In-vivo stretch of term human fetal membranes☆

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ABSTRACT

Introduction: Fetal membranes (FM) usually fail prior to delivery during term labor, but occasionally fail at preterm gestation, precipitating preterm birth. To understand the FM biomechanical properties underlying these events, study of the baseline *in-vivo* stretch experienced by the FM is required. This study's objective was to utilize high resolution MRI imaging to determine *in-vivo* FM stretch.
Methods: Eight pregnant women (38.4 ± 0.4wks) underwent abdominal-pelvic MRI prior to (2.88 ± 0.83d) caesarean delivery. Software was utilized to determine the total FM *in-vivo* surface area (SA) and that of its components: placental disc and reflected FM. At delivery, the SA of the disc and FM in the relaxed state were measured. *In-vivo* (stretched) to delivered SA ratios were calculated. FM fragments were then biaxially stretched to determine the force required to re-stretch the FM back to *in-vivo* SA.
Results: Total FM SA, *in-vivo* vs delivered, was 2135.51 ± 108.47 cm^2 vs 842.59 ± 35.86 cm^2; reflected FM was 1778.42 ± 107.39 cm^2 vs 545.41 ± 22.90 cm^2, and disc was 357.10 ± 28.08 cm^2 vs 297.18 ± 22.14 cm^2. The ratio (*in-vivo* to *in-vitro* SA) of reflected FM was 3.26 ± 0.11 and disc was 1.22 ± 0.10. Reflected FM re-stretched to *in-vivo* SA generated a tension of 72.26 N/m, corresponding to approximate pressure of 15.4 mmHg. FM rupture occurred at 295.08 ± 31.73 N/m corresponding to approximate pressure of 34 mmHg. Physiological SA was 70% of that at rupture.
Discussion: FM are significantly distended *in-vivo*. FM collagen fibers were rapidly recruited once loaded and functioned near the failure state during *in-vitro* testing, suggesting that, *in-vivo*, minimal additional (beyond physiological) stretch may facilitate rapid, catastrophic failure.

1. Introduction

Normal pregnancy requires that the physical integrity of the fetal membranes (FM) be maintained until term delivery. However, premature failure of the FM-aka preterm premature of fetal membranes is responsible for nearly 40% of all preterm births. Preterm birth is a major global public health problem with an estimated 14.9 million babies being born premature in 2010 worldwide [1]. Prematurity is responsible for 35% of the world's 3.1 million annual neonatal deaths and is the second largest direct cause of deaths in children less than 5 years [2]. Preterm birth rate in the US is high at 11.39% resulting in US being one of the ten countries globally with highest number of preterm births [1,3].

The physiological mechanisms which cause membranes to fail, term or preterm, are not completely understood. In the past, weakening and rupture of the FM were attributed to the mechanical stresses of labor contractions. This is clearly inconsistent with the 10% of term and 40% of preterm births in which FM rupture precedes contractions. It is now established that FM weaken in late gestation as a result of biochemical changes: extracellular matrix remodeling and apoptosis in late gestation [4]. Many groups have

confirmed that these biochemical changes are focused at the para-cervical zone of FM overlying the cervix [4–18]. We have further demonstrated that this biochemically remodeled, para-cervical FM region is physically weaker than other FM regions and have termed it "physiological weak zone" [4,12,13]. The spontaneous rupture of FM initiates in this physiological weak zone but it is not clear how this occurs.

The FM is a collagenous soft tissue that surrounds the fetus with a major role in supporting the fetus and amniotic fluid, and in tolerating local deformation associated with fetal movements during gestation [19,20]. The FM is a complex structure with two components; the choriodecidua which is relatively thick and cellular, and the amnion which is thinner and stronger. The amnion accounts for approximately 20% of the FM thickness but dominates the mechanical response of the FM [21,22]. In-vitro, the amnion undergoes a decrease in thickness with substantial collagen fiber alignment in response to minimal loads [23]. Type I collagen in the compact sublayer of the amnion is responsible for much of the mechanical strength of the FM.

Individual collagen fibers are initially undulated (crimped) in a stress-free state [24]. Undulated collagen fibers do not carry load,
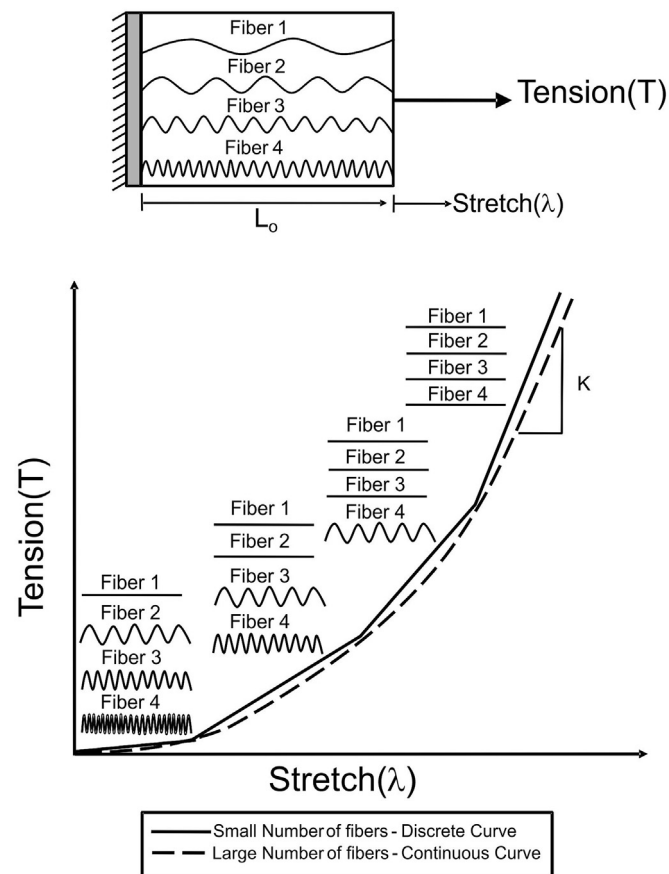

Fig. 1. FM collagen fiber recruitment: Traditional recruitment modeling assumes that a collagen fiber does not bear load until the collagen fiber is straightened, where K is the elastic constant. Gradual recruitment of collagen fibers results in a non-linear tension-stretch relationship. Initially all collagen fibers are crimped and do not bear load, which results in the toe region. With increased stretch, the fiber with the least amount of crimp, fiber 1 in this schematic, will bear load and become straightened. With increased stretch, fibers 2 and 3 will become straightened, causing the tension-stretch curve to increase in a non-linear manner. As stretch continues to increase, all collagen fibers (1, 2, 3, and 4) will bear load and become straightened resulting in a highly linear region on the tension-stretch curve. When a small population of fibers is present, the tension-stretch curve is discrete (indicated by the solid line), but when a large population of fibers exists, the tension-stretch curve is continuous (dashed line).

but once a fiber has been stretched to a "straightened" state, the fiber will transmit load in a linearly elastic manner (Fig. 1). Stretch can be defined as: $\lambda = L/L_o$, where L is the current (stretched) length of the collagen fiber and L_o is the original length of the collagen fiber before being stretched (Fig. 1). FM collagen fibers are recruited at different stretch levels due to various degrees of initial collagen crimping (Fig. 1). While one fiber may be fully stretched, another may still be crimped, resulting in a non-linear tension-stretch curve (Fig. 1). Once all collagen fibers are straightened, the tension-stretch curve becomes linear. In this region, membrane tension can be defined as force/unit length (Fig. 1). When a small number of fibers are present, the various regions of the tension-stretch curve (i.e. "toe" region, transition regions between the "toe" and linear regions, highly linear region) are discrete (Fig. 1; solid line). However, when a large population of fibers is present, the tension-stretch curve is continuous, as demonstrated previously by Oyen et al. (Fig. 1; dashed line) [19–21].

Tissue failure (rupture), which occurs when the FM can stretch no further, is a unique event in normal human physiology. Other tissue failures (e.g. bone fracture, skin tears, vascular aneurysms) are all pathological processes. Because tissue failure typically occurs as a result of a pathological process and not normal function, the biomechanics of soft tissue failure is generally poorly understood. The importance of biomechanics in FM failure is reflected in the relation between biomechanical and biochemical processes. Millar et al. have suggested that distention of the FM extracellular matrix (ECM) normally stimulates remodeling of the ECM, resulting in an increase in membrane surface although there is minimal growth of the FM during the final weeks of pregnancy as demonstrated by decreased mitosis [25]. Excessive distension or inadequate remodeling, could result in preterm premature rupture of the FM and preterm birth [26]. We have demonstrated in both clinical human FM specimens, and from our in-vitro human FM weakening model, that FM remodeling associated with collagen degradation and cellular apoptosis plays an important role in FM weakening [4,12,13,27–33]. Biochemically pre-weakened FM more readily fail as a result of the mechanical forces of uterine contractions at the onset of labor. This is supported by the known interplay between mechanical deformation and collagen degradation in other collagenous soft tissues both of which perhaps works synergistically to induce tissue failure [34–37]. Ellsmere et al. demonstrated that tensile loading accelerates the proteolysis of bovine pericardium subjected to collagenase, while another study demonstrated that the degradation rate of collagen increased with stretch [34,36].

In order to develop a rational basis for treatment and prevention of premature FM failure, a better understanding of FM structural and mechanical behavior at near/full term under both sub-failure and failure conditions is necessary. In order to quantify the intact FM (FM with amnion adherent to the choriodecidua) mechanical behavior under sub-failure and failure conditions from full term tissues, it is necessary to perform this ordered sequence: (1) Accurately measure baseline FM stress in-vivo in order to determine its effects on growth and failure of the FM; (2) Quantify the FM mechanical properties under biaxial stretch; (3) Use the biaxial stretch data to develop a structural (mathematical) model to derive intrinsic fiber properties; and (4) Use the model to gain insight into the micromechanical mechanisms of failure. To initiate this, intra-uterine physiological tension of the FM was determined utilizing a two-step procedure. First, the areal stretch, the ratio of the surface area of the FM in-vivo (at term just prior to delivery) vs in-vitro surface area (immediately after delivery) was determined. Second, fragments of FM were biaxially stretched with a novel membrane inflation device to provide tension-areal stretch data from sub-failure to failure. The surface area ratio obtained in the first step