Multiaxial mechanical behavior of human fetal membranes and its relationship to microstructure

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Received: 5 April 2012 / Accepted: 30 August 2012 / Published online: 13 September 2012 © Springer-Verlag 2012

Abstract This study was directed to the measurement of the mechanical response of fetal membranes to physiologically relevant loading conditions. Characteristic mechanical parameters were determined and their relation to the microstructural constituents collagen and elastin as well as to the pyridinium cross-link concentrations analyzed. 51 samples from twelve fetal membranes were tested on a custom-built inflation device, which allows mechanical characterization within a multiaxial state of stress. Methods of nonlinear continuum mechanics were used to extract representative mechanical parameters. Established biochemical assays were applied for the determination of the collagen and elastin content. Collagen cross-link concentrations were determined by high-performance liquid chromatography measurements. The results indicate a distinct correlation between the mechanical parameters of high stretch stiffness and membrane tension at rupture and the biochemical data of collagen content and pyridinoline as well as deoxypyridinoline concentrations. No correlation was observed between the mechanical parameters and the elastin content. Moreover, the low stretch stiffness is, with a value of $105 \pm 31 \times 10^{-3}$ N/mm much higher for a biaxial state of stress compared to a uniaxial stress configuration. Determination of constitutive model equations leads to better predictive capabilities for a reduced polynomial hyperelastic model with only terms related to the second invariant, $I_2$, of the right Cauchy-Green deformation tensor. Relevant insights were obtained on the mechanical behavior of fetal membranes. Collagen and its cross-linking were shown to determine membrane’s stiffness and strength for multiaxial stress states. Their nonlinear deformation behavior characterizes the fetal membranes as $I_2$ material.

Keywords Fetal membrane · Biaxial response · Collagen · Elastin · Cross-links

1 Introduction

The fetal membrane (FM) is a membranous structure that surrounds and protects the developing fetus during gestation and is composed of two layers, called amnion and chorion. During pregnancy, the membrane deforms as a consequence of internal pressure as well as fetal movements. The rupture of the FM is an integral part of term delivery but has serious complications when it happens prior to term. The spontaneous preterm premature rupture of the FM (PPROM) affects 3% of all pregnancies worldwide (Calvin and Oyen 2007;
Mercer 2003) and is associated with a high risk for perinatal morbidity and mortality. In addition, iatrogenic PPROM is the Achilles heel in the field of developments for minimally invasive fetal surgery, which occurs in about 30 % of all treatments (Beck et al. 2011). The development of future treatments to prevent premature rupture requires understanding the mechanical behavior and failure of fetal membranes and its relationship to membrane’s microstructure.

The macroscopic anatomy of the FM distinguishes two layers, amnion and chorion, where amnion is the inner layer facing the amniotic fluid and chorion the outer layer facing the uterine wall (Ilancheran et al. 2009). Membranes at term present an amnion layer with thickness in the range of 100 μm and significantly higher stiffness and strength, as compared with the chorion, which is 300–500 μm thick, compliant and extensible. Amnion and chorion are further subdivided into sub-layers of different cellular content and fibrous components of the extracellular matrix (Ilancheran et al. 2009). The mechanical properties of the membrane are commonly related to the type and distribution of collagen in the connective tissue. Amnion contains fibrils forming collagen types I and III in all layers below the amniotic epithelium, along with filamentous collagen types V and VI, which contribute to connecting the fibrillar components to the surrounding connective tissue (Malak et al. 1993); collagen type IV is present in the amniotic basement membrane and contributes to an anchoring zone between amniotic mesoderm and epithelium (Bachmaier and Graf 1999). Chorion contains fibrillar collagen in the reticular layer, interfacing amnion, and collagen type IV in the trophoblast layer. The latter provides the scaffold for the assembly of other non-collagen structural proteins (laminin, entacin, and proteoglycan), (Bryant-Greenwood 1998). Measurement of the FM total collagen content has been reported, providing contents of 4–20 % (Hampson et al. 1997; Jabareen et al. 2009) of dry weight. Not only fibrillar collagen quantity, but also its cross-linking and fibrils arrangement determines tissue’s strength and stiffness. Protocols for quantitative determination of pyridinium cross-links (relevant to fibril forming collagen) are available as part of an established method for the diagnosis of osteoporosis. So far, measurements were taken in one single study of human amnion. Stuart et al. (2005) report values of the pyridinoline (PYD) and deoxypyridinoline (DPD, in Avery and Bailey (2008) referred to as hydroxylysil- pyridinoline) concentrations and conclude that collagen cross-linking is not involved in the etiology of PROM. Meinert et al. (2001) attributed a relevant role in the mechanical stability of amnion to the proteoglycan decorin, which binds to collagen type I and III, and facilitates the lateral alignment or organization of collagen fibrils.

Elastin also contributes to the deformation behavior of FM. Determination of the elastin content in FM is challenging and has only been achieved in recent studies. The published values ranging from 0.08 % of fat-free dry weight (Hieber et al. 1997) to approximately 2 % of wet weight (Jabareen et al. 2009), up to 36 % of (total) wet weight of fresh amnion (Wilshaw et al. 2006) point at the difficulties involved in the quantitative determination of elastin in fetal membranes.

Information on the ultrastructure of the membrane is available from electron microscopy studies. Scanning electron micrographs of the amnion in cross-section demonstrated a composite layered structure (Oyen et al. 2005). Distinct layers can be observed, including a surface cell layer and a collagen network with varying density, with greatest density immediately beneath the cell layer (compact layer). The collagen network appears to be mainly oriented in the membrane plane. Electron microscope images of amnion in Hollenstein (2011) show individual collagen fibrils approximately 50 nm in diameter, organized in somewhat loose bunches, which appears randomly interwoven without perceivable directionality. Assembly of the collagen fibrils into higher-order bundles has not been observed.

In the last decades, many experiments were conducted to understand FM mechanics and failure and its relation to membranes structure and morphology (Moore et al. 2006; El Khwad et al. 2005). Most studies were aimed to the determination of the rupture strength to get insight into the mechanisms leading to PROM (Artal et al. 1976; Lavery and Miller 1979). Other studies were performed in order to determine the different mechanical properties of amnion and chorion (Oxlund et al. 1990). Three types of mechanical test setups were used for these purposes: (i) uniaxial tensile test (Oxlund et al. 1990; Helmig et al. 1993; Jabareen et al. 2009), which is rather simple to perform and to analyze but does not represent the physiological conditions of loading, (ii) puncture testing (Joyce et al. 2009 and references therein), where a spherical metal probe is used to deflect the cramped circular membrane specimen, and (iii) inflation or burst testing (Al-Zaid et al. 1980; Lavery and Miller 1979; Polishuk et al. 1962; Wittenberg 2011), where a circular membrane is deformed with the aid of pressurized water or air. Puncture testing allows characterizing a large number of samples in each FM, but the local force application leads to a state of deformation that differs from the in vivo loading condition. Nevertheless, Schober et al. (1994b) has shown that results obtained by puncture testing can be related to results from inflation tests. Membrane inflation best mimics the physiological loading situation and the in vivo mechanical deformation. Burst tests were accomplished to acquire data on rupture properties of FM, such as burst pressure (MacLachlan 1965) or elevation at rupture (Parry-Jones and Priya 1976). Other studies compared the rupture properties of term membranes with those of preterm ruptured membranes (Lavery and Miller 1979; Al-Zaid et al. 1980) or the difference in the rupture properties between vaginally delivered membranes and membranes...