration below dye saturation levels for the Sircol dye step. Samples were taken out 12 at a time, 100μL sample was combined with 1mL

Sircol dye and shaken for 30 min. at 200RPM (Sarstedt TPM2 orbital shaker), then centrifuged for one hour at 14,000xg. The supernatant was drained, and any remaining drops were swabbed with cotton tips. The pellet was resuspended in 1mL Sircol alkali solution by shaking overnight. Four 200uL aliquots from each sample, as well as 200µL aliquots of Sircol alkali blanks, were placed in a 96-well plate and absorbance measured at 540nm with a BioTek Powerwave XS equipped with Gen5 scanning software (Winooski, VT). Dyed samples were covered at all times to avoid photobleaching.

The enzymatic activity, k_{cat}, was calculated to be $0.16s^{-1}$ using an exponential fit of remaining collagen concentration to calculate a maximum reaction velocity. This is in good agreement with published data. Collagen undergoes fibrillogenesis at 37°C, with complete gelation typically occurring in 1 hour. The collagen degrading in this experiment, only pre-heated for 15 min. to avoid cooling BC upon combination, can be assumed to be at most weakly gelled, with a large percentage of collagen molecules still in solution. To adjust for the differences between degradation rate of monomers in solution and insoluble collagen fibrils, k_{cat} is multiplied by 20% (representative of 20% of the monomers in corneal collagen fibrils being available on the surface), giving a predicted k_{cat} for intact bovine corneal collagen of $0.0334s^{-1}$ [3].

1. Mallya, S. K.; Mookhtiar, K. A.; Van Wart, H. E., Kinetics of hydrolysis of type I, II, and III collagens by the class I and II Clostridium histolyticum collagenases. *J Protein Chem* **1992,** 11, (1), 99-107.
2. Lindy, S.; Sorsa, T.; Suomalainen, K.; Lauhio, A.; Turto, H., Hyperchromic effect of collagen induced by human collagenase. *Eur J Biochem* **1986,** 156, (1), 1-4.
3. Welgus, H. G.; Jeffrey, J. J.; Stricklin, G. P.; Roswit, W. T.; Eisen, A. Z., Characteristics of the action of human skin fibroblast collagenase on fibrillar collagen. *J Biol Chem* **1980,** 255, (14), 6806-13.