Experimental and computational studies of the time-dependent mechanical behavior of cellular and acellular collagen hydrogels

Kim Busenhart*[1], Alexander E. Ehret[1,2], Havar Junker , Simon Gruber , and Edoardo Mazza†[1,2]

[1]ETH Zurich, Department of Mechanical and Process engineering, Institute for Mechanical Systems – Switzerland

[2]Empa, Swiss Federal Laboratories for Materials Science and Technology – Dübendorf, Switzerland

Abstract

Collagen hydrogels are often employed as in vitro model systems for mechanobiological investigations of soft biological tissues, and also serve as tissue replacements in tissue engineering applications (1,2,3). We developed experimental protocols to comprehensively characterize their complex non-linear, anisotropic, time-dependent deformation behavior. At the same time, we formulated enhanced numerical models to gain deeper understanding of the underlying deformation mechanisms, e.g., (4).
Similar to soft collagenous tissues, the mechanical properties of collagen hydrogels are characterized by a load-bearing network of collagen, swollen to contain a large amount of water, which physically interacts with the collagen and adds an isotropic, mainly hydrostatic stress contribution to the elsewise anisotropic and non-affine mechanical behavior of the material. The control over composition and structure, and hence reduced variability in the observed mechanical characteristics allows systematic studies. We use an established protocol to produce collagen hydrogels (2), including a gel compression step that yields a 16-fold increase in the collagen concentration, and induces structural anisotropy in the material (4). In uniaxial tensile tests, the gels exhibit pronounced stiffening, with tangent stiffness increasing by an order of magnitude over an applied strain range of 10%. This is accompanied by significant lateral contraction, reflected in tangent Poisson's ratios up to 8. The mechanical properties are also highly sensitive to the fabrication protocol (6), as demonstrated by a comparison with another type of collagen hydrogel (5), that shares the same volume fraction of collagen but was prepared following a different protocol. This gel shows an order of magnitude lower tangent stiffness with less stress stiffening, has more rubber-like characteristics, and features a Poisson's ratio of 0.45 at 10% strain, in stark contrast to the gels prepared according to (2,4).

For many relevant applications of collagen hydrogels, their time-dependent behavior plays a critical role. To explore these characteristics, we therefore extended our experimental analysis to stress relaxation and creep experiments, and tests at different loading rates. Significant stress relaxation was observed, highlighting persistent viscoelastic effects, likely driven by fiber rearrangements and poroelastic effects due to fluid movement. Notably, the pronounced stress relaxation occurs even at very low loading rates (), where the stress reduces to just 40% of its peak value within 10 minutes of the relaxation phase. Moreover, the

*Speaker

†Corresponding author: mazza@imes.mavt.ethz.ch

influence of embedded cells on these time-dependent properties was investigated, as cellular activity is expected to dynamically alter the hydrogels' microstructure and mechanical response over time. This information may therefore be key for developing more representative in vitro models for soft collagenous tissues and understanding their behavior under physiological conditions.

To interpret the experimental observations, we first developed an enhanced computational model capable of capturing the complex mechanical behavior of collagen hydrogels in uniaxial tensile tests under static loading conditions, and able to rationalize the observed differences between hydrogels prepared according to different protocols. The model builds on a fully 3D discrete network of fiber connectors, integrated within a continuous compressible neo-Hookean type matrix. To shed light on the origin of time-dependent phenomena seen in the relaxation and creep tests, we extended the model and explored viscoelastic formulations for the fibers' mechanical behavior to represent time-dependent fiber sliding and crosslink dynamics.

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