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On the deformation behavior of human amnion

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ABSTRACT

Recently renewed interest for the mechanical behavior of fetal membranes is related to the problem of iatrogenic preterm rupture, limiting the effectiveness and applicability of minimally invasive fetal surgery. This study aimed at characterizing and modeling the deformation behavior of the amnion layer, the highly deformable and tough membrane that surrounds the amniotic fluid and the growing fetus in the uterine cavity. Uniaxial tension tests have been performed on samples obtained immediately after cesarean section, and the deformation field has been analyzed by digital image correlation. The results show that the kinematic response of human amnion is highly reproducible and that the incremental Poisson's ratio is, with a value of up to 8, higher than any previously reported value for biological or synthetic materials. This unique behavior is related to the characteristic architecture of amnion's microstructure and can be rationalized by mechanisms of rotation, stretching and buckling of collagen fibers. Simple constitutive equations have been selected based on this interpretation, which lead to a model with excellent predictive capabilities for the uniaxial and equibiaxial mechanical response of human amnion. Relevant insights were gained on the role of collagen fibers in determining the deformability and toughness of soft biological tissue.

1. Introduction

The fetal membrane (FM) surrounds and protects the fetus during pregnancy and is adjacent to the uterine wall. The FM is composed of two main layers, called amnion and chorion. During pregnancy, it has to resist deformations due to internal pressure and fetal movements. Rupture of FM is a natural event for term delivery but has serious complications when it happens prior to term. The so called spontaneous preterm premature rupture of FM (PPROM) affects 3% of all pregnancies worldwide (Calvin and Oyen, 2007; Mercer, 2003) and is associated with a higher risk of perinatal morbidity and mortality. Moreover, preterm rupture after fetal surgery, called iatrogenic PPROM (iPPROM), is the Achilles heel for developments in this field and occurs in about 30% of all treatments (Beck et al., 2011). The development of methods to avoid FM preterm rupture requires a better understanding of their mechanical behavior.

Only few attempts were reported for the determination of a constitutive model of FM (Miller et al., 1979; Prevost, 2004; Jabareen et al., 2009; Joyce, 2009). Most previous studies focused on the extraction of parameters to quantitatively characterize FM rupture and on the effects leading to PPROM (Artal et al., 1976; Lavery and Miller, 1979; Al-Zaid et al., 1980). Other investigations were aimed at determining differences in mechanical properties of amnion and chorion (Polishuk et al., 1962; Oxlund et al., 1990; Helmig et al., 1993). It is known from these foregoing studies that amnion is stiffer and stronger than chorion and is therefore considered the mechanically dominant layer of FM. Oyen has investigated the time and history dependence of amnion's deformation behavior (Oyen et al., 2004, 2005) and has shown that amnion is more sensitive to chemical and mechanical changes that occur during gestation (Oyen et al., 2006).

Amnion is the inner layer of the FM, facing the amniotic fluid. Membranes at term have an amnion layer with a thickness in the range of 100 μm (Ilancheran et al., 2009). Next to the amniotic epithelium and the basement membrane, the so called “compact layer” is made predominately of collagens type I and III that form a dense network (Malak et al., 1993; Parry and Strauss, 1998). The adjacent fibroblast layer is the thickest layer of amnion and is characterized by collagens forming a looser network. The outermost layer of amnion is the “intermediate layer”, which contains a non-fibrillar network of mostly type III collagen (Parry and Strauss, 1998), it lies between amnion and chorion and accommodates relative movement between the layers. Fig. 1 shows scanning electron micrograph (SEM) images of a FM sample. It illustrates the crimped collagen fibres, forming a random network in the membrane plane, with no distinct directionality.

Chorioamniotic membrane separation leads to a premature rupture of the membranes in 63% of the cases after surgery (Sydorak et al., 2002). The separation of the FM layers has been observed in puncture experiments (Arikat et al., 2006). We also consistently observed a separation of amnion and chorion in uniaxial tension tests on intact FM, see Fig. 2. In this case

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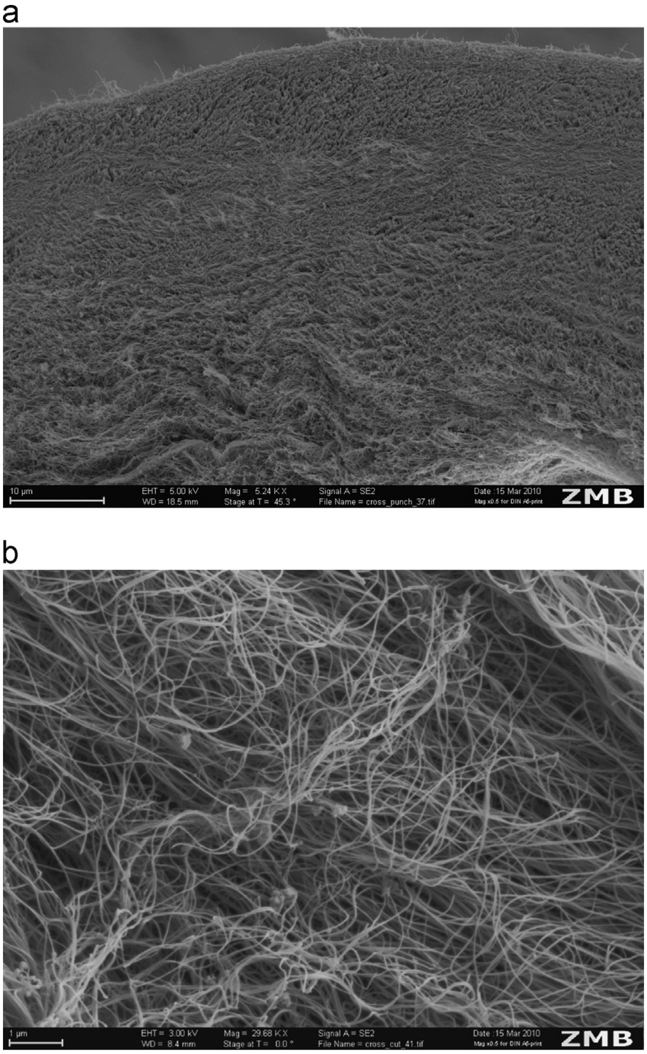

Fig. 1. Scanning electron micrograph of a human fetal membrane sample. Cross-sectional view (a) and top view (b) show an irregular network of singe collagen fibers with no distinct directionality. With permission from Hollenstein et al. (2010).

separation is caused by different contraction behavior (Poisson's effect) of the two layers, with amnion displaying a much greater lateral contraction.

Unusually large contraction of amnion in tensile tests had never been reported. This motivated a series of tensile tests on human amnion with quantitative determination of lateral contraction. Experimental observations along with considerations on mechanisms of rotation, stretching and buckling of fibers in a random network (Stylianopoulos and Barocas, 2007; Kabla and Mahadevan, 2007; Picu, 2011) led to the selection of a constitutive model formulation. Model parameters were determined to simultaneously match tension–stretch and contraction–elongation curves of tensile tests. Model response to equibiaxial tension was finally compared with corresponding data from previous measurements (Haller, 2012).

2. Experimental methods

2.1. Sample harvesting and preparation

Fetal membranes were collected from patients with single child pregnancies who underwent elective cesarean section between 37 and 39 weeks of gestation. Patients were recruited for this study with informed written consent according to a protocol approved by the ethical committee of the district of Zurich (study Stv22/2006). The patients were randomly selected after thorough serologic testing to exclude HIV, hepatitis B, and streptococcus B. The selected patients had no history of diabetes, connective tissue disorders, or chromosomal abnormalities.

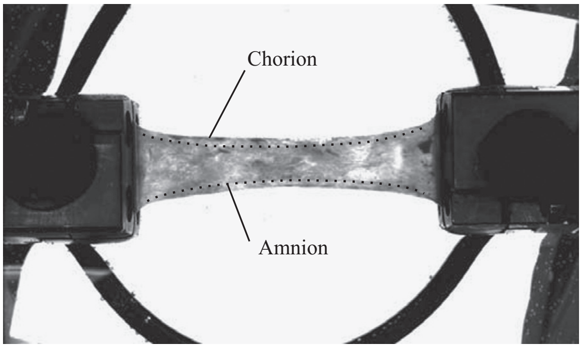

Fig. 2. Image of an amnion–chorion FM sample during tension test. The constitutive layers of the FM start to separate at a certain level of deformation. The dotted line indicates the border of the amnion layer after separation.

After cesarean section the FM was cut approximately 2 cm away from the placental disk and stored in saline solution until mechanical testing, within a few hours after delivery. Amnion and chorion were separated manually by gently pulling the layers apart. Amnion was spread on a lubricated plastic mat and a die cutter (see Hollenstein et al., 2010) was used to create rectangular samples with the dimension of 80 × 15 mm. Small markers of India Ink were applied on the surface of the sample with the aid of a brush, which allows better optical tracking of the deformation field.

2.2. Mechanical experiments

In order to test the FM samples in a uniaxial stress configuration, a custom built experimental setup (Hollenstein et al., 2010) was used. This consists mainly of computer controlled hydraulic actuators equipped with force sensors (20 N capacity) and a CCD camera system which allows to record image series during the experiment. The tensile tests were performed as monotonic tension to failure with an elongation rate of 0.5% nominal strain per second, starting from an initial free length of 60 mm. No preconditioning was applied. All tests were performed at room temperature in a bath filled with saline solution to avoid drying or swelling of the sample.

Data on the response of amnion in a biaxial state of stress were obtained using a custom built inflation device (Haller, 2012). The inflation setup, the experimental protocol, and the procedures for extraction of biaxial tension–stretch curves are described in Buerzle et al. (2012).

2.3. Analysis of the deformation field in uniaxial experiments

The recorded image series serves as basis for the determination of the kinematic response of the amnion layer. The digital image correlation (DIC) software VEDDAC 4.0 (Chemnitzer Werkstoffmechanik GmbH, Chemnitz, Germany) is used to trace a set of user defined measurement points corresponding to the markers in the central region of the sample, see Fig. 3. The resulting discrete displacement filed is used for the approximation of the in-plane stretches by the use of element based interpolation functions, as applied in the finite element (FE) method.

The displacements of the measurement points are considered as nodal displacements of a linear four node element. This approach allows calculating the in-plane components of the element specific deformation gradient $\mathbf{F}_{el}$ as a function of the associated nodal displacements $\hat{\mathbf{u}}$ and the linear shape functions N

$$\mathbf{F}_{el} = \mathbf{I} + \sum_{a=1}^{4} \hat{\mathbf{u}}^{(a)} \otimes \frac{\partial N^{(a)}}{\partial \mathbf{X}}. \tag{1}$$

All common measures of deformation can be calculated based on the deformation gradient. For the load cases considered in this work, only the two in-plane principal stretches λ_1 and λ_2 are determined. Averaging over the entire region, in this case a 2 × 3 element scheme, provides the kinematic response of the amnion layer. The typical variability of elongation values in different elements was in the range of 5%.

The Poisson's ratio is defined as the ratio of lateral strain (contraction, in direction 2) and longitudinal strain (elongation, in direction 1). It is strictly defined only for infinitesimal deformations. For large strain behavior, an incremental Poisson's ratio or Poisson's function, according to the definitions of Alderson et al. (1997) and Smith et al. (1999) can be used, which is based on the ratio of