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Characterization of irreversible physio-mechanical processes in stretched fetal membranes

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ABSTRACT

We perform bulge tests on live fetal membrane (FM) tissues that simulate the mechanical conditions prior to contractions. Experimental results reveal an irreversible mechanical behavior that appears during loading and is significantly different than the mechanical behavior that appears during unloading or in subsequent loading cycles. The irreversible behavior results in a residual strain that does not recover upon unloading and remains the same for at least 1 h after the FM is unloaded. Surprisingly, the irreversible behavior demonstrates a linear stress–strain relation.

We introduce a new model for the mechanical response of collagen tissues, which accounts for the irreversible deformation and provides predictions in agreement with our experimental results. The basic assumption of the model is that the constitutive stress–strain relationship of individual elements that compose the collagen fibers has a plateau segment during which an irreversible transformation/deformation occurs. Fittings of calculated and measured stress–strain curves reveal a well-defined single-value property of collagenous tissues, which is related to the threshold strain ε_{th} for irreversible transformation. Further discussion of several physio-mechanical processes that can induce irreversible behavior indicate that the most probable process, which is in agreement with our results for ε_{th}, is a phase transformation of collagen molecules from an α-helix to a β-sheet structure. A phase transformation is a manifestation of a significant change in the molecular structure of the collagen tissues that can alter connections with surrounding molecules and may lead to critical biological changes, e.g., an initiation of labor.

Statement of Significance

This study is driven by the hypothesis that pre-contraction mechanical stretch of the fetal membrane (FM) can lead to a change in the microstructure of the FM, which in turn induces a critical biological (hormonal) change that leads to the initiation of labor. We present mechanical characterizations of live FM tissues that reveal a significant irreversible process and a new model for the mechanical response of collagen tissues, which accounts for this process. Fittings of calculated and measured results reveal a well-defined single-value property of collagenous tissues, which is related to the threshold strain for irreversible transformation. Further discussion indicates that the irreversible deformation is induced by a phase transformation of collagen molecules that can lead to critical biological changes.

1. Introduction

Preterm labor is the leading cause of neonatal morbidity and mortality, accounting for 70% of perinatal mortality and nearly 50% of long-term neurological morbidity, including neurodevelopmental handicaps, chronic respiratory problems, infections and ophthalmological problems. Preterm birth puts infants at increased risk of death during infancy and contributes to developmental delays.

The risk of preterm labor is particularly high in pregnancies associated with uterine over-distention and particularly in multiple pregnancies, which account for 3% of all births but 17% of births before 37 weeks' gestation and 23% of births before 32 weeks [1].

Infants born before 32 weeks' gestation account for nearly half of all long-term neurological morbidity attributable to prematurity

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[2]. This information indicates a possible relation between preterm labor and mechanical stresses that are applied on different uterine organs/tissues and motivates a study of the mechanical behavior of uterine organs/tissues.

During the gestation period, the fetus is enclosed by the fetal membrane (FM), which undergoes several mechanical adjustments to support the loads of the fetus and the amniotic fluid as well as to tolerate local deformation associated with fetal movement and growth [3]. The FM is surrounded by the thick uterus muscle that bears the mechanical loads, except for a small region of the FM located above the cervix [4,5]. The intrauterine pressure induces bi-axial mechanical stretching of the FM in that region. It is therefore of particular importance to study the mechanical behavior of the FM during stretching.

The FM is a bilayer structure composed of the amnion and chorion layers. The chorion layer is thicker than the amnion (413 μm versus 111 μm), but the latter is stiffer and stronger and thus dominates the mechanical response of the FM, even though it only accounts for approximately 20% of the FM thickness [3,6]. The amnion is avascular and comprises various sub-layers, including a compact layer composed of collagen, predominantly Types I and III [7]. The relatively high stiffness and strength of the amnion is attributed to the distribution of collagen in the connective tissue.

Previous studies have focused on the mechanical failure of the FM, which leads to preterm rupture of the membrane [3,4,7–13]. For example, Oyen et al. [3,12] performed a biaxial puncture test on the amnion and chorion layers of the FM and found that the chorion layer ruptures first. During the rupture of the chorion, the force decreased slightly before increasing again prior to failure, indicating that the amnion layer bears most of the stress.

A rupture of the FM is part of the process of labor, but it is not always the cause for the initiation of preterm labor. In most of the cases, labor begin with uterine contractions that finally lead to the rupture of the FM. Biological changes are induced in many cases as a reaction to a change in the environment. One possibility for such a reaction may be related to a change in the molecular structure of the tissue—in this case, of the FM. Thus, it is possible that in some cases the precontraction mechanical stretch of the FM leads to a change in the molecular structure of the FM, which in turn induces a critical biological change that leads to the initiation of labor. Such a process can explain the correlation between preterm labor and an overdistended uterus. As a first stage of exploring this hypothesis, it is important to study irreversible changes in the FM that are induced by mechanical stretching. The focus in this case is on the effect of moderate mechanical stresses, comparable to stresses that exist before labor, rather than on large stresses typical of contractions.

Soft materials usually exhibit a conditioning effect [14–22] in which few loading–unloading cycles are necessary to reach a repeatable response. Most of the previous studies were performed on preconditioned specimens of FM tissues, which were subjected to several cycles of loading and unloading before the test began. In these cases, the first cycle, during which most of the irreversible deformation takes place, may have been overlooked. For example, Jabareen et al. [6] performed a uniaxial tension test on a preconditioned specimen and found that the hysteresis loops shift vertically downward along the stress axis and that the difference between two consecutive cycles decreases as the number of cycles increases. They did not report any permanent deformation in the first cycle. The results of Perrini et al. [13] and Oyen et al. [8] demonstrated a large hysteresis loop and irreversible deformations during the first cycle, followed by a much smaller hysteresis for subsequent cycles at the same strain level. However, no explanation has been given for this phenomenon.

In most biological materials, the internal architecture and the atomistic and microstructural arrangement of molecules determine the mechanical behavior more than the chemical composition does. The mechanical behavior of the FM resembles that of other soft tissues rich in Type I collagen, which provides structural integrity to collagenous tissues. These tissues are typically based on collagen fibrils as elementary building blocks, which are assembled in a complex hierarchical way into macroscopic structures. Several levels of fiber structures are observed. The lowest level is collagen molecules, which are triple helical protein chains. The collagen molecules are assembled in parallel into fibrils. The fibrils are joined by a matrix into fiber forms. Fibers are bounded together by a fine layer of connective tissue called the endotenon [23].

There have been several experimental investigations regarding the mechanical response of single collagen fibrils [24–30]. All of them report a linear force–displacement relation, although a small hysteresis has been observed in some (e.g., Refs. [24,25,28,29]). Shen et al. [25] observed a yield effect at very high stress values, which resulted in residual plastic deformation that remained when unloading the fibrils. After prolonged rest times at zero load, part of the residual deformation was recovered. It should be mentioned that in all studies of single collagen fibrils, samples were stored in a wet environment before the test, but the tests were performed in a dry environment.

Microscopic observations of FM tissues, as well as other collagenous tissues, show that most of the collagen fibers are undulated (not fully straight) and that each fiber has a different amount of undulation [9,23,31–36]. These observations, together with the information obtained from mechanical tests of single fibrils, led to the following commonly accepted model for the mechanical response of collagenous tissues [9,13,37]. In a group of collagen fibers, each fiber has a different amount of undulation and is thus straightened at a different level of stretch. Initially, the collagen fibers of the FM are undulated. Upon stretching, the fiber that has the lowest amount of undulation straightens first, followed by the fiber with the second lowest amount of undulation and so on. Thus, as the stretching strain increases, the number of straightened fibers increases. It is assumed that only the straightened fibers carry the load and therefore the stiffness increases as the strain increases (Fig. 1a). Once all of the collagen fibers are straightened, the stress–strain curve evolves into a linear region. For a collagenous tissue composed of numerous collagen fibers, the effect of individual fibers cannot be resolved and a smooth stress–strain curve as illustrated in Fig. 1b is obtained. Under a cyclic load, some amount of hysteresis is usually observed (Fig. 1c) due to viscous effects in the collagen fibers or the matrix [13,38,39].

Recently, the above described model has been elaborated in light of new findings on correlations between parameters characterizing the mechanical response and parameters quantifying the microstructural constituents. Buerzle et al. [37,40,41] found a relation between membrane's strength as well as stiffness at large deformations and the collagen content as well as its cross-linking. Mauri et al. [38,39], found significant volume reduction which was associated with dehydration of the FM during loading. This effect was mostly recovered during unloading. Therefore, the hydration change is expected to have a significant effect on the viscoelastic hysteresis, as observed by Mauri et al. [38]. The above mentioned model description and experimental results are not focused on mechanisms for irreversible deformation that may occur during loading and not recovered during unloading.

In this study, we performed mechanical tests on live FM tissues, which were collected from women who underwent Caesarean sections. Because these tissues were not subjected to contractions, they can be considered non-preconditioned. Our experimental results show that after a few cycles (i.e., after preconditioning), the mechanical behavior of the FM tissues is in accordance with the prediction of the common model, as illustrated in Fig. 1.