Characterization and modeling of the mechanical behavior of human amnion

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Summary: The mechanical properties of human amnion were characterized in creep and relaxation tests, under uniaxial and multiaxial loading conditions. A hyperelastic-viscoelastic constitutive model formulation was selected based on the observed in-plane and out-of-plane kinematic response. The model allows rationalizing the complex monotonic and time dependent mechanical behavior of amnion.

Introduction

Preservation of pregnancy requires intact fetal membranes (FMs). In normal pregnancy, FMs are progressively deformed due to the increased intrauterine pressure. FMs are further stretched when the fetus moves and pushes against the uterine wall. It is astonishing to think that the life of the fetus depends on the integrity of a thin biological layer (few hundred micrometers thick) exposed to severe mechanical loads and large deformations. The FM layer has particular mechanical properties that make the event of preterm premature rupture of membrane (PPROM) unlikely. PPROM is not frequent but represents a devastating complication of pregnancy that accounts for 30 to 40% of preterm deliveries and is associated with a high risk of fetal morbidity and mortality.

The FM is composed of two layers, amnion and chorion [1,2]. The chorion is a thick (120-400 μm) mainly cellular layer connecting the FM to the maternal tissue (decidua), whereas the amnion is a thin biological membrane of 60-100 μm thickness that includes a relatively dense collagenous "compact layer", a thicker "fibroblast layer" and the "spongy layer", which forms the interface with chorion [2]. The higher stiffness and strength of amnion compared to chorion suggest that the mechanical properties of amnion play a critical role for the incidence of PPROM [3]. Improved physical understanding and quantitative mathematical description of the mechanical behavior of amnion could contribute to understanding clinical problems related to both spontaneous and iatrogenic PPROM. Mechanical characterization of amnion has thus been the objective of several experimental studies (e.g. [4-8]).

We have recently generated comprehensive data sets on the time and history dependent mechanical response of FM tissue under uniaxial and biaxial loading conditions [11,12]. Based on these observations and information from in-situ testing in a multiphoton microscope, we have developed model equations representative of the deformation behavior of human amnion under physiological conditions of mechanical loading.

Experimental methods

Determination of constitutive model equations for soft bio-membranes requires the combination of data from different stress states, and (depending on modeling purposes) consideration of the time and history dependence of the mechanical response. We have applied a wide range of testing procedures for the characterization of amnion, including stress states ranging from uniaxial to "strip biaxial", to equibiaxial, see Figure 1. Control profiles were defined for monotonic or cyclic loading, in force or displacement control (at different strain rates). Details of the loading protocols were reported elsewhere [7, 8, 10-13].

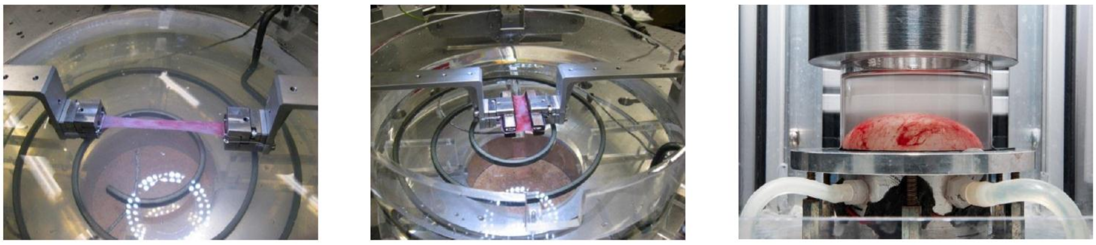

Figure 1: Set-up for uniaxial tension (left), strip biaxial (center) and inflation (right) experiments on FM tissue.

Local deformations were quantified using image analysis and correlation algorithms. FM samples were collected after elective caesarian sections and tested within a few hours after delivery. Experiments were performed with test pieces submerged in physiological saline solution to avoid dehydration. In order to gain insights into the mechanisms of deformation underlying the mechanical response of amnion, we performed mechanical experiments in situ, in a multiphoton microscope, which allows visualization of the whole thickness of amnion (see Figure 2) and quantitative determination of the local in-plane and out-of plane deformations, as well as of the collagen fiber orientation [9, 11, 13].

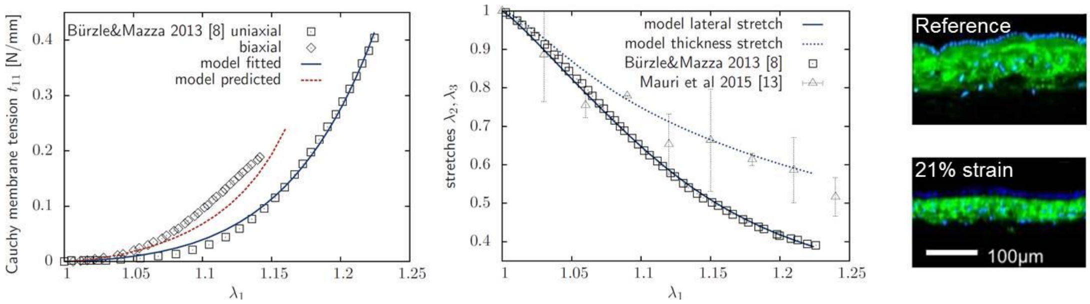

Figure 2: Uniaxial and equibiaxial tension stretch curves, mean experimental data [8,13], and model (left). In-plane and out-of plane contraction in uniaxial tension tests (middle). Thickness reduction was obtained through multiphoton microscopy (right, adapted from [13]).

Experimental observations and model equations

Comparison of tension stretch curves of uniaxial and biaxial experiments revealed that the uniaxial compliance of amnion is much larger than the biaxial one. The analysis of the in-plane response in uniaxial tension tests showed that the kinematic response of human amnion is highly reproducible and that the incremental Poisson's ratio in-plane and out-of plane is very large (of up to 8), see Figure 2. This behavior was rationalized by mechanisms of rotation, stretching and buckling of the very thin fibers which constitute the collagen network of amnion [8].

Creep and relaxation responses of amnion were characterized in uniaxial tension, biaxial tension and biaxial inflation configurations [11]. The amnion showed a large tension reduction during relaxation and a small inelastic strain accumulation in creep, see Figure 3. The short-term relaxation response was related to a concomitant in-plane and out-of-plane contraction leading to large volume reduction, but no change of volume was measured long-term. Tension-strain curves normalized with respect to the maximum strain were highly repeatable in all configurations. The data indicate that the dissipative behavior of human amnion is related to two mechanisms: (i) volume reduction due to water outflow and (ii) long-term dissipative behavior without macroscopic deformation and no systematic global reorientation of collagen fibers.

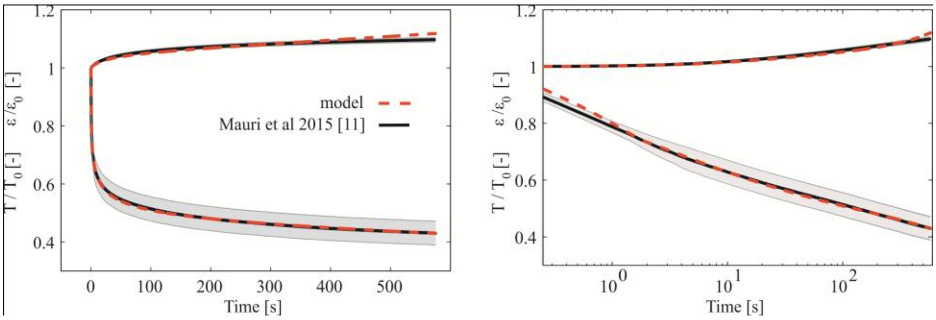

Figure 3: Time history of tension and strain in relaxation and creep experiments. The model (cf. [14]) well reproduces the experimental data presented in [11].

A modified version of the constitutive model in [8, 10] was formulated which is able to rationalize the response in uniaxial and biaxial states of tension, and considers two viscoelastic mechanisms with two distinct time scales. The collagen network is modelled by N representative and mechanically equivalent fiber families that are uniformly distributed in the membrane plane but slightly inclined out of plane, and embedded in a compressible matrix. The free energy of the resulting Rubin-Bodner-type model [15] takes the form

$$\Psi = \frac{\mu_0}{2q}(e^{qg} - 1), \qquad g = g_1(J_e) + g_2(I_1, J) + g_3\left(|\boldsymbol{m}_\mathrm{e}^i|\right), \qquad i = 1,2, \dots, N,$$

where $I_1 = \mathrm{tr}\left(\mathbf{F}^\mathrm{T}\mathbf{F}\right)$, $J = \det\mathbf{F}$, $\mathbf{F}$ is the deformation gradient, and $\boldsymbol{m}_\mathrm{e}^i$ are the current fiber vectors. μ_0 and q represent material parameters. The variables J_e and $\boldsymbol{m}_\mathrm{e}^i$ account for viscoelasticity and result from the evolution equations

$$\dot{J}_e = J_e \mathrm{tr}\mathbf{d} - \Gamma_\mathrm{M}(J_e), \qquad \dot{\boldsymbol{m}}_\mathrm{e}^i = \mathbf{l}\boldsymbol{m}_\mathrm{e}^i - \Gamma_\mathrm{M}\left(|\boldsymbol{m}_\mathrm{e}^i|\right)\boldsymbol{m}_\mathrm{e}^i, \qquad i = 1,2, \dots, N,$$

where $\mathbf{l} = \dot{\mathbf{F}}\mathbf{F}^{-1}$ is the spatial velocity gradient and $\mathbf{d}$ is its symmetric part. Γ_M and Γ_F, determine the rates of dissipation and were chosen such that the dissipation inequality is satisfied and the model is thermodynamically consistent. Moreover, the functions g_1, g_2, g_3 were defined in accordance with classical formulations in tissue biomechanics. As shown in Figures 2 and 3, the model accurately captures the uniaxial and biaxial monotonic response, as well as the time history of tension in relaxation and strain in creep experiments (cf. [14]).

Conclusions and outlook

A model formulation was identified that is able to rationalize the complex mechanical response observed in multiaxial relaxation and creep experiments. The phenomenological model was selected based on the deformation mechanisms governing the kinematic response of amnion. We applied our model to analyze consistent discrepancies between observations in inflation experiments and puncture tests [10], and were able to reconcile our observations of stiffness and rupture with those reported by other groups [16]. Current investigations focus on factors influencing the toughness of amnion and the constitutive model is used to design experimental protocols for quantitative determination of fracture properties of amnion as schematically illustrated in Figure 4.

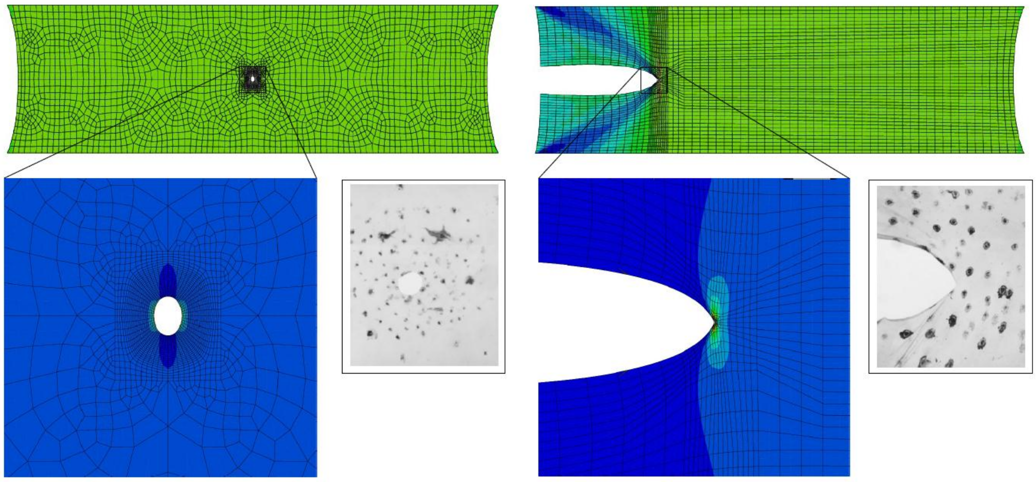

Figure 4: Development of experimental protocols for quantitative evaluation of toughness of amnion: finite element models for a hole (left) and a sharp notch (right), and images from tests (markers for near field analysis are visible).

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