NON AFFINE FIBER KINEMATICS IN COLLAGEN GELS UNDER UNIAXIAL EXTENSION

Preethi L. Chandran, Victor H. Barocas

Department of Biomedical Engineering
University of Minnesota
Minneapolis MN 55455

INTRODUCTION

It is well known that macroscopic mechanics of fibrous tissues are governed by the arrangement and properties of their microstructure (1). The term 'macroscopic' is used to refer to the observable millimeter scale, the net result of activity at the micron or 'microscopic' scale. The challenge in modeling such systems is determining the level at which governing equations must be formulated. Tissues and tissue equivalents are commonly modeled as a continuum or as a collection of independently-acting and oriented fibers, embedded in a continuous matrix. In such a setting, information about boundary deformation is conveyed to each fiber solely by the surrounding matrix. Thus, when the connection between fibers and the matrix is slip-free, fibers deform as though a continuous part of it (i.e., affine fiber kinematics, 2-6).

In most connective tissues and tissue equivalents, however, the fibers are extensively entangled, if not covalently crosslinked to each other. For example, the mechanical response of skin specimens was found to be independent of the integrity of the matrix (7). The articular cartilage has a tensed network of collagen fibers that resists the swelling pressure of a water-retaining matrix (8). On the collagen gel side, it shown that connectivity of the fibrous microstructure was important to sustain load. All these observations suggest the existence of a fiber phase, capable of independently supporting a stress field, because of its interconnections. In other words, the fiber phase behaves like a network, transmitting forces mostly from fiber to fiber, rather than fiber to matrix. Lanir argues (2) that 'affine deformation is intuitively justified by multiplicity of interconnections'.

METHODOLOGY - THEORY AND SIMULATION

A first test of the affine kinematics assumption was to simulate a fibrous network and probe the fiber response to boundary strain. The network was sufficiently large to be representative and to minimize boundary effects. Both the macroscopic and the microscopic levels were interrogated. At the macroscopic level, net fiber orientation and the net fiber density tensor were used to characterize the network. These 'fabrics' can be related to the experimentally observable birefringence and stress-strain behavior and are functions of microstructure rearrangement. At the microscopic level, the final fiber orientation and strain versus initial orientation were studied.

RESULTS - THEORY AND SIMULATION

The simulated network was subjected to uniaxial extension, and the evolution of the macroscopic and microscopic states noted. The orientation fabric showed small but inconclusive differences for both models. However, the network stress fabric (9) showed non-monotonic behavior. This suggested a break point up to which fiber reorientation dominates over fiber lengthening as a strain-bearing mechanism. The microscopic level gave additional insight, as seen from Figure 1 on the next page. Although the sample was stretched to a macroscopic engineering strain of 20%, very few fibers experienced that much strain, including those fibers completely aligned with the direction of the stretch - network rearrangement accommodated the large macroscopic strains with relatively small microscopic strains. The non-affine rearrangement occurred for fibers that were equally stiff in tension and compression. When the nonlinearity of fiber behavior, the effect became extremely pronounced. Some of the fibers in the network of Figure 1 are actually in compression for a macroscopic tension because the overall energy cost for the system is reduced.

METHODOLOGY - EXPERIMENT

Experiments were conducted to complement the theoretical studies and investigate macroscopic and microscopic level tissue-equivalent (TE) behavior. TEs were fabricated as follows. Collagen monomers were polymerized under physiological conditions into a meshwork of long continuous fibrils, typically 200 to 400 nm diameter (10,11). Trapping contractile cells (fibroblasts in this case) resulted in gel compaction and exclusion of the interstitial fluid, giving a tissue-like

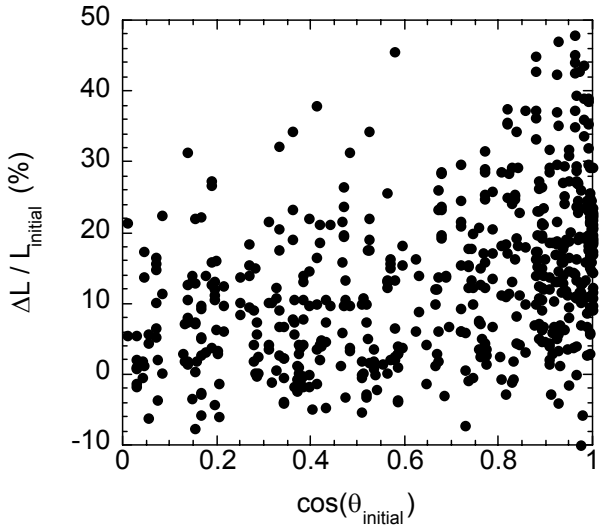

Figure 1. Fiber Strain Distribution for 20% Macroscopic Strain. An affine model would predict no strain in the fibers aligned perpendicular to the direction of stretch (cosθ = 0) and 20% strain in fibers parallel to the stretch (cosθ = 1). The microstructural results were much more complex.

structure (11). Beads (200 micron polystyrene, Polysciences Incorporation) incorporated during the gelation phase allowed the mapping of the macroscopic strain field. A two-week incubation was given to allow the TE to stiffen by forming crosslinks and to minimize viscoelastic effects. Since square molds were used, no net fiber alignment was expected in the TE.

In a tensile test, the stress strain behavior is a macroscopic function of the fiber deformation state as well as the fiber constitutive equation. Simultaneous interrogation of the stretching sample with polarized light gave birefringence data (cf. 12). Modeling segments of collagen fibers as rigid rods (13), birefringence data was related to the average refractive index and to the fiber polarizabilities, concentration, and net orientation (14). From these, with appropriate algebra, an estimate of the macroscopic network fabrics was made. The affine fabrics were obtained directly from the macroscopic strain field.

The TE was subjected to uniaxial extension on an Instron Planar Biaxial Extensometer. A rotating polarizer imaging setup (15, 16) was used to obtain birefringence data. A low strain rate was used to minimize TE movement during polarizer rotations. This was also to ensure that the equilibrium behavior, after possible relaxation was being studied. A controlled stress relaxation experiment, performed at different strain levels, allowed determination of the effect of viscoelastic relaxation on fiber orientation and retardation. The homogeneity of the macroscopic field within each strain element was checked. A Weibull distribution of fiber orientation was assumed.

The microscopic level restructuring was examined using confocal microscopy. Within a strain field defined by the incorporated beads, the movement of the individual fibers was traced. The orientation verses strain correspondence was obtained. The average microscopic state was checked against that estimated for the fabrics.

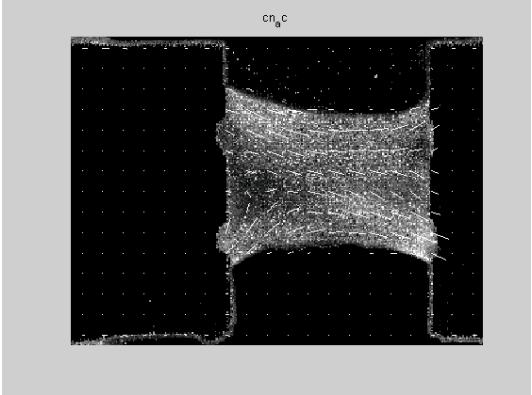

Figure 2. Birefringence imaging of a collagen in uniaxial extension. The arrows give the local strength and direction of the fiber alignment.

RESULTS - THEORY AND SIMULATION

Fig 2 shows preliminary birefringence imaging of a compacted collagen gel. The micrtostructural analysis is still in progress at the time of this abstract but will be discussed in the presentation.

REFERENCE

1 Silver, F.H. *Biological Materials: Structure, Mechanical properties and Modeling of Soft Tissues*, New York, New York University Press
2 Lanir Y. *Journal of Biomechanics*. 12(6):423-36, 1979
3 Lanir Y. *Journal of Biomechanics.* 16(1):1-12, 1983
4 Billiar KL. Sacks MS. *Journal of Biomechanical Engineering.* 122(4):327-35, 2000
5 Billiar KL. Sacks MS. *Journal of Biomechanics.* 30(7):753-6, 1997
6 Schwartz MH. Leo PH. Lewis JL. *Journal of Biomechanics.* 27(7):865-73, 1994
7 Harkness M.L.R and Harkness R.D. *Nature*, 183,1982
8 Maroudas A. Ziv I. Weisman N. Venn M. *Biorheology*. 22(2):159-69, 1985
9 Bell E. Ivarsson B. Merrill C. *PNAS USA.* 76(3):1274-8, 1979
10 Mehrabadi, Nemat – Nasser,Oda, *International Journal for Numerical and Analytical Methods in Geomechanics*,6,95-108,1982
11 Tranquillo RT. *Biochemical Society Symposia.* 65:27-42, 1999
12 Wolman M. Kasten FH. *Histochemistry*. 85(1):41-9, 1986.
13 Roska FJ. Ferry JD. *Biopolymers*. 21(9):1811-32, 1982
14 Collett E, *Fundamentals of Polarized Light*, New York Press, New York
15 Tower T Tranquillo *Biophysical Journal*, 81,2964-2971.2000
16 Glazer AM *Proc. R. Soc. Lond. A* ,452,2751-2761,1996