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Mechanical characterization of liver capsule through uniaxial quasi-static tensile tests until failure

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Accidentology data showed that liver is often injured in car crashes; three types of injuries occur: hematoma, laceration and vessel failure. This paper focuses on surface laceration, which involves liver capsule and hepatic parenchyma. Liver capsule behavior has been studied but its failure properties are still unclear, particularly on a local point of view. In the present study, tensile quasi-static tests are run on parenchyma and capsule samples until failure to characterize capsule failure. Normalized load as well as failure properties—ultimate load per width unit and ultimate strain—are determined. Digital image correlation is used to measure the full local strain field on the capsule. Mean values of failure characteristics for hepatic capsule are $47 \pm 29\%$ for the ultimate local strain and 0.3 ± 0.3 N/mm for the ultimate load per width unit. A comparison between human and porcine tissues is conducted based on Mann–Whitney statistical test; it reveals that capsule characteristics are close between these two species; however, freezing preservation significantly affects porcine capsule failure properties. Therefore using porcine instead of human tissue to determine failure characteristics of liver capsule seems satisfactory only on fresh tissues.

1. Introduction

A large number of studies have been conducted to analyse how human body gets injured in car accidents. Based on data collected in various states during various periods, they revealed that abdominal organs are involved in 3–10% of the vehicle crashes (Tinkoff et al., 2008; Welsh et al., 2007). Despite this relatively small frequency, abdominal organs account for a much larger amount of the serious injuries: in vehicle to vehicle side crashes, 49% of the AIS 3+ concern the chest and abdomen (Augenstein, 2000; Elhagediab and Rouhana, 1998) revealed that 8% of the AIS 3 injuries are abdominal injuries, this rate growing up to 16.5% for AIS 4 and 20.5% for AIS 5, with a high fatal risk. Liver is concerned by 38% of the AIS 3 to AIS 5 injuries. Therefore an important attention is paid to liver in the protection of vehicle passengers. This organ made of three main components—parenchyma, capsule and a dense vascular network—has three main types of injury: hematoma, laceration and vascular failure (Tinkoff et al.,

2008). The present study focuses on surface laceration, that involves capsule and parenchyma. The hypothesis of the authors is that this type of injury occurs because of an important tension on the liver surface during an impact. Liver is indeed full of blood under pressure, so an impact would lead to an excessive pressure under the capsule. A first step to understand this failure mechanism is to study how the capsule tears up.

Liver tissues have already been widely studied in different ways. First, impact tests on whole livers are close to the reality of crash: Yamada (1970) and Melvin et al. (1973) conducted impact tests on in-vivo rabbit and rhesus monkey livers, respectively; Sparks et al. (2006) ran impact tests on ex-vivo perfused human and porcine livers and Cheynel et al. (2006) conducted free-fall and hard deceleration tests on ex-vivo human livers. They confirmed the occurrence of surface laceration on livers during an impact. Besides, perfusing is a way to remain close to living conditions, as a living liver is pressurized with blood (around 25% of its weight); it was used in indentation tests (Kerdok et al., 2006; Jordan et al., 2009). Some studies involving various abdominal organs were specifically focused on the organ capsule: quasi-static uniaxial tension tests were conducted on capsules of porcine kidney (Farshad et al., 1999), bovine liver (Hollenstein et al., 2006) and human liver and spleen (Stingl et al., 2002). Dynamic torsional tests were conducted on bovine and porcine

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liver capsule (Valtorta and Mazza, 2005). Snedeker et al. (2005) investigated strain-rate dependent mechanical properties of human and porcine kidney capsule through quasi-static and dynamic uniaxial tension tests, while Dan (1999) conducted relaxation tests on human liver capsule, revealing its non-linear behavior. Failure properties such as ultimate stress and ultimate tensile strain were determined, but the strain measurement was only global; besides there is still a lack of information on failure properties of human liver capsule that the present study aims at filling.

Finally, testing biological tissues requires special care for their preservation and sampling. Freezing can ease these steps but its influence on the mechanical properties of the tissues is not precisely known. Some studies on ligaments (Woo et al., 1986) and porcine liver (Tamura et al., 2002) concluded that thawed tissues had the same properties as fresh tissues, while studies on rabbit tibialis anterior muscle (Van Ee et al., 1998) and bovine liver (Santago et al., 2009b) concluded that they had significantly different properties. The variability of the apparent constitution of the studied tissues and of the mechanical loading can explain these different conclusions.

A series of uniaxial tensile tests are conducted on porcine and human liver samples. The aims of this paper are the characterization of the failure of liver capsule, particularly local strain, a comparison between porcine and human tissues and an assessment of freezing influence on the mechanical behavior of hepatic tissues.

2. Methods

2.1. Preparation of the samples

Seven adult pig livers were obtained from the local butchery, within 4 or 5 days after euthanasia. Whole livers or parts of them were either stored in the freezer until the cutting phase (from 24 h to a few days, $-18\text{ }^{\circ}\text{C}$), or immediately cut into samples (Table 1).

Four livers were removed from post-mortem human subjects (PMHS) from the Department of Anatomy of University of Lyon through the French voluntary corpse

donation to science program; their age and gender are given in Table 1. The first was entirely frozen, while the three others were divided in two parts, which were, respectively, kept fresh and frozen. Parts kept fresh were tested within 5 days after death. In the following, "fresh" will refer to never frozen tissue and "frozen" to thawed tissue.

Attempts to unstick capsule from parenchyma failed on porcine liver although the authors tried methods from Snedeker et al. (2005) (cutting the capsule and getting it on a paper) and Dan (1999) (bubble of water below the capsule); mechanical characterization of the capsule was therefore carried out by using bimaterial samples made of parenchyma and capsule (Fig. 1a) (as in Chui et al., 2007) for both human and porcine livers, to ensure the similarity of the experimental procedure between species. Description of the way to use this bimaterial is given in a following part.

From frozen samples, a 5 mm-thick slice was cut using a butcher electrical slicer, parallel to the liver surface. From fresh samples, the slice was cut with a butcher knife, as regularly as possible. I-shaped samples were then cut with a scalpel (Fig. 1b) along a pattern; parenchyma was indeed too compliant to be cut with a stamp. As mechanical properties of parenchyma were proved to be transversely isotropic (Chui et al., 2007), no cutting direction was favored. Prepared samples were constituted of capsule and parenchyma.

Prior to testing, and after checking the complete defrosting of the frozen samples, the capsule face of the samples was covered with a random pattern of white dots made of matt paint with a toothbrush. This pattern will be used to perform local measurement of the strain field on the capsule using the digital image correlation (DIC) described in Jacquemoud et al. (2007). Samples were kept under moist paper to ensure their hydration, and then tested within 5 min at room temperature; this is consistent with the conclusions of Santago et al. (2009a) about bovine liver, which mechanical properties are not affected by the testing temperature between room and body temperature.

2.2. Experimental device

The two ends of the sample were clamped; sliding was prevented by using sand-paper stuck on the clamps. Clamps were set up vertically on a Deltalab[®] EM550 electromechanical testing machine. Samples were loaded with a displacement speed of 0.5 mm/s, which implied a range of longitudinal strain rate of 10^{-3} – 10^{-2} s^{-1} depending on the sample initial length, remaining in quasi-static domain. Elongation of the sample was recorded with a Visario[®] 1500 Speed Cam digital video camera.

2.3. Measurements

Clamp-to-clamp displacement and tensile load on the upper clamp were measured by using a cable actuated position sensor and a 3daN-single point load

Table 1
Subjects description and number of samples in each liver.

Origin	Subject number	Fresh samples	Frozen samples
Porcine	1	2	2
Porcine	2	5	3
Porcine	3	8	5
Porcine	4	5	7
Porcine	5	4	0
Porcine	6	0	5
Porcine	7	0	3
Human (91 years old, male, no illness)	159	0	8
Human (86 years old, male, no illness)	240	3	5
Human (64 years old, female, no illness)	140	5	4
Human (85 years old, female, no illness)	229	5	5

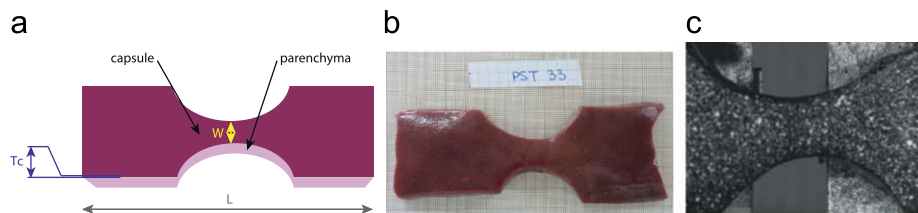


Fig. 1. Sample for tensile tests: (a) description: L =length; T_c =capsule thickness; W =sample minimum width, (b) liver sample, (c) sample with random pattern fixed onto the machine.

cell. The data acquisition frequency was 100 Hz. The digital high-speed video camera positioned perpendicularly to the sample, recorded the tensile test, so as to follow the random pattern and to measure the current dimensions of the sample at any time, with an accuracy of ± 0.3 mm. The picture acquisition frequency was 10 per second.

After processing the video, the DIC was applied to determine the full local strain field on liver capsule, thanks to the Icasoft[®] software (Mguil-Touchal et al., 1997). Local strains were computed at the center of patterns of 9×9 pixel² corresponding to 1.2×1.2 mm² areas with an accuracy of 1/100 pixel. Contrary to a global measurement of strain, the DIC allows the determination of the full strain field with no contact with the sample, and for the displacement really applied to the sample ; it can be different from the displacement applied by the machine to the clamps, due to possible sliding.

Due to the softness of the tissue, the initial state of the sample must be stated during post-processing; the correlation can be conducted if the tissue is slightly stretched on the reference picture. The initial state was therefore set at a load of 0.2 N. This load was higher than the sample weight (4–11 g), so the initial state of compression mentioned in Gao and Desai (2010) is overcome by straightening the sample.

The average strain is computed on the area where failure occurs, called area of interest (cf first picture on Fig. 4), for each time step. When failure emerges, there is a localization of the strain field; local measurement of the strain is therefore more representative than global measurement to quantify the risk of failure. That is why the area of interest is limited to a small surface around failure.

Fig. 3 displays a load–displacement curve until the end of failure. It includes drops representing capsule and parenchyma failures. This failure mechanism (Fig. 2) allows to break down the load measurement into parenchyma load and capsule load. Measuring the load before and after the first drop allows to determine the ratio of load that is sustained by the capsule and by the parenchyma. The method to determine the constitutive law for liver capsule is described in Fig. 3. Green–Lagrange longitudinal strain is measured with DIC.

The “normalized load” is defined as the slope of the curve representing the load per width unit versus the longitudinal strain. Failure parameters for the capsule are the ultimate strain—picked up just around the localization area—and the ultimate load per width unit, rather than ultimate longitudinal stress, since capsule thickness is in the order of 100 μ m and its uniformity not precisely quantified. The ultimate load per width unit is calculated as the ratio of the load

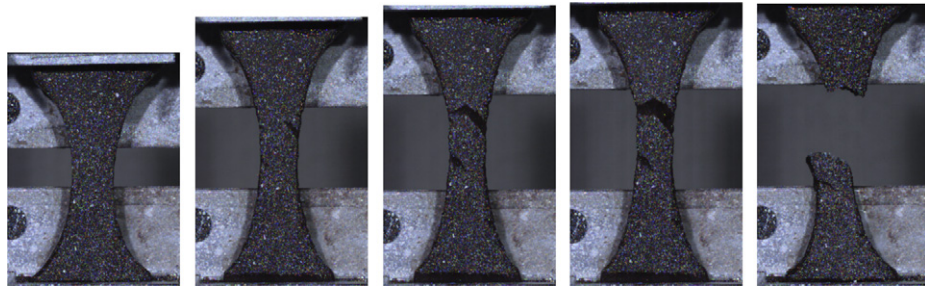


Fig. 2. Tensile test on parenchyma and capsule sample. Failure emerges on capsule first, then on parenchyma.

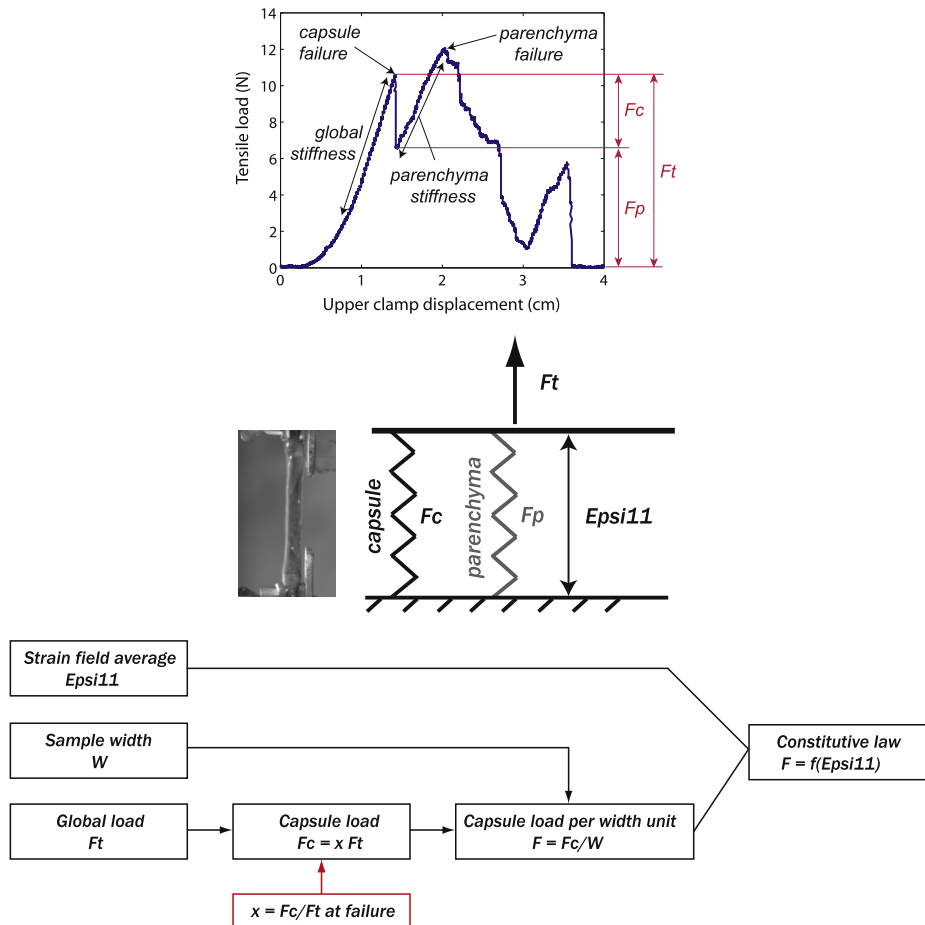


Fig. 3. Load–displacement curve and capsule constitutive law determination. The model for the bimaterial consists in two springs in parallel: once one spring (capsule) is broken, the other bears the entire tensile load. x is the ratio of global load born by the capsule before its failure; it is determined at capsule failure.

sustained by the capsule by the current width around the localization area. It is therefore a “true” load per width unit.

However, capsule thickness measurement is required to compare the results of loads to the values found in the literature, which are often given in MPa. It was conducted on histological sections sampled on one of the human and one of the porcine livers. The 23 measurements were conducted on the human liver, and 19 on the porcine liver. Then, values of true modulus and ultimate true stress are obtained by dividing, respectively, the normalized load and the ultimate load per width unit by the mean thickness of human and porcine capsule.

2.4. Statistical analysis

Statistical computing is required to know whether variables really influence mechanical properties. For small samples sizes (around 20), non-parametric Mann-Whitney test (Mann and Whitney, 1947) can be used. If $p \leq 5\%$, there

are 95% chances for a variable to be significant. The 5% is the threshold used in this paper.

The main variables studied in this paper are the biological origin (porcine or human) and the preservation method (freezing or not). Parameters are the normalized load and failure properties.

3. Results

3.1. Capsule behavior and failure properties

Calculation of the full local strain field shows that failure emerges in the region of the strain localization on the capsule (Fig. 4). Capsule behavior is displayed on Fig. 5; it becomes linear

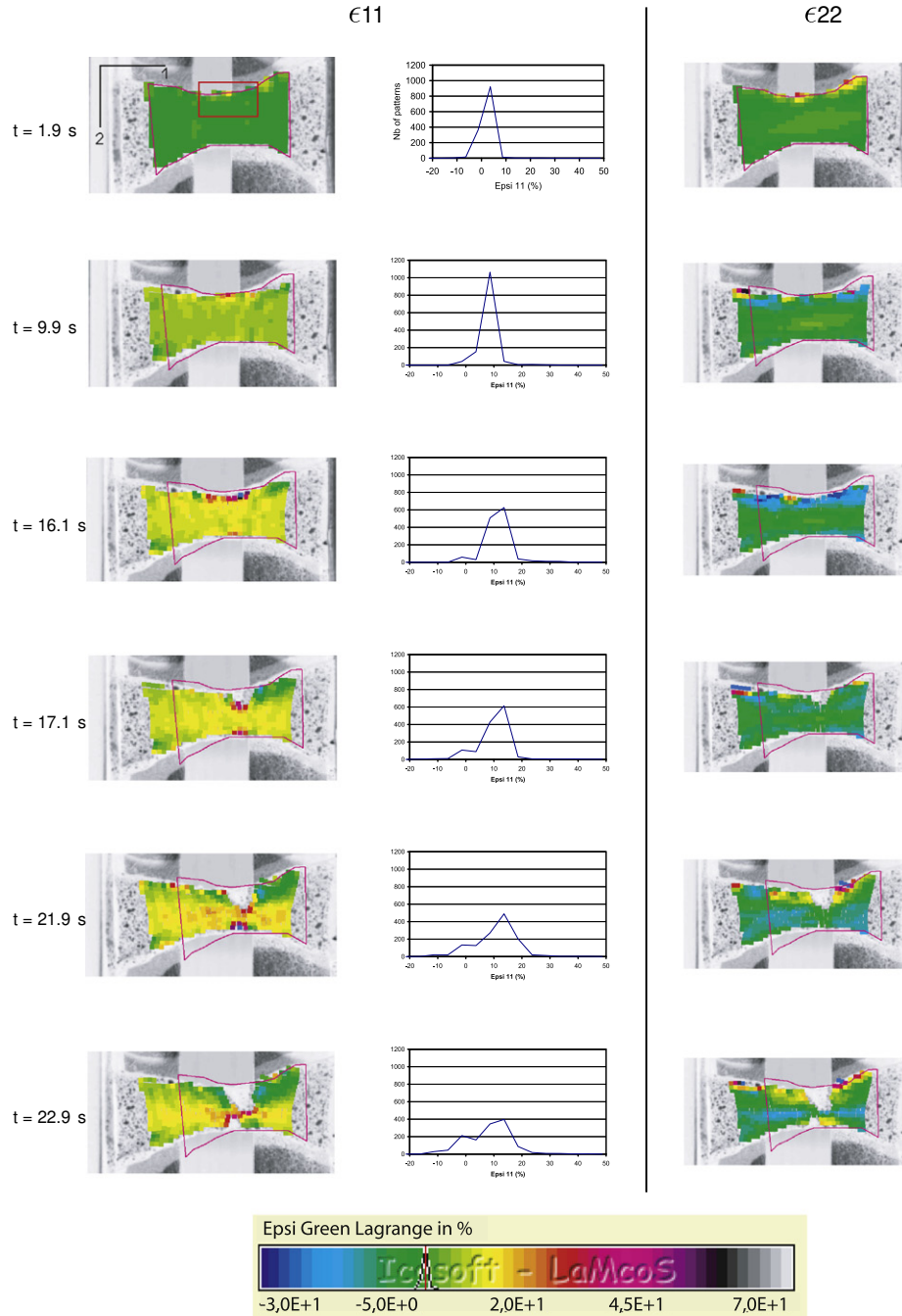


Fig. 4. Tensile test on frozen porcine tissue sample: longitudinal strain field, distribution of its values (number of patterns versus ϵ_{11} value) and transversal strain field at different instants. Failure emerges where there is a localization of ϵ_{11} , while ϵ_{22} remains small (second column). Red rectangular on the first picture represents the area of interest. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

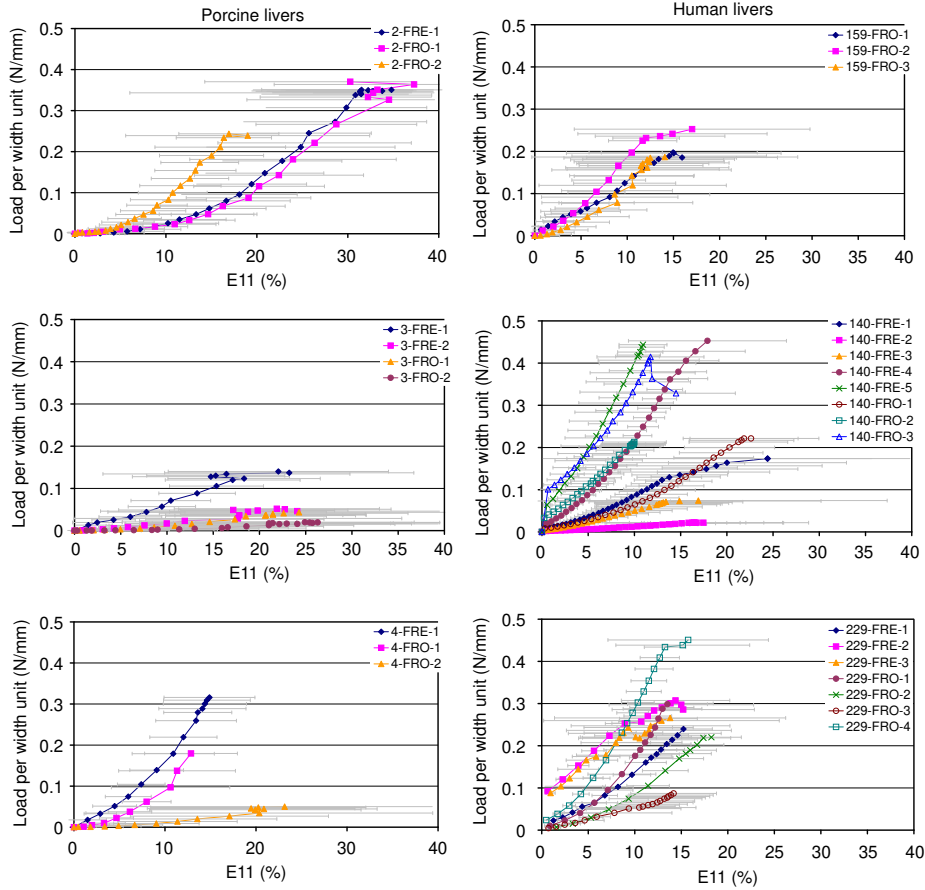


Fig. 5. Constitutive law for hepatic capsule: load per width unit versus longitudinal Green-Lagrange strain. First column: illustrations on porcine livers labeled 2, 3 and 4. Second column : illustrations on human livers labeled 159, 140 and 229. FRE=fresh, FRO=frozen. Error-bars: SD of the strain field in the area of interest.

as longitudinal strain increases. A bilinear behavior, observed in previous studies, is observed for part of the tests, but not for all, so only the linear part occurring at large strains is considered here. The normalized load of the linear part is 1.5 ± 1.9 N/mm ($n=29$) and 2.8 ± 2.3 N/mm ($n=27$) for porcine and human tissues, respectively. Error-bars represent the standard deviation of strain values in the area of interest (around failure) and show how it becomes heterogeneous when failure occurs: they indeed grow until failure.

The first histogram in Fig. 6 displays the values of local ultimate strain obtained on porcine and human capsule. The second displays values of ultimate load per width unit. Values of true modulus and ultimate true stress are obtained using human and porcine capsule mean thicknesses: $119 \mu\text{m}$ ($\pm 25 \mu\text{m}$) and $197 \mu\text{m}$ ($\pm 30 \mu\text{m}$), respectively (see Table 2).

Post-processing reveals that the initial state of the sample, due to a tensile load of 0.2 N, corresponds to an initial load per width unit of $3.3 \pm 1.9\%$ of the ultimate load per width unit, and a longitudinal strain of $1.3 \pm 1.2\%$ of the ultimate strain (extrapolated using the normalized load results).

3.2. Synthesis and statistical analysis

Table 2 displays the mean value and standard deviation of the mechanical parameters determined in the present study, as well as the number of samples of each type tested.

The table includes results of Mann-Whitney statistical analysis for two variables: biological origin and preservation method. Colored cells show indeed properties that are significantly influenced by one of these variables.

4. Discussion

Uniaxial tensile tests were performed to determine the failure properties of human and porcine hepatic tissue. Load per width unit and longitudinal strain on liver capsule were measured.

The values of initial load per width unit and initial strain, calculated from the initial global load on the samples, signify that the initial state of load per width unit/strain remains in a negligible range compared to the ultimate values and should not be a source of variability in the results.

Characterizing liver capsule properties was carried out by using samples composed of parenchyma and capsule. This method seems satisfactory as capsule breaks first but standard deviation on the results is quite high, which is a limitation of this study. Using the DIC allowed the determination of the ultimate local strain; it gives a higher precision than a global strain measurement as failure occurs with a strain localization. Values of normalized load found on human fresh capsule in this paper are close to the one given by Dan (1999) which is 2.13 N/mm.

Measurements of the capsule thickness were conducted to calculate its modulus and compare it to the values from the literature. They were consistent with thicknesses from Snedeker et al. (2005) on human and porcine kidney (55 ± 4 and $42 \pm 6 \mu\text{m}$, respectively) and Hollenstein et al. (2006) for bovine liver ($93 \pm 6 \mu\text{m}$).

The true modulus found in the present study is therefore 16.9 ± 9.9 and 27.5 ± 22.7 MPa for human fresh and frozen liver capsule, respectively, and 11.6 ± 19.2 and 7.8 ± 10.5 MPa for porcine fresh and frozen capsule. Part of the literature about capsule shows a bilinear behavior for the capsule and establishes

two Young's moduli (one for small strains, one for large strains), among which the second is the one to be compared to the present modulus. Snedeker et al. (2005) found a large strain-Young's

modulus of 41.5 ± 11.1 and 35.9 ± 13.3 MPa for human and porcine kidney capsule, respectively; Hollenstein et al. (2006) found a value of 38.5 ± 4.9 Mpa on bovine liver. They all are in the same order as the modulus found in this study.

Ultimate stress and ultimate strain values from the literature are displayed in Table 3. The values from this paper are in the same range. For the ultimate stress, the order of 10 found between Stingl et al. (2002) and this paper on human liver capsule cannot be explained as the experimental protocol is not described in Stingl et al. (2002). Using a local measurement for the strain implies a higher standard deviation for the ultimate strain than in previous studies, but the range of values is comparable to those found in the literature.

The main result of the statistical analysis is that the behavior and failure properties of liver capsule depend significantly on the biological origin once the capsule has been thawed. Besides, porcine capsule ultimate strain being significantly affected by freezing tends to show that porcine capsule is more damaged by freezing than human capsule. On many different soft biological tissues, the literature provides different conclusions about the influence of freezing on the mechanical properties (Van Ee et al., 1998; Woo et al., 1986). Concerning liver parenchyma, Tamura et al. (2002) and Santago et al. (2009b) have opposite results on porcine and bovine tissues, respectively. As shown in the literature, freezing may affect differently various types of tissues (muscle, ligaments, parenchyma and capsule) probably due to their very different constitutions and possibly to different mechanical loadings. In the present study, we observed that freezing can affect differently the tensile behavior of capsules from various biological origins, despite their apparently similar constitution. Further analysis of the human and porcine capsule microscopic structure could explain this disparity.

Perspectives on this work are to study hepatic parenchyma failure properties to be able to fully described liver laceration. Concerning capsule, individual testing will be possible for human liver as capsule sticks less to parenchyma on human than on porcine liver; dynamic biaxial tensile tests are closer to real loading during impact and could give further information on capsule mechanical properties and failure mechanism.

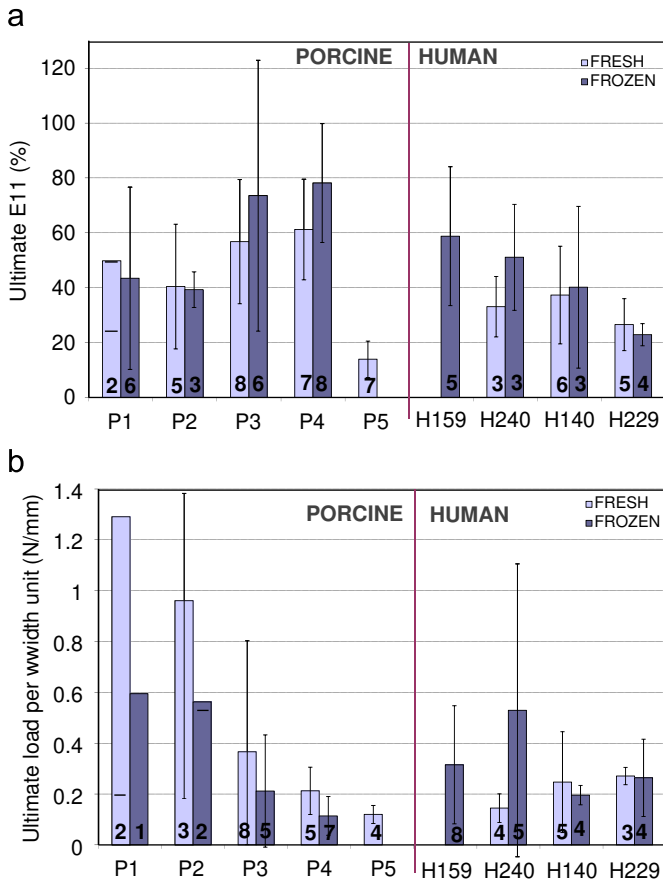


Fig. 6. Failure properties of liver capsule: (a) ultimate longitudinal strain, (b) ultimate load per width unit, P: porcine tissue; H: human tissue. Number in bar: number of samples. Error-bars: standard deviation. When there are two samples, min and max values are displayed. When there is one sample, the unique value is displayed.

Table 2

Mechanical parameters of liver capsule (value \pm SD), number of samples in each case and results of Mann-Whitney statistical test.

	Fresh	Nb	Frozen	Nb
Normalized load (N/mm)				
Human	2.02 \pm 1.18	11	3.27 \pm 2.70^A	16
Porcine	1.44 \pm 1.76	15	1.53 \pm 2.08^A	14
True modulus (MPa)				
Human	16.9 \pm 9.9	11	27.5 \pm 22.7	16
Porcine	11.6 \pm 19.2	15	7.8 \pm 10.5	14
Ultimate load per width unit (N/mm)				
Human	0.22 \pm 0.14	12	0.33 \pm 0.32^B	21
Porcine	0.40 \pm 0.48	22	0.24 \pm 0.22^B	15
Ultimate true stress (MPa)				
Human	1.85 \pm 1.18	12	2.77 \pm 2.69	21
Porcine	2.03 \pm 2.44	22	1.22 \pm 1.12	15
Ultimate strain (%)				
Human	32.6 \pm 13.8	14	43.9 \pm 24.2^D	15
Porcine	43.3 \pm 25.4^C	29	62.9 \pm 35.4^{C,D}	23

Values in Bold with the same superscript letter are significantly different with respect to the changing variable.

Table 3

Ultimate stress and ultimate longitudinal strain in the capsule—Comparison to values from the literature.

Reference	Biological origin	Organ	Ultimate stress (MPa)	Ultimate strain (%)
Farshad et al. (1999)	Pig	Kidney	6–14	18–45
Hollenstein et al. (2006)	Cow	Liver	9.2 ± 0.7	35.6 ± 5.2
Snedeker et al. (2005)	Pig	Kidney	10.9 ± 4.4	41.5 ± 7.7
	Human	Kidney	9.0 ± 2.9	33.4 ± 6.5
Stingl et al. (2002)	Human	Liver	0.203 ± 0.133	
Current study	Human	Liver	1.85 ± 1.18	32.6 ± 13.8
	Pig	Liver	2.03 ± 2.44	43.3 ± 25.4

Conflict of interest statement

All authors disclose any financial interest and personnel relationship with other people and organizations that could inappropriately influence their work on the article “Mechanical characterization of liver capsule through uniaxial quasi-static tensile tests until failure” submitted to the *Journal of Biomechanics* in November, 2009.

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