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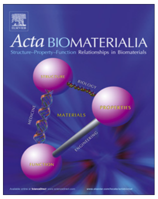

Time-dependent mechanical behavior of human amnion: Macroscopic and microscopic characterization

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ABSTRACT

Characterizing the mechanical response of the human amnion is essential to understand and to eventually prevent premature rupture of fetal membranes. In this study, a large set of macroscopic and microscopic mechanical tests have been carried out on fresh unfixed amnion to gain insight into the time-dependent material response and the underlying mechanisms. Creep and relaxation responses of amnion were characterized in macroscopic uniaxial tension, biaxial tension and inflation configurations. For the first time, these experiments were complemented by microstructural information from nonlinear laser scanning microscopy performed during in situ uniaxial relaxation tests. The amnion showed large tension reduction during relaxation and small inelastic strain accumulation in creep. The short-term relaxation response was related to a concomitant in-plane and out-of-plane contraction, and was dependent on the testing configuration. The microscopic investigation revealed a large volume reduction at the beginning, but no change of volume was measured long-term during relaxation. Tension–strain curves normalized with respect to the maximum strain were highly repeatable in all configurations and allowed the quantification of corresponding characteristic parameters. The present data indicate that dissipative behavior of human amnion is related to two mechanisms: (i) volume reduction due to water outflow (up to ~20 s) and (ii) long-term dissipative behavior without macroscopic deformation and no systematic global reorientation of collagen fibers.

1. Introduction

The fetal membrane (FM) surrounds the growing fetus and ensures its environment during gestation. Preterm premature rupture of the membrane affects about 3% of all pregnancies and increases the risk of morbidity in the newborn [1]. The etiology of preterm premature rupture of the membrane is complex and not completely understood. Repeated mechanical loading, such as that occurring as a result of fetal movement and labor, was recently shown to affect the microstructure of the membrane [2] and to reduce its toughness [3]. These results suggest that the time- and history-dependent behavior of FM tissue plays a critical role. To understand this behavior, detailed analysis of both macroscopic stress and kinematic responses and the microstructural mechanisms is required.

The FM is a multilayered structure [4] with two main components, the amnion and the chorion, which are connected by an interface called spongy layer. The amnion is the inner layer of the FM facing the amniotic liquid. This thin membrane has a mean thickness of about 60–120 μm [5–8] and is composed of a monolayer of epithelial cells, a compact layer of collagen and a layer of collagen fibers containing fibroblast cells [4]. The amnion is considered to be the load-bearing layer of the FM [9], and has thus become the focus of mechanical investigations. In addition to its essential physiological function, it was also proposed as a promising candidate to be used as a scaffold material for tissue engineering applications [10,11].

The mechanical response of the intact FM and of the separated amnion has been investigated in uniaxial tensile tests [7,12–18], biaxial tensile tests [16,19], puncture tests [9,17,20–24] and inflation tests [3,25–31]. These studies focused primarily on the quasi-static monotonic deformation and rupture behavior, with only a few works investigating the time-dependent response of the intact membrane [26,28,29] or of the amnion alone [15,16,32]. Lavery

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and Miller [26] analyzed creep and relaxation phenomena in the inflated intact membrane, identifying conditions of non-recoverable deformation and quantifying a pressure-dependent rate of relaxation. The authors showed that preterm membranes were less affected by strain hardening and underwent thickness thinning to a greater degree than term membranes [28], and that membrane integrity reduced more in labored than in unlabored membranes [29]. Stress-relaxation and cyclic experiments on human amnion showed a stress-level-dependent response and, surprisingly, lower dissipation at higher strain levels, which could indicate an intrinsic coupling of strain- and time-dependency [15,16].

Stress relaxation in soft biological tissues arises from microstructural mechanisms, such as relaxation of single collagen fibrils [33,34], global rearrangement of collagen microstructure [34–36], progressive failures of crosslinks [37–39], liquid phase rearrangement or dehydration [40,41], and may depend on the stress level reached [42]. The specific mechanisms determining the mechanical time dependence of amnion have not yet been identified.

Bürzle et al. [18] observed extremely large lateral contraction of the amnion in uniaxial tension tests. In contrast to the common large inter- and intra-membrane variability of the tension–stretch curves, this kinematic response was highly repeatable. In the present work, repeatable features of the time-dependent behavior will be investigated at macroscopic and microscopic length scales. A new in situ experimental setup that allows for macroscopic deformation while simultaneously performing multiphoton microscopy was developed to gain microstructural insights. By this means, for the first time, thickness measurements, collagen orientation and microscopic in-plane kinematics were quantified for fresh, unfixed and hydrated human amnion during relaxation experiments. Macroscopic uniaxial tension tests with free or constrained lateral contraction were performed. A new normalization procedure is introduced, which extracts highly repeatable features of the time-dependent behavior of the amnion from the scattered experimental data typical for soft biological tissue samples, providing a valuable basis for the development and validation of corresponding constitutive models.

2. Material and methods

2.1. Amnion samples

Fresh FMs were collected from patients who underwent elective caesarean sections between 37 and 39 gestational weeks. Patients were recruited with informed written consent using a protocol approved by the Ethical Committee of the District of Zürich (Stv22/2006 and Stv07/07). The selected pregnancies had no labor contractions prior to delivery, no preterm rupture of the membrane and no diabetes mellitus, and were negative for streptococcus B, HIV, hepatitis A and B, chlamydia and cytomegaly. Immediately after collection, the amnion was gently separated from the chorion and stored in physiological solution (NaCl 0.9%) for about half an hour. Samples were cut with a razor blade and stored in saline solution at room temperature until testing, which took place within a few hours after delivery. A series of mechanical tests (Section 2.2) were performed to characterize creep and relaxation response in uniaxial and multiaxial stress states. Other mechanical tests (Section 2.3) were performed within the multiphoton microscope for microstructural characterization during relaxation. A total of 26 specimens from eight different membranes were investigated, and test duration ranged between 10 min and 2 h. Corresponding sample geometries and testing configurations are summarized in Table 1.

2.2. Macroscopic experiments

Test configurations include both the biaxial tension state, representative of physiological FM loading, and the uniaxial tension state, representative of the stress state at holes or defects in the amnion. Relaxation is typical of deformation-controlled loading of the FM supported by the uterine wall, while creep, i.e. loading at constant stress or pressure, might occur in the cervical region after ripening. All tests were performed at room temperature.

2.2.1. Relaxation experiments

Relaxation tests (R) were performed with our custom experimental setup [18,43], which consists of two hydraulic actuators with calibrated 20 N load cells, a video extensometer system and a buffered saline solution bath. Amnion was gently positioned on a plastic sheet, sprayed with saline solution and marked in the central region with a water-resistant pen (GEOCollege Pigment Liner 0.05). With the help of a sandpaper jig, specimens were clamped while immersed in saline solution to minimize dehydration and artifacts arising from the high surface tension of the amnion. Relaxation experiments were carried out in two testing configurations. Uniaxial tension (U) was achieved by elongating a long, narrow specimen (free dimensions: 60 × 15 mm) in the direction of the long axis and allowing free contraction in the other directions to reach stress-free boundaries. For uniaxial extension with constrained contraction, specimens with a large width-to-length ratio (free dimensions: 15 × 60 mm) were used, so that lateral (but not thickness) contraction was strongly restrained by the clamping and a planar biaxial state of tension (B) was obtained (see e.g. Ref. [44]).

The reference configuration and the reference length L_{ref} were defined by a force threshold of 0.01 N (U) and 0.04 N (B), respectively, corresponding to an equivalent reference membrane tension T_{ref} of $0.0006\ \mathrm{N\,mm^{-1}}$. By this means, the nominal strain was defined as $\varepsilon = \Delta L / L_{ref}$ and the loading was performed at a fixed nominal strain rate of $0.2\ \mathrm{s^{-1}}$. The dwell phase started after reaching a target force of 0.8 N (U) and 2.4 N (B), corresponding to a membrane tension T_0 of $0.054\ \mathrm{N\,mm^{-1}}$ in both cases. This value was chosen as a significant loading level with a membrane tension of the order of that generated by early contractions [3], while still being beyond a critical value potentially causing membrane rupture. To investigate the influence of loading history, the loading protocol was repeated with the same specimen after a recovery phase of 100 min in the unloaded clamped state. Force and displacement signals were recorded at 8 Hz, while images of the

Table 1
Summary of all experiments.

Samples	No. of specimens	Testing configuration	Specimen dimensions	Holding time	Repeated loading
R-U	$n = 5$	Uniaxial tension relaxation	60 mm × 15 mm	10 min	After 100 min
R-U-M	$n = 3$	Uniaxial tension relaxation	60 mm × 15 mm	10 min	–
R-B	$n = 4$	Biaxial tension relaxation	15 mm × 60 mm	10 min	After 100 min
C-U	$n = 5$	Uniaxial tension creep	60 mm × 15 mm	10 min	After 100 min
C-I	$n = 4$	Inflation creep	∅ = 50 mm	10 min	After 100 min

R: relaxation; C: creep; U: uniaxial; B: biaxial; I: inflation; M: microscope.