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Influence of osmolarity and hydration on the tear resistance of the human amniotic membrane

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ABSTRACT

The amnion is considered to be the load-bearing part of the fetal membranes. We investigated the influence of osmolarity of the testing medium and hydration on its fracture toughness. Mode I fracture tests revealed that physiological variations in the bath osmolarity do not influence the tear resistance of amnion, while larger changes, i.e. from physiological saline solution to distilled water, lead to a significant reduction of the fracture toughness. Uniaxial tensile tests on collagen hydrogels confirmed the reduction in toughness, suggesting that lower bath osmolarity triggers changes in the failure properties of single collagen fibers. Prenatal surgeries, in particular fetoscopic procedures with partial amniotic carbon dioxide insufflation, might result in dehydration of the amnion. Dehydration induced a brittle behavior; however, subsequent rehydration for 15 min resulted in a similar tear resistance as for the fresh tissue.

1. Introduction

Preterm delivery occurs in around 3% of all pregnancies, often associated with preterm premature rupture of the membranes (PPROM) (Mercer et al., 1999). The latter is defined by membrane rupture prior to 37 weeks of gestation (Mercer et al., 1999), which is the case in one fourth of all preterm deliveries, potentially leading to neonatal death or morbidity (Mercer, 2003). To better understand the causes of PPROM, the strength and deformation properties of fetal membranes have been widely investigated (Mauri et al., 2015b; Perrini et al., 2015; Calvin and Oyen, 2007; Oyen et al., 2006; Buerzle, 2014; Oyen et al., 2004; Oyen et al., 2005; Koh and Oyen, 2012; Koh, 2013; Mauri et al., 2015a; Ehret et al., 2017; Bircher et al., 2017; Pensalfini et al., 2018; Ernest et al., 1989; Schober and Kusy, 1995; Puthiyachirakkal et al., 2013; Kumar et al., 2011). The fetal membranes are constituted by an amniotic (AM) and a chorionic membrane and may undergo modifications due to both biochemical and biomechanical processes (Puthiyachirakkal et al., 2013; Kumar et al., 2011). Previous studies contributed to an improved understanding of the mechanisms of deformation and fracture of fetal membranes (Bircher et al., 2019). Our previous work (Bircher et al., 2019a, 2019b; Ehret et al., 2017) rationalized the favorable fracture properties of fetal membranes and addressed the potentially detrimental influence of suturing the membranes, which is required at the time of fetal surgeries. The AM was shown to be stiffer, tougher and stronger in uniaxial and multiaxial biomechanical studies compared to chorion (Bircher et al., 2019), even though the thickness of the AM is only 60–100 μm (Buerzle, 2014; Halaburt et al., 1989) compared to the thicker chorion (300–400 μm (Buerzle, 2014; Halaburt et al., 1989)). The superior mechanical resistance is attributed to the higher collagen content of AM (Halaburt et al., 1989), making it the load-bearing and fracture-resisting constituent of the fetal membranes (Bircher et al., 2019; Calvin and Oyen, 2007; Oyen et al., 2006; Buerzle, 2014). The present study thus focuses on the amniotic membrane.

AM consists mainly of collagen (types I, III, IV, V and VII), cells, proteoglycans, water and minor quantities of elastin and other extracellular matrix proteins (Mauri et al., 2016; Mauri et al., 2015a; Mauri et al., 2013; Buerzle, 2014; Bourne, 1962). The thin collagen fibers (diameter around 100 nm (Mauri et al., 2016; Oyen et al., 2005)) form a higher-order network around a com-

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pressible, hydrated matrix. Water-binding proteoglycans contribute to the deformation behavior of the AM, in particular to the dependence on the chemical potential of its environment (Ehret et al., 2017). AM consists of several layers, and the outermost is a monolayer of epithelial cells, which is connected through the basement membrane to the collagenous and densely packed compact layer (Mauri et al., 2015a; Buerzle, 2014; Bourne, 1960). The adjacent thicker fibroblast-rich layer is linked to the spongy layer, which is the interface between AM and chorion (Mauri et al., 2015a; Buerzle, 2014; Bourne, 1960).

In an attempt to identify factors leading to PPROM, Ernest et al. (1989) and Gleeson et al. (1989) reported that an increased vaginal pH is indicative of an elevated risk for PPROM. Other studies investigated the biochemical composition of the amniotic fluid during gestation, primarily focusing on potassium, calcium and sodium chloride concentrations (Gillibrand, 1969; Doran et al., 1970; Johnell and Nilsson, 1971; Andersen and Weber, 1985), reporting sodium concentrations in the range of 134–138 mmol/L with a reduction to around 128–130 mmol/L towards the end of the pregnancy. Schober and Kusy (1995) demonstrated a reduction of strength, stiffness and toughness with increasing amniotic fluid pH, while sodium concentration was shown to affect tissue thickness and hydration; however, no significant effect on the strength was observed. Note that the influence of sodium concentration was tested within a range of 100–150 mmol/L, in line with concentration values reported in literature (Gillibrand, 1969; Doran et al., 1970; Johnell and Nilsson, 1971; Andersen and Weber, 1985). More recently, Ehret et al. (2017) reported a substantial influence of the environmental osmolarity on the deformation behavior of the AM when modifying the surrounding fluid from saline solution (0.9% NaCl) to distilled water. Previous investigations analyzed the influence of the bath osmolarity on the fracture toughness of another soft collagenous tissue, i.e. the bovine Glisson's capsule (Bircher et al., 2019b). The experiments revealed significant reduction of tear resistance and critical elongation when the testing medium was changed from saline solution to distilled water. In our previous work (Bircher et al., 2019b), we investigated the fracture toughness of collagenous tissues using a 3D discrete fiber network model coupled with chemo-elastic volume elements. Interestingly, at large strains close to the critical elongation, the influence of bath osmolarity on the network state of deformation was shown to become negligible. Premature failure might therefore be due to osmolarity-dependent failure mechanisms between fibers (interfiber crosslinks) or within the hierarchical structure of fibers (Bircher et al., 2019b). To further study the effect of osmotic pressure on the failure behavior of collagen networks, in the present work we performed uniaxial tension to failure (UA) experiments with collagen gels in distilled water and in saline solution.

Improvement of technology with better ultrasound machines and earlier diagnosis of fetal diseases offers the option of prenatal surgery, which improves postnatal outcome in selected fetuses with congenital malformations. The surgery can be done either by opening the uterus and membranes or by minimally invasive access (or fetoscopy) to the amniotic cavity. Despite the smaller trauma, fetoscopic surgeries seem to increase membrane rupture rate, hence premature delivery (Adzick et al., 2011; Wilson et al., 2004). In those interventions, the amniotic fluid is partially drained and replaced with carbon dioxide (CO_2), a technique called partial amniotic CO_2 insufflation (PACI), to increase work space and visibility, facilitate hemostasis and immobilize the fetus (Kohl et al., 2010). At the end of the procedure, the CO_2 is removed and amniotic fluid volume is restored by adding physiological saline solution at body temperature (Kohl et al., 2010). Several factors increasing membrane rupture rates have been suggested, such as insufflation pressure, tissue dehydration and prolonged operating times (Skinner et al., 2018), which are reported to be in a range of 100–500 minutes (Menon and Richardson, 2017). Herein, we investigate the influence of dehydration on the fracture toughness of AM, by mimicking the above surgical condition by first inducing a strong dehydration, as well as the subsequent rehydration.

This evidence formed the basis for the present study which applied the method described in Bircher et al. (2019b) to analyze two potential risk factors for PPROM, i.e. the possible detrimental influence on AM toughness of reduced environmental osmolarity and of dehydration associated with prenatal surgery.

2. Methods

2.1. Sample collection and basic preparation

Fetal membranes were obtained from patients who had elective primary caesarean section at around 38 gestational weeks. Written consent was provided by each patient (ethical approval KEK-StV-Nr. 07/07). All chosen pregnancies had no maternal hepatitis B or HIV, chlamydia and no preterm rupture of the membrane. The AM was gently separated from the chorion on a plastic foil (Mauri et al., 2015a). Samples were continuously kept hydrated in saline solution (PS, 0.9% NaCl, osmolarity 308 mosmol/L, corresponding to 154 mmol/L of sodium) or, if specified, in distilled water (DW) or physiological solution (PLS, osmolarity of 270 mosmol/L, corresponding to 135 mmol/L of sodium) (Gillibrand, 1969; Doran et al., 1970; Johnell and Nilsson, 1971; Andersen and Weber, 1985). Sample preparation and all testing was performed at room temperature (20 °C), and all experiments were performed on freshly harvested membranes within few hours after the delivery.

Acellular, plastically compressed collagen gels (CG) were produced at the Tissue Biology Research Unit in the University Children's Hospital Zurich, following the protocol of Braziulis et al. (2012). CG testpieces were prepared using a dog-bone-shaped stamp (ISO 37 Type 4, 2 mm width, initial free length $L_0 = 20$ mm) in order to perform UA tests.

Mode I fracture tests investigating the influence of the bath osmolarity were performed on rectangular AM testpieces (Fig. 1), cut with a scalpel to dimensions of 30 mm × 60 mm in total length and width ($L_0 = 10$ mm, notch depth $c = 15$ mm, ligament width $b = 45$ mm) (Bircher et al., 2019a, 2019b). The notch is created by cutting from the sample edge, using a scalpel, and resulting in a predefined notch depth c, see Fig. 1. Circular AM samples were cut to a diameter of 70 mm with a scalpel (cf. Fig. 2a) for use in a custom-made inflation device (cf. reference (Buerzle, 2014)). Analysis of the in-plane deformation field in both mode I fracture and UA tests was facilitated by carefully inducing black marks on the tissue surface with a commercial waterproof black pen (GeoCollege Pigment Liner 0.005). Experiments were not performed along a specific direction, as AM is generally considered in-plane isotropic (Buerzle, 2014; Mauri et al., 2016; Oyen et al., 2005).

2.2. Protocols for investigating the influence of hydration

Circular AM samples were dehydrated while subjected to a moderate insufflation in the inflation device. AM samples were placed with the epithelial layer facing downwards and fixed using sandpaper rings between a cover ring with an inner diameter of 50 mm and an inflation cylinder with six screws (Fig. 2b). Pressure was applied by pumping air into the cylinder using a syringe pump (Standard Infuse/Withdraw PHD Ultra Syringe Pumps, Harvard Apparatus, Holliston, MA, USA) controlled with a custom algorithm in LABVIEW (National Instruments, Huntsville, AL, USA). The pressure p in the cylinder was prescribed and measured with a pressure sensor (digital manometer, LEX 1, Keller, Switzerland).

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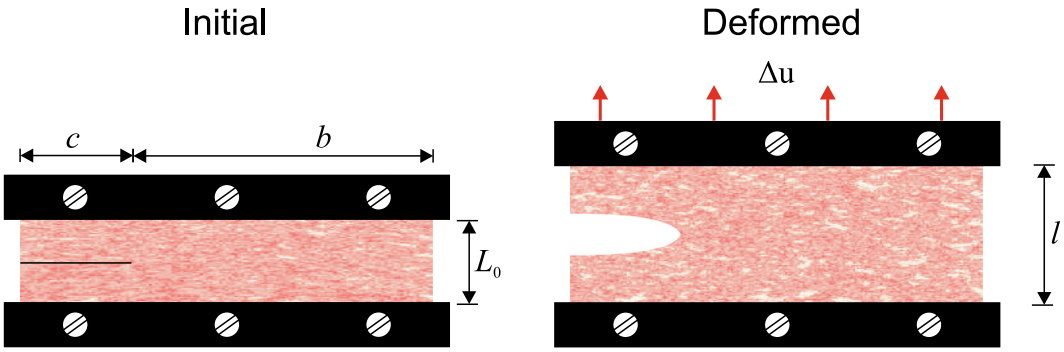

Fig. 1. Schematic of mode I fracture tests in the initial and deformed state.

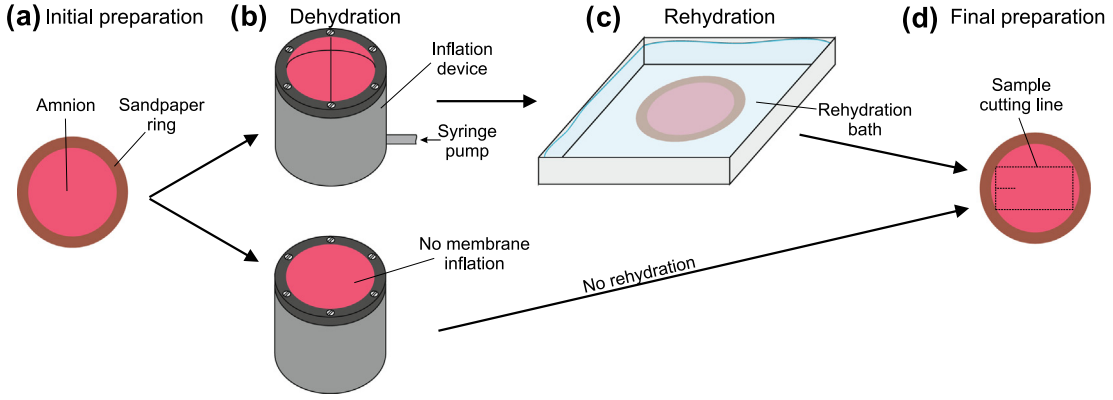

Fig. 2. Illustration of sample preparation for testing the influence of dehydration. Note that dehydration time and pressure in the inflation device was adapted depending on the protocol.

Two dehydration conditions were analyzed: samples were insufflated for 15 minutes at 0.1 mbar inflation pressure or for 120 minutes at 8 mbar (Fig. 2b). Note that the pressure levels of 0.1 mbar and 8 mbar were selected small enough to prevent mechanical damage during insufflation, but large enough to deform the AM. Based on (Buerzle, 2014), 8 mbar corresponds to a stretch of about 1.05. Subsequently, samples were removed from the inflation device and rehydrated for 15 minutes in PS (0.9% NaCl, Fig. 2c). An additional set of experiments was performed on samples with a dehydration time of 120 minutes and no subsequent rehydration (Fig. 2b,d), without the application of an insufflation pressure.

Similar as to the standard mode I fracture experiments, samples were cut to free dimensions of 30 mm × 40 mm in total length and width (L_0 = 10 mm, c = 10 mm) on a plastic foil with a surgical scalpel and black markers were applied on the tissue surface (Fig. 2d).

2.3. Mode I fracture tests

Mode I fracture tests were performed in a tensile setup (Buerzle and Mazza, 2013; Bircher et al., 2019b), which is composed of hydraulic actuators with force sensors (MTS Systems, Eden Prairie, MN, USA, force range: up to 100 N), and of a CCD camera (Pike F-100B Allied Vision Technologies GmbH, Stadtroda, Germany) equipped with a 0.25× telecentric lens (NT55-349; Edmund Optics GmbH, Karlsruhe, Germany). This setup is capable of simultaneously recording top-view images of the specimens at 4 Hz, while acquiring force F and clamping displacement Δu at 10 Hz. Sacrificial clamping jigs were produced with sandpaper and a plastic foil for the clamping process (Bircher et al., 2019b). The jaws of the clamps were covered with sandpaper to minimize tissue slippage and tightly closed with screws (Mauri et al., 2015b; Bernardi et al., 2017). Clampings and samples were immersed in PS or, if specified in DW or PLS. Note that tests of dehydrated samples without rehydration were performed in room air. Strain rate was set to 0.3%/s in order to reduce time-dependent effects and to capture the response representative of the long-term behavior of the tissue.

Tests investigating the influence of bath osmolarity were either started in DW, PLS or PS (Bircher et al., 2019b). After the incident of crack propagation initiation, samples were unloaded to the initial condition (Bircher et al., 2019b). Subsequently, the bath was left unchanged (control), or the bath was changed either from PS to DW, from DW to PS, from PS to PLS or from PLS to PS, and the specimens were loaded again up to crack propagation initiation (Bircher et al., 2019b). This procedure was repeated up to 3 times.

Nominal tension was defined as $T = F/b$, where b is the ligament width of the sample, i.e. the total width minus the notch depth c (cf. Fig. 1). Data analysis was based on a specified reference configuration based on a tension level of 7.0×10^{-4} N/mm (Bircher et al., 2019a). The corresponding sample length L_{ref} was used to calculate the nominal stretch in the direction of the applied loading, defined as $\lambda_N = \frac{L_{ref}+\Delta u}{L_{ref}}$ (cf. Fig. 1) with $l = L_{ref} + \Delta u$ denoting the current length. Local stretches in the direction of loading (λ) and perpendicular to it (λ_2) were computed with a custom algorithm (Hopf et al., 2016), which tracks the markers in top-view images and reconstructs the affine in-plane deformation field. The nominal tension vs. local stretch curves were used to calculate the secant stiffness at a certain value of T which corresponds to the end of the toe region in the mechanical response of fresh AM (i.e. for T=0.05 N/mm), defined as $K_{sec} = \frac{\Delta T}{\Delta \lambda}$. The incident of crack propagation initiation was identified from analysis of top-view images (Bircher et al., 2019a, 2019b; Pensalfini et al., 2018), enabling the

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determination of failure tension T_{crit} and stretch λ_F. The approach originally developed by Rivlin and Thomas (1953) to quantify the tearing energy of elastomers was adapted for the characterization of the fracture toughness of soft tissues (Bernardi et al., 2017; Pensalfini et al., 2018; Bircher et al., 2019b). Specifically, the sample-geometry specific membrane tearing energy was calculated as $\Gamma_a = \gamma_c L_{ref} \int_1^{\lambda_F} T d\lambda$. Note that γ_c is a factor accounting for possible tissue slippage at the clamps, and is calculated as $\gamma_c = \lambda_N/\lambda$ at crack propagation (Bernardi et al., 2017). The characteristic energy of tearing is defined as $\overline{\Gamma} = \Gamma/t_0$, where t_0 denotes the initial thickness of the tissue and Γ the stabilized value of Γ_a for large enough samples (cf. reference (Bircher et al., 2019a, 2019b)).

2.4. Uniaxial tension tests

UA tests on CG were performed in the tensile setup used for mode I fracture tests. The goal of the experiments was to quantify the difference in uniaxial strength depending on bath osmolarity. To this end, samples were loaded at a nominal strain rate of 0.3%/s up to failure. Postprocessing of the data was similar as in mode I fracture tests: local in-plane stretches (λ and λ_2) and nominal tension $T^{UA} = F/w$ were extracted from the recorded force signal F, the sample width in the reference state w and analysis of the top-view images. Subsequently, critical tension T^{UA}_{crit}, critical stretch λ^{UA}_F and toughness τ were extracted, defined as $\tau = \int_1^{\lambda^{UA}_F} T^{UA} d\lambda$. Additionally, the nominal tension vs. λ curves were used to calculate the secant stiffness for a predefined stretch level (i.e. $\lambda = 1.1$), as $K^{UA}_{sec} = \frac{\Delta T}{\Delta \lambda}$.

2.5. Statistical analysis

Data were analyzed and tested for statistical significance using Python. Values are expressed as boxplots or as mean±standard deviation. Significance between two different groups was tested with the Mann-Whitney U test. More than two groups were analyzed with Kruskal-Wallis and subsequent Dunn's post hoc test. Significance was defined for p-values of less than 0.05.

3. Results

Membrane tearing energy Γ_a of all mode I fracture tests performed on fresh, untreated AM in PS are shown in Fig. 3 as boxplots. Present data are compared with previous measurements, i.e. from Koh (2013) and Pensalfini et al. (2018). A good correspondence with the results from Pensalfini et al. (2018) is observed, while the data from Koh (2013) lead to larger tearing energy values.

The influence of osmolarity of 270 mosmol/L (PLS) and 308 mosmol/L (PS) on the fracture toughness is shown in Fig. 4 in terms of Γ_a values (based on the first loading) and change in critical elongation $\Delta\lambda_F$ in consecutive loadings. No significant differences between the tests in PLS and PS were observed, neither in terms of $\Delta\lambda_F$ nor in terms of Γ_a.

Similar experiments were then performed to investigate the influence of a more drastic change in the osmolarity, i.e. between PS and DW. Γ_a from first loading of each sample is reported in Fig. 5a, and changes of the critical elongation in subsequent loadings in Fig. 5b, both revealing a significant influence of the bath osmolarity on the fracture behavior of AM.

In order to rationalize the results of Fig. 5, the strength of a collagen hydrogel was considered. Results of corresponding UA tests are reported in Fig. 6. The deformation behavior of collagen gels in UA tensile tests is unaffected by the bath osmolarity, as shown

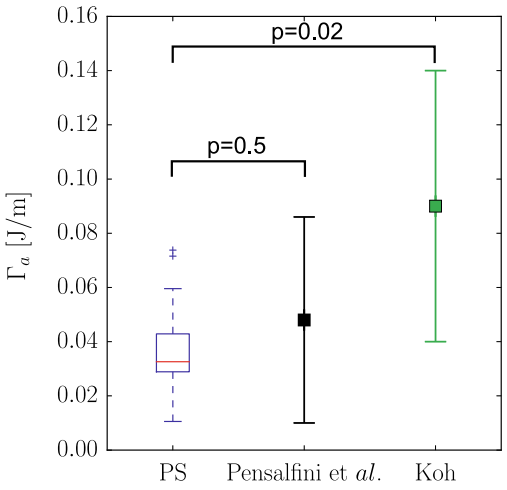

Fig. 3. All experimental results in terms of the membrane tearing energy Γ_a of AM, tested in 0.9%NaCl (PS) of the present study (in boxplot, $n = 14$), as well as values from literature, i.e. from Pensalfini et al. (2018) and adapted from Koh (2013). Differences between the present results and the data from the literature are evaluated with a t-test, as the latter are presented using mean±standard deviation. p-levels are indicated in the figure.

in Fig. 6a, reporting the secant membrane stiffness and the lateral contraction at a stretch of $\lambda = 1.1$. Importantly, the critical tension as well as the toughness of CG are larger for experiments performed in PS than in DW (Fig. 6b). Note however, that for the current sample number, significant differences are obtained only for the toughness values but not for the critical tension.

The results of the experiments analyzing the influence of AM dehydration (with or without subsequent rehydration) on critical elongation λ_F, tension T_{crit} as well as on the membrane tearing energy Γ_a are reported in Fig. 7b-d. Corresponding tension vs. stretch curves were used to calculate the secant stiffness K_{sec} (Fig. 7a), revealing a significantly stiffer behavior for the non-rehydrated samples. For rehydrated samples, no significant differences were observed, even after 2 hours of dehydration at an elevated insufflation pressure of 8 mbar. On the other hand, without rehydration critical elongation and fracture toughness are strongly reduced. Interestingly, the critical tension T_{crit} is not significantly different between any of the testing conditions.

4. Discussion

The influence of the chemical conditions of the medium to which soft collagenous tissues (e.g. AM, bovine Glisson's capsule and porcine pericardium) are exposed to on the deformation behavior and fracture toughness has been previously investigated (Schober and Kusy, 1995; Ehret et al., 2017; Bircher et al., 2019b). The tearing energy reported in Koh (2013) is expected to significantly overestimate the fracture resistance of AM, due to the absence of correction of slippage at the clamps, as discussed in Bernardi et al. (2017), which explains the larger values reported in Koh (2013) compared to the present study. The variability of Γ_a from mode I fracture tests on AM is in line with the large scatter typically observed in mechanical tests on soft collagenous tissues for both deformation and fracture properties. The present results are in line with previous observations of reduced fracture toughness with decreasing bath osmolarity (Fig. 5). This indicates a possible impact of environmental conditions on AM fracture behavior. However, this effect becomes negligible for small variations in the chemical potential, in the range of sodium concentration variations of 135–154 mmol/L (Fig. 4), which covers the physiological values of osmolarity fluctuations in amniotic fluid

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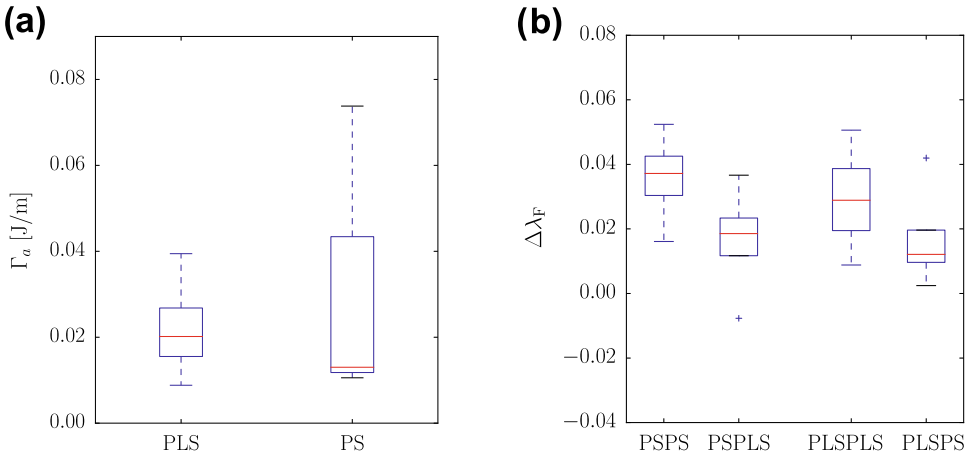

Fig. 4. **(a)** Membrane tearing energy Γ_a (initial sample length L_0 = 10 mm) of AM tested in PS (308 mosmol/L, n = 4) and PLS (270 mosmol/L, n = 4). **(b)** Sample-specific change in critical elongation $\Delta\lambda_F$ for consecutive tests in same (control) or different bath (n = 4 each for PS-PS, PS-PLS, PLS-PLS and PLS-PS, resp.). Results are reported with boxplots and significant differences between groups are indicated with ∗ (p<0.05, none in these cases, Mann-Whitney U).

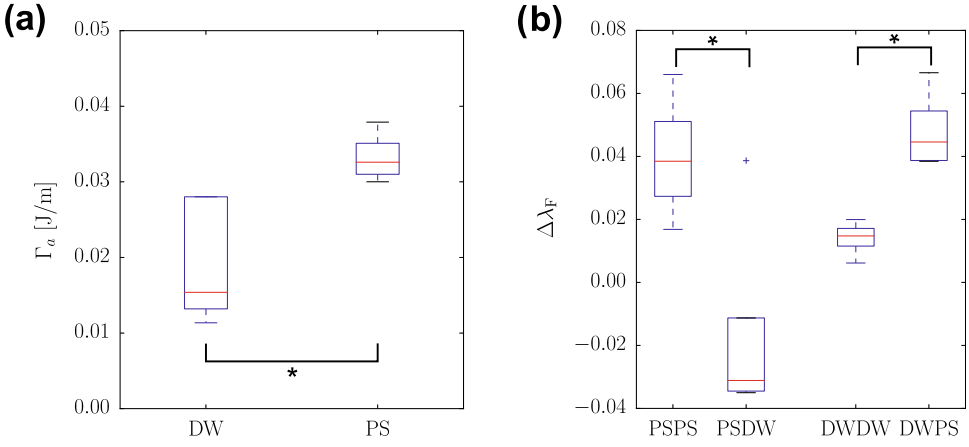

Fig. 5. **(a)** Membrane tearing energy Γ_a (initial sample length L_0 = 10 mm) of AM tested in PS (308 mosmol/L, n = 5) and DW (n = 4). **(b)** Sample-specific change in critical stretch for subsequent tests in same (control) or different bath (n = 4 each for PS-PS, PS-DW, DW-DW and DW-PS, resp.). Results are reported with boxplots and significant differences between groups are indicated with ∗ (p<0.05, Mann-Whitney U).

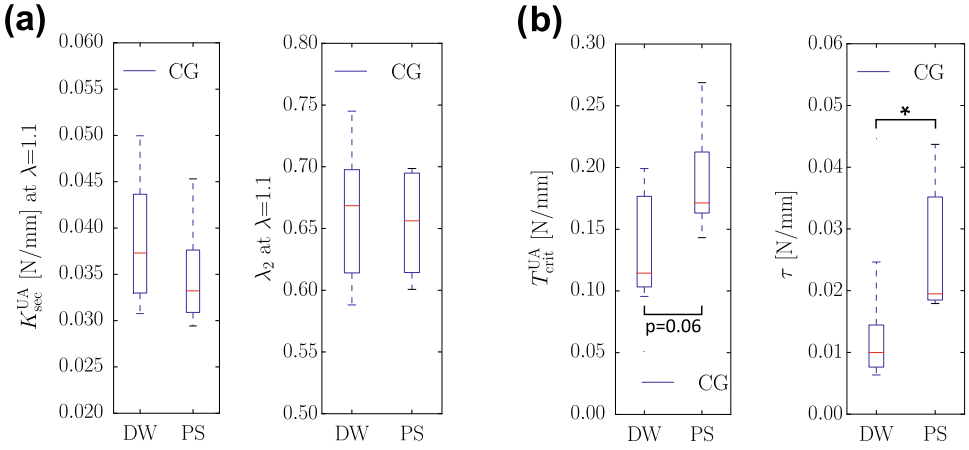

Fig. 6. **(a)** Deformation behavior in uniaxial tension (UA) tests on collagen gels (CG). Data are reported for λ = 1.1 and corresponding secant stiffness K_{sec}^{UA} and λ_2 values are shown, from tests in PS (n = 5) and DW (n = 5). **(b)** Critical tension T_{crit}^{UA} and toughness τ, i.e. the area under the tension vs. stretch curve, of UA tests of CG performed in PS and DW. Results are reported with boxplots and significant differences are indicated with ∗ (p<0.05, Mann-Whitney U).

currently reported in literature, and which are also present in clinically used amnio-infusion fluids (Gillibrand, 1969; Doran et al., 1970; Johnell and Nilsson, 1971; Andersen and Weber, 1985; Evrard et al., 1997). In contrast to the difference observed between PS and DW, such small osmolarity changes are not expected to influence the risk of PPROM since no systematic differences in

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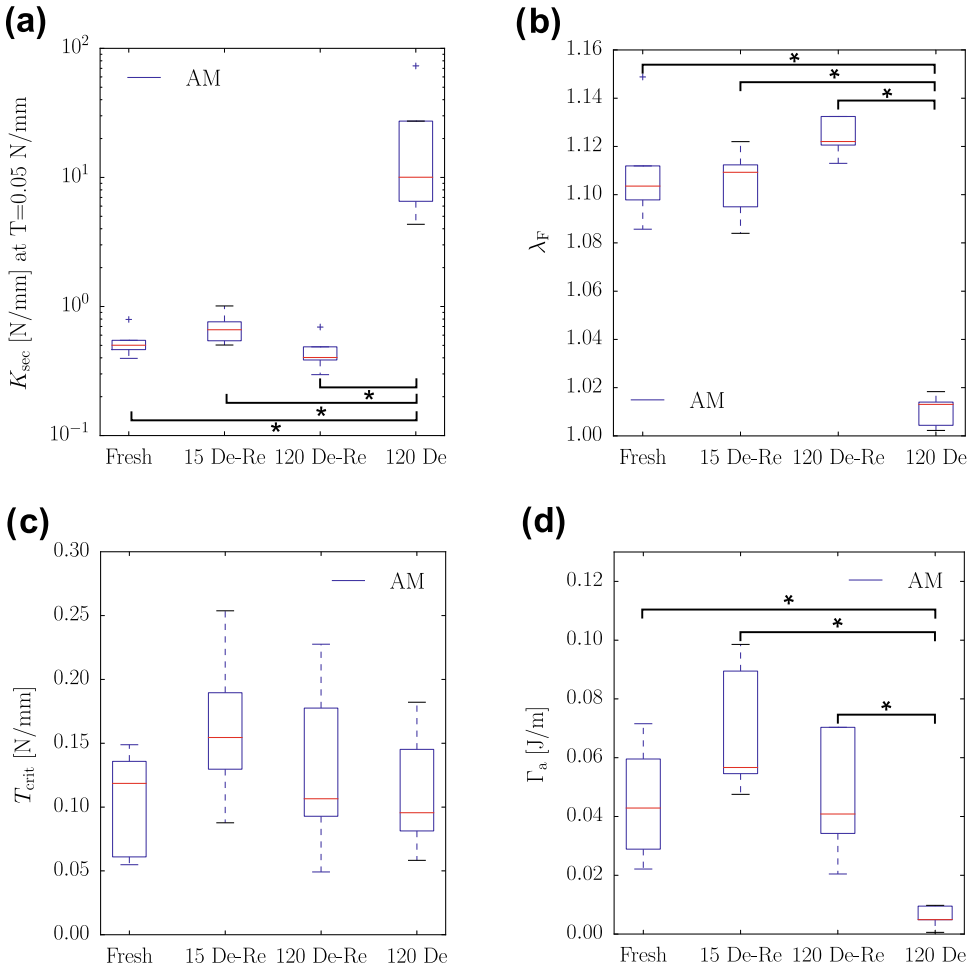

Fig. 7. **(a)** Experimental results based on the tension vs. stretch curves in mode I fracture tests investigating the influence of dehydration and rehydration. Data were analyzed in terms of the secant stiffness K_{sec} for $T = 0.05$ N/mm. **(b,c,d)** Corresponding values of critical elongation λ_F (**b**), critical tension T_{crit} **(c)** and membrane tearing energy Γ_a **(d)**. Samples were tested fresh after extraction ($n = 5$), after 15 minutes of dehydration followed by rehydration (15 De-Re, $n = 5$), after 120 minutes of dehydration followed by rehydration (120 De-Re, $n = 5$) and after 120 minutes of dehydration without rehydration (120 De, $n = 5$). Results are reported with boxplots. Significant differences between groups are indicated with $*$ ($p<0.05$, Kruskal-Wallis).

the fracture properties of AM was observed between PLS and PS (cf. Fig. 4). Conversely, a potential effect on the mechanical properties of the AM is the pH of the amniotic fluid, which was earlier named as a factor influencing the risk of PPROM (Gleeson et al., 1989; Ernest et al., 1989) and does affect the mechanical integrity of fetal membranes (Schober and Kusy, 1995). The pH of the solution used for this study was about 5.5 (Reddi, 2013), i.e. lower than the physiological value of amniotic fluid, which is around 7 (Johnell and Nilsson, 1971). Thus, while the present fracture experiments provide insights on the relative influence of bath osmolarity, the absolute values of tearing energy are expected (based on (Schober and Kusy, 1995)) to be slightly larger than for *in-vivo* conditions.

In the present experiments AM was exposed to a bath of reduced osmolarity for a relatively short time, i.e. 10 minutes. Based on the data reported in Ehret et al. (2017), this time-scale is considered sufficient to achieve the level of liquid uptake required for equilibrium in the chemical potential of the extracellular fluid, due to the small thickness of the tissue. On the other hand, the exposure might be too short for corresponding biochemical reactions at the molecular level to occur, which might influence tissue toughness on the long term. Thus, we cannot exclude that relatively small changes in osmolarity, representative of physiological conditions during gestation, might affect the tear resistance of amnion at longer time-scales. Furthermore, prolonged exposure time to changes in bath osmolarity might influence cell behavior and thus induce remodeling processes in the extracellular matrix, which might affect the tear resistance of the AM. Corresponding investigations could be performed by culturing AM samples for several days in a modified bath, similar to the approach applied in Puthiyachirakkal et al. (2013), Kumar et al. (2011), Kumar et al. (2014) and Kumar et al. (2018) for the analysis of the influence of specific enzymes and cytokines on the strength of fetal membranes. In Kumar et al. (2014) and Kumar et al. (2018) it has been shown that inflammation as well as bleeding at the maternal-fetal interface induces cells in the decidua to produce the glycoprotein GM-CSF and this is followed by enhanced proteases secretion, eventually leading to AM weakening. It might be hypothesized that the level of amniotic fluid osmolarity influences this pathway. Corresponding experiments could be performed based on the two-side multilayer (amnion +choriodecidua) tissue culturing set-up developed by J.J. Moore (Kumar et al., 2014; Kumar et al., 2016), followed by fracture experiments according to the procedure applied in the present work.

Results from Bircher et al. (2019b) indicate that the deformation behavior of the collagen network might be independent of the bath osmolarity for near-critical stretches. This might suggest that osmolarity changes influence directly the failure properties of col-

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lagen fibers. In order to investigate this hypothesis, we performed experiments on collagen hydrogels, whose deformation behavior was not dependent on bath osmolarity. The fact that their failure behavior was indeed affected by an osmolarity change (Fig. 6) points at osmolarity dependence of the fracture conditions, governed by modifications in the failure properties of collagen fibers. This observation is further in line with results reported in literature, suggesting effects of environmental conditions on collagen fiber mechanics (Andriotis et al., 2018; Grant et al., 2008; Gautieri et al., 2011). In fact, several studies indicate an increase in single fiber stiffness associated with an increased osmotic pressure (Andriotis et al., 2018; Grant et al., 2008; Gautieri et al., 2011) and attribute it to the reversible swelling of the fibers. Collagen overall possesses many acceptor and donor sites for H-bonds, and when fibers are submerged in an aqueous bath, water is incorporated into the fibrillar structure, causing an increase of mean intermolecular distance, thus explaining the swelling of fibers (Andriotis et al., 2018). Critical strain for molecular uncoiling was also observed to decrease with increasing hydration and osmotic pressures in computations, as weaker and fewer non-covalent interactions are present due to the increased distance between collagen molecules, resulting in a less constrained deformation of the molecules when tensioned (Andriotis et al., 2018). Such changes in the failure behavior of collagen fibers might be reflected in observable differences in the fracture surfaces of mode I fracture experiments, see e.g. (Comley and Fleck, 2010). Corresponding investigations should be considered for future studies.

Dehydration of 120 minutes without subsequent rehydration leads to substantial changes in the mechanical response of the AM, possibly caused by inhibited fiber kinematics, leading to significantly reduced tear resistance and critical stretches (Fig. 7). Interestingly, fibers might still possess the ability to carry load, as the critical tension is not affected by dehydration; however, the tension-stretch curve becomes almost linear and substantially stiffer (Supplementary Fig. 1). No significant influence of the dehydration time or inflation pressure was observed for the experiments with subsequent rehydration, demonstrating a fully reversible fracture behavior of AM (Fig. 7). This might indicate that the mechanical integrity of the AM is not compromised once rehydrated at the end of fetoscopic surgeries. However, perioperative AM embrittlement associated with mechanical loading from pressure fluctuations might represent concerning aspects. Operation times up to 500 minutes were reported (Menon and Richardson, 2017), and a prolonged duration was associated with a higher risk of PPROM (Skinner et al., 2018; Wilson et al., 2004). Interestingly, using warm and humidified CO_2 during fetoscopic surgeries (Skinner et al., 2018; Skinner et al., 2018), although done for metabolic reasons, was associated with reduced occurrence of PPROM (21% (Cortes et al., 2019) vs. 85–100% (Pedreira et al., 2016; Degenhardt et al., 2014)), and no significant change in apoptosis between membranes from patients that underwent fetoscopic surgery and the control groups was found. We acknowledge that the membrane loading conditions resulting from the applied insufflation pressure might differ from the *in-vivo* scenario. In fact, the effective *in-vivo* loading on the membrane is unknown, as the uterine wall and the chorion contribute to the load-bearing function and the effective stress state in AM depends on both the internal pressure and the tissue curvature. In addition, insufflation with CO_2 might cause different metabolic effects on AM as compared to air. Despite these limitations, the present results indicate that dehydration might cause enhanced brittleness, so that the tissue might experience mechanical damage. This study thus motivates future investigations analyzing the risk of AM dehydration and its contribution to PPROM.

5. Conclusion

Two factors potentially affecting AM toughness, namely altered environmental osmolarity and tissue dehydration, were analyzed based on mode I fracture experiments. The tear resistance of AM was shown to significantly depend on the osmolarity of the bath medium, as demonstrated by experiments carried out in 0.9% NaCl and distilled water. However, for sodium concentrations in the amniotic fluid within physiological ranges for the pregnancy (Gillibrand, 1969; Doran et al., 1970; Johnell and Nilsson, 1971; Andersen and Weber, 1985), this influence becomes negligible and is not expected to contribute to PPROM. Tests on collagen hydrogels indicated that the dependence of the tear resistance on the bath osmolarity might be governed by alterations in the failure properties of collagen fibers or their crosslinks.

Likewise, tissue dehydration, potentially occurring during prenatal surgeries, was shown to significantly alter the mechanical response of amnion and reduce its tear resistance and critical elongation. Interestingly, subsequent rehydration leads to a full recovery of the tear resistance. Future studies will focus on the influence of insufflation conditions (nature of gas mixture and saturation, temperature, duration, pressure fluctuation, AM tension) on the mechanical integrity of fetal membranes, with the aim to reduce the risk of PPROM associated with prenatal fetoscopic surgery.

Data availability

All relevant data are available from the authors upon request, and/or are included within the paper.

Declaration of Competing Interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jbiomech.2019.109419.

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