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The mechanical response of human liver and its relation to histology: An in vivo study

Edoardo Mazza [a,b,*], Alessandro Nava [a], Dieter Hahnloser [c], Wolfram Jochum [d], Michael Bajka [e]

[a] Department of Mechanical Engineering, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland
[b] Swiss Federal Laboratories for Materials Testing and Research (Empa), 8600 Dübendorf, Switzerland
[c] Department of Visceral and Transplantation Surgery, University Hospital Zurich, 8091 Zurich, Switzerland
[d] Department of Pathology, University Hospital Zurich, 8091 Zurich, Switzerland
[e] Clinic of Gynecology, Department of OB/GYN, University Hospital Zurich, 8091 Zurich, Switzerland

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Abstract

The mechanical response of human liver is characterized in vivo by means of intra-operative aspiration experiments. Mechanical characterization is combined with histological evaluation of liver tissue biopsies obtained from the resected liver at the site of mechanical testing. This procedure enables a quantitative analysis of the correlation between mechanical response and tissue micro-structure of normal and diseased liver.

Ten organs were tested in vivo at multiple locations, as well as ex vivo immediately after resection. Biopsies were analyzed in terms of pathology and percentage of connective tissue content. The change of the mechanical parameters from in vivo to ex vivo has been determined, with an increase of 17% of the proposed stiffness index. The relationship between mechanical parameters and various pathologic conditions affecting the tissue samples has been quantified, with fibrosis leading to a response up to three times stiffer as compared with normal tissue. Increased stiffness can be detected by digital palpation (increased "consistency") and may suggest the presence of a tumor. The present observations suggest that stiffness increase cannot be attributed to the tumoral tissue itself, but rather to the fibrotic stroma that often arise within or adjacent to the tumor. Variation of the mechanical parameters as a function of connective tissue content has been evaluated based on the histological examinations and the results confirm a direct proportionality between stiffness index and connective tissue percentage. The approach described here might eventually lead to a diagnostic procedure and complement other clinical methods, like palpation and ultrasound examination of the liver.

Keywords: Liver; Histology; Mechanical properties; In vivo experiment; Diagnosis

1. Introduction

The mechanical properties of human organs depend on their tissue micro-structure. Histological modifications lead to changes in the mechanical behavior of organs. Investigations of this interplay are relevant for the development of biomedical products and for medical applications. Histological and mechanical analysis have been combined in a number of recent studies to (i) investigate the mechanics of organs and their pathological states (e.g. Li et al., 2006; Kerdok et al., 2006; Sairyo et al., 2005; Wulandana and Robertson, 2005; Ghadiali et al., 2004; Holzapfel et al., 2004), (ii) model growth, re-modeling and healing processes in tissues (e.g., Weaver and Haut, 2005; Hewitt et al., 2005), and (iii) study tissue engineering (e.g., Wilshaw et al., 2006; Juncosa-Melvin et al., 2006).

* Corresponding author. Present address: Institute of Mechanical Systems, CLA J21.1, Department of Mechanical Engineering, ETH Zentrum, 8092 Zürich, Switzerland. Tel.: +41 44 6325574; fax: +41 44 6321145.
E-mail address: emazza@ethz.ch (E. Mazza).

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The present study reports experimental data on the in vivo mechanical behavior of human liver combined with the histological evaluation of biopsies obtained from the site of mechanical testing. These data allow a quantitative analysis of the relationship between mechanical response and modifications of tissues micro-structure.

One main motivation for investigating the relationship between mechanical behavior and histology is the potential improvement of diagnostic procedures. Qualitative evaluation of the mechanical properties of soft tissues through palpation is an established practice in medicine. Specifically the palpation of the liver represents a standard screening procedure for the identification of hepatic diseases. Intra-operative ultrasonography of the liver is combined with palpation to detect metastases or primary liver tumors (Knol et al., 1993). Another example of mechanical evaluation for diagnostic purposes is elastography (Manduca et al., 2001; Ophir et al., 1991). This method complements the information from ultrasound examination by providing qualitative (in some cases quantitative) data on differences in stiffness between tissues of internal organs. In this way lesions or diseases can be identified that do not possess echogenic properties. A recent study has applied dynamic magnetic resonance elastography for the detection of liver fibrosis (Huwart et al., 2006), confirming the diagnostic potential of mechanical parameters.

Evaluation of the mechanical response in terms of stiffness, viscosity, time and history dependence of the resistance to mechanical deformation might lead to more accurate tissue classification and early detection of diseases. Quantitative local measurements of mechanical properties of tissues are required to this end. The first quantitative mechanical measurements on internal human organs in vivo were performed by Carter et al. (2001) with indentation experiments on human liver. Different procedures for quasi-static in vivo tissue testing of human and animal soft organs have been recently proposed. They are based on indentation, aspiration or shear testing (see, e.g., Hendriks et al., 2003; Kalanovic et al., 2003; Kauer et al., 2002; Miller et al., 2000; Nasseri et al., 2002; Ottensmeyer, 2002; Tonuk and Silver-Thorn, 2004; Zheng and Mak, 1996).

Recently, an improved version of the so-called "aspiration device", originally developed by Vuskovic (2001), has been used for intra-operative measurements on human uterine cervix and human liver (Mazza et al., 2006; Nava et al., submitted for publication). The experimental procedure, using this aspiration device, controls the kinematic and kinetic boundary conditions so that identical aspiration experiments can be repeated for the same tissue sample or for different locations in one or several organs (Nava et al., 2004). The aspiration test allows the assessment of the mechanical response of internal organs under sterile conditions without harm to the tested tissue.

Here, we report on the combination of mechanical testing and histological analysis of normal and diseased human liver. Results are presented and discussed with respect to (i) differences between in vivo and ex vivo behavior, (ii) mechanical response for different pathologies, and (iii) dependence of the mechanical response on connective tissue content. Our results suggest that the histological analysis after quantitative mechanical testing in vivo represents a novel and useful contribution to biomechanics research. The approach described in this paper might eventually lead to the development of a diagnostic procedure, which complements clinical methods, like palpation and ultrasound examination, in the evaluation of patients with liver diseases.

2. Experimental details

2.1. Aspiration device

The working principle of the aspiration test is briefly introduced here. Further information on the device, on the design of each component, on the image analysis procedure, on the control algorithms, and on the sterilization process are reported in Nava (2007). An evaluation and discussion of the experimental uncertainties associated with the aspiration test is omitted here and can be found in Mazza et al. (2006) and Nava (2007).

The working principle of the device is illustrated in Fig. 1 and is based upon the pipette aspiration technique, Aoki et al. (1997). The device consists of a tube in which the internal pressure can be controlled according to a desired pressure law. The tube is closed on one extremity by a disc containing the circular opening for tissue aspiration. The experiment is performed by (i) gently pushing the

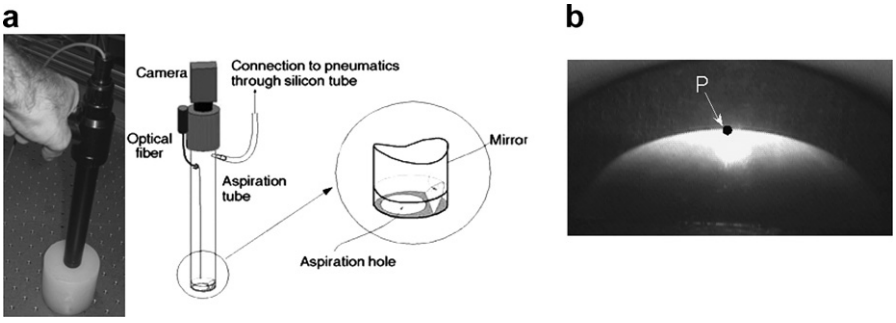

Fig. 1. (a) Picture of the aspiration device (in contact with a silicon sample) and principle of working. (b) Example of an image grabbed by the digital camera on a liver tissue, with the highest point of the profile indicated (P).

tube against the tissue to ensure a tight initial contact, and (ii) creating a (time variable) vacuum inside the tube so that the tissue is sucked through the aspiration hole (with a diameter of 10 mm), see Fig. 1.

For an isotropic and homogeneous tissue, a complete description of the tissue deformation field can be obtained by monitoring the side-view profile of the tissue during the vacuum change. An optical fiber, which is connected to an external source of light, provides the necessary illumination in the inner part of the tube. The images of the side-view are reflected by a mirror and are captured by a digital camera. The grabbed images are analyzed off-line in order to extract the profiles of the deformed tissue.

The duration of the loading and unloading cycles is about 15 s and the magnitude of the vacuum (maximum of 800 mbar absolute pressure or 200 mbar negative relative pressure, see Fig. 2) is selected in order to avoid tissue damage due to excessive deformation. Preservation of an intact capsule is particularly important in case of tumoral tissue to avoid spreading of tumoral cells. Time histories of measured pressure and deformation profiles constitute the input data used to evaluate the mechanical properties of the tissue.

2.2. Mechanical data analysis

The deformation profiles obtained from the aspiration experiments are processed to extract the displacement history of the highest point of the profile (point P, Fig. 1). Fig. 2 shows representative plots of the measured displacement history of point P. Specific points of the deformation history are identified. These are:

Point A: displacement immediately before the pressure decreases.
Point B: displacement at which minimum pressure is reached.
Point C: maximum displacement in the cycle (before pressure increases again).

The initial displacement is typically within the range of 1–2 mm, and is due to the compressive force exerted when pushing the tube against the tissue. Typically, the maximum relative displacement in each cycle (difference between the displacement at A and C, Fig. 2) is within the range of 1–2 mm.

The curve between the levels B and C is interpolated by an exponential function, with the origin shifted to location B, i.e.

$$f(t) = A_0(1 - \exp(-t/\tau)), \text{ with}$$
$$A_0 = (\text{displ. at C} - \text{displ. at B})/(1 - \exp(-t_0/\tau)),$$

where τ is the characteristic time (in s) of the exponential function (referred to as "rising time"), and t_0 is the time between the points B and C ($t_0 = 8$ s.).

In addition to the rising time τ, two additional index are introduced to characterize the displacement histories, called "stiffness" and "creep" (originally proposed in Mazza et al. (2006)), defined as follows:

$$\text{Stiffness}: \quad \eta = p_{\min}/(\text{displ. at C} - \text{displ. at A}) \ (\text{bar/mm})$$
$$\text{Creep}: \quad \delta = (\text{displ. at C} - \text{displ. at B})/(\text{displ. at B} - \text{displ. at A}).$$

where $p_{\min}$ is the lowest applied pressure in the cycle (200 mbar negative pressure).

The measured parameters η, δ and τ are analyzed, calculating mean values and standard deviations and assuming a Gaussian distribution of the data. The data collection however is non-homogeneous, with only few samples for each liver (and each type of pathology). For this reason no statistical analysis is performed. The values of standard deviation are considered as indicative measure of data variability.

The proposed parameters characterize the overall mechanical response, as measured with the aspiration device. They represent phenomenological quantities used to compare the deformation curves obtained from the different experiments. They cannot be considered as material parameters: i.e. δ and τ cannot be directly associated with the creep compliance of the material, and no elastic constant can be calculated from η. This approach enables direct characterization of the mechanical behavior of the tissue without going through computationally expensive numerical procedures for the solution of the inverse

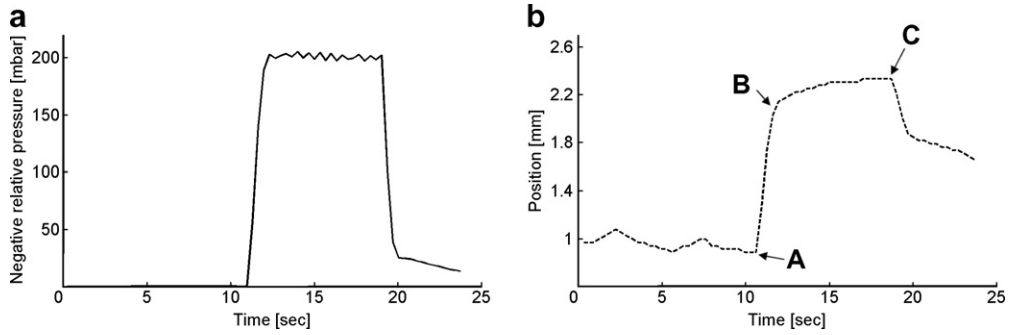

Fig. 2. (a) Applied negative pressure history. (b) Example of measured deformation history of point P (see Fig. 1). Specific locations of the deformation history are indicated.

problem, see Nava et al. (submitted for publication). These parameters can be evaluated quickly (almost ''on-line'') with possible advantages for applications for diagnosis.

2.3. Testing procedure and nomenclature

The study was approved by the ethical commission of the Canton of Zurich. Informed consent was obtained from each patient who participated in the study. The majority of tests were performed on diseased liver segments undergoing subsequent resection. Measurements were performed by the surgeon on the normally perfused liver (in vivo, Fig. 3) and on the resected specimen (ex vivo, Fig. 4) within 10 min after removal. Before the ex vivo measurements, the resected specimen was gently pressed against a surgical towel to collect blood (for further investigations) and in order to record the geometry of the specimen. For the aspiration experiments the specimen was then placed on a sterile table and held in position by hand.

In vivo and ex vivo measurements were performed at the same location of the liver surface. In case of neoplastic liver diseases, measurements were carried out on both tumor tissue and surrounding non-neoplastic liver tissue. Multiple tests were conducted at the same location in order to evaluate the variability of the results with a time interval of 20–40 s between each aspiration run. The aspiration tests did not significantly prolong the surgical procedure. Sometimes, the dimensions (small) or the shape (irregular) of the resected area were not suitable for the ex vivo experiments. A minimum size of approximately $50 \times 50 \times 50$ mm was required for testing. In fact, finite element simulations of the aspiration experiment (Nava, 2007) have shown that tissue deformation is not influenced by the boundary conditions (free or constrained) applied at a distance of approximately three times the diameter of the aspiration hole.

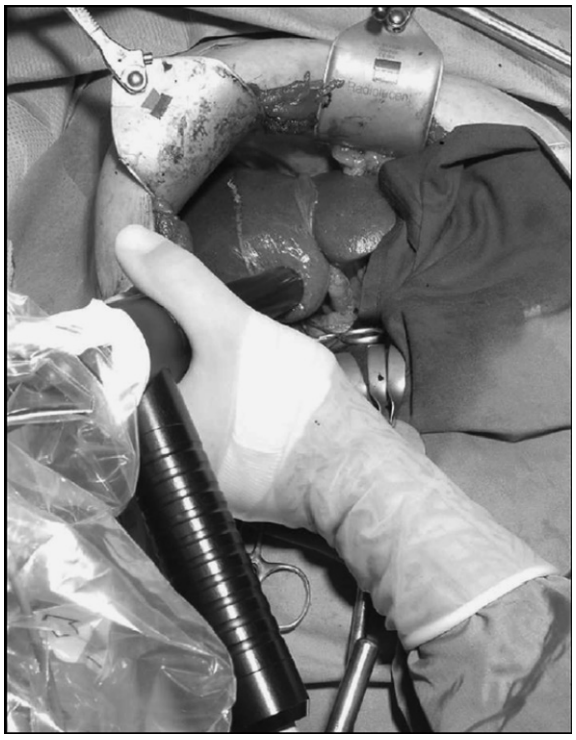

Fig. 3. In vivo aspiration experiment: the device is placed on the right lobe of the liver by the surgeon.

Fig. 4. Ex vivo aspiration experiment: the resected part of the liver is held in place by hand and the measurement is performed at the same site as in vivo.

Ten livers have been tested in the present study. Each organ is identified by letters (from A to L). For each liver usually experiments were performed at two locations, indicated by numbers. For example, sample C2, represents liver C in location 2.

In Table 1 the number of tests performed for each organ and for each location (in vivo and ex vivo) is summarized. It was not always possible (for medical reasons) to test the tissue both in vivo and ex vivo for all the organs and in the same location: (i) samples A2 and F1 were tested only in vivo because they were not in the resected portion of the organ; (ii) sample E1 was tested only in vivo because no pathology was evident and therefore no tissue was resected; (iii) samples G1 and H1 were tested only ex vivo because the surgeon preferred not to perform measurements during the intervention.

All the other samples were tested both in vivo and ex vivo, the number of tests performed was determined by the time constraints, given by the surgical procedure and according to the surgeons and their assistants. The total number of experiments performed is 72: 37 in vivo and 35 ex vivo.

2.4. Biopsies, histological analysis

After ex vivo mechanical testing, liver tissue (including the adjacent organ capsule) was excised from the site of measurement using a surgical scalpel. The site of measurement was identified by the circular mark on the liver surface induced by the aspiration device. Tissues were immersion-fixed in 4% neutral-buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin

Table 1
Nomenclature and number of tests in vivo and ex vivo

Organ	A		B		C		D		E	F		G	H	I		L	
Sample nr.	A1	A2	B1	B2	C1	C2	D1	D2	E1	F1	F2	G1	H1	I1	I2	L1	L2
In vivo	2	3	4	1	3	2	2	3	3	2	2	0	0	2	2	3	3
Ex vivo	3	0	3	1	2	1	3	2	0	0	3	3	3	2	3	3	3

and eosin using standard histological techniques. Sections were analyzed for the presence of normal liver parenchyma, tumor tissue, necrosis (dead tissue), connective tissue (fibrosis) and fatty changes of liver cells (steatosis). The relative area percentage of these changes was estimated on haematoxylin and eosin stained sections. To quantify the proportion of connective tissue in the liver tissue samples, serial sections were stained with Sirius red which specifically labels collagen in the extracellular matrix of connective tissue. Stained sections were digitized and the relative area percentage staining positive for Sirius red was determined using the analySISD image analysis software (Olympus, Volketswil, Switzerland). For each tissue sample, 2–3 tissue sections were analyzed.

3. Results

3.1. Mechanical parameters

The results of the aspiration tests are presented as mean values and standard deviations of the mechanical parameters η, δ and τ. Due to the small number of data determined for each testing location, the corresponding values of standard deviation are reported as indicative measure of data variability.

Figs. 5–7 report the measured mechanical parameters for the experiments on 15 and 14 samples performed in vivo and ex vivo, respectively. The mean stiffness data (Fig. 5) differ up to one order of magnitude, with values between 0.06 bar/mm and 0.6 bar/mm. The mean values of rising time vary between 1.3 s and 3.8 s, and the creep parameter between 0.08 and 0.35. The latter two parameters show modest changes between in vivo and ex vivo. The variability is lower for the stiffness, and in particular for the η data from in vivo measurements (with standard deviation always lower than 20% of the corresponding mean value).

3.1.1. Histological analysis

Normal liver tissue was found in four samples (B1, C1, I2, L1). Two samples were composed of liver tissue with a normal architecture, but prominent fatty changes (steatosis) (A1, D2). Five samples were predominantly (>50%) formed of carcinoma tissue (D1, F2, G1, H1, L2). One

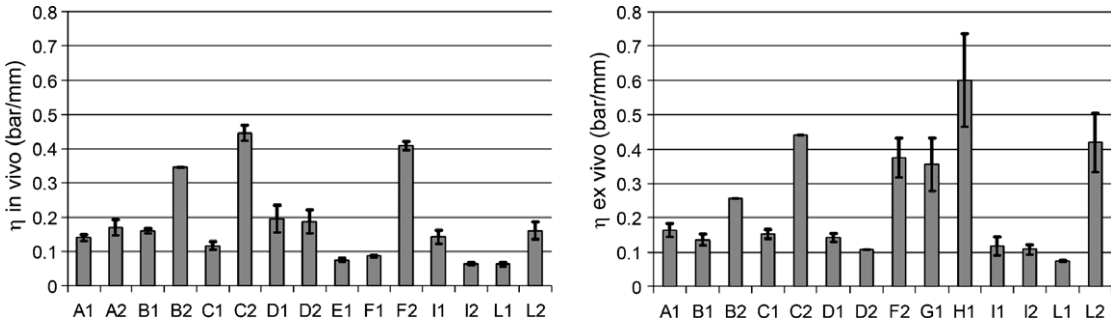

Fig. 5. Stiffness parameter η, bar/mm, measured in vivo (left) and ex vivo (right).

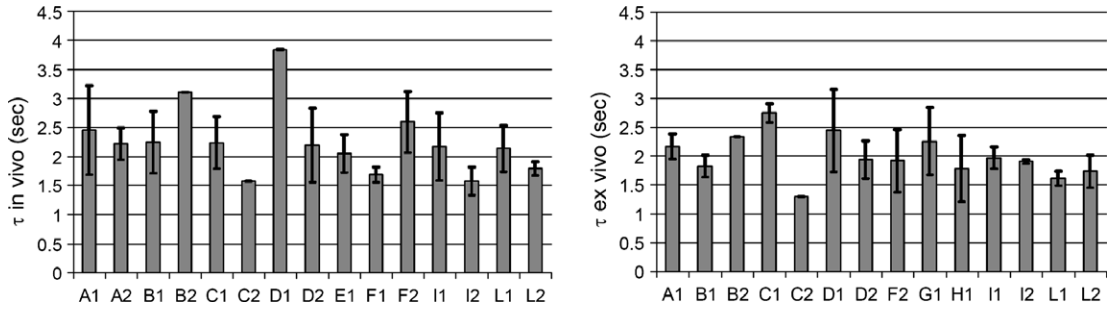

Fig. 6. Rising time τ, s, measured in vivo (left) and ex vivo (right).

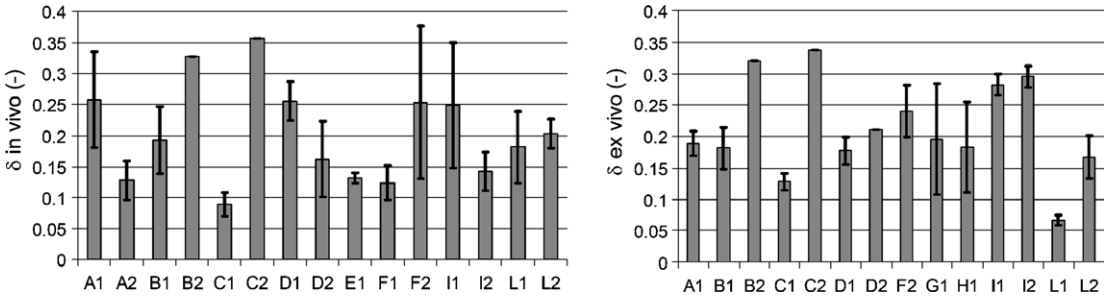

Fig. 7. Creep parameter δ (–) measured in vivo (left) and ex vivo (right).

Table 2
Pathological classification

Sample	Biopsy analysis
A1	Prominent steatosis 100%
A2	No biopsy
B1	Normal liver tissue 100%
B2	Fibrosis 70%, necrosis 20%, normal liver tissue 10%
C1	Normal liver tissue 100%
C2	Fibrosis 80%, carcinoma 20%
D1	Carcinoma 100%
D2	Prominent steatosis 100%
E1	No biopsy
F1	No biopsy
F2	Carcinoma 95%, normal liver tissue 5%
G1	Carcinoma 95%, normal liver tissue 5%
H1	Solid carcinoma 50%, fibrosis 50%
I1	Cavernous hemangioma 95%, normal liver tissue 5%
I2	Normal liver tissue 100%
L1	Normal liver tissue 100%
L2	Carcinoma 50%, normal liver tissue 50%

sample was a cavernous hemangioma (I1), and two samples were largely composed of fibrotic areas (B2, C2). For each sample, the relative percentage of each histological category was estimated on H&E stained sections (Table 2).

To more quantitatively assess the content of connective tissue in the samples, sections were also stained with Sirius Red, which specifically stains collagen, the major component of the extracellular matrix in connective tissue. The relative area occupied by connective tissue was determined using image analysis (Table 3). Fig. 8 depicts Sirius Red stained sections of samples L1 (normal liver tissue with 3.3% connective tissue on average) and F2 (carcinoma, with 17% connective tissue on average). In the normal liver tissue (Fig. 8a), connective tissue is located in the organ capsule (arrow head) and in portal tracts (asterisk). In sample F2 a large area of connective tissue was observed between the organ capsule and the carcinoma tissue (arrows, Fig. 8b).

4. Discussion

The variability of the results obtained from repeated experiments on one liver at the same location is due to uncertainties related to the aspiration test. The main source of experimental uncertainties is the unknown compressive force exerted by the surgeon during the measurements in order to ensure a good initial contact between aspiration device and liver surface. This unknown compressive force induces an initial bulge. The initial displacement is in the range of 1 mm (see Fig. 2). In Nava (2007), experiments on silicone phantoms were performed to evaluate the relationship between compressive force magnitude and maximum relative displacement in an aspiration run. From these results, it can be concluded that about 10% of the variability of the stiffness index can be attributed to the variability of the compressive force. The aspiration experiment is performed with a hand held device. This facilitates significantly (or even enables) the intra-operative application as compared with alternative set-ups (e.g. indentation) for which a mechanical fixation and positioning system is needed, and thus require additional space at the surgery table and longer time during the operation. The "price" of the simple measurement is the uncontrolled compressive force. Several "corrections" are currently evaluated for future applications of the aspiration device.

4.1. Comparison of in vivo and ex vivo measurements

Ex vivo measurements were performed immediately (<10 min) after resection. The differences in the mechanical behavior (in vivo vs. ex vivo) can thus be attributed to the change in perfusion and possibly to immediate biological

Table 3
Histological classification (connective tissue content in %)

	B1	B2	C1	C2	D1	D2	F2	G1	H1	I1	I2	L1	L2
Section 1	3.2	9.0	1.8	95	1.7	5.8	15	3.2	5.0	24	2.6	5.7	3.7
Section 2	4.2	8.5	1.1	96	1.5	6.0	18	6.0	6.6	20	1.8	0.9	4.4
Section 3	–	–	1.5	64	–	–	–	–	–	–	–	–	7.6
Average	3.7	8.7	1.5	85	1.6	5.9	17	4.6	5.8	22	2.2	3.3	5.2

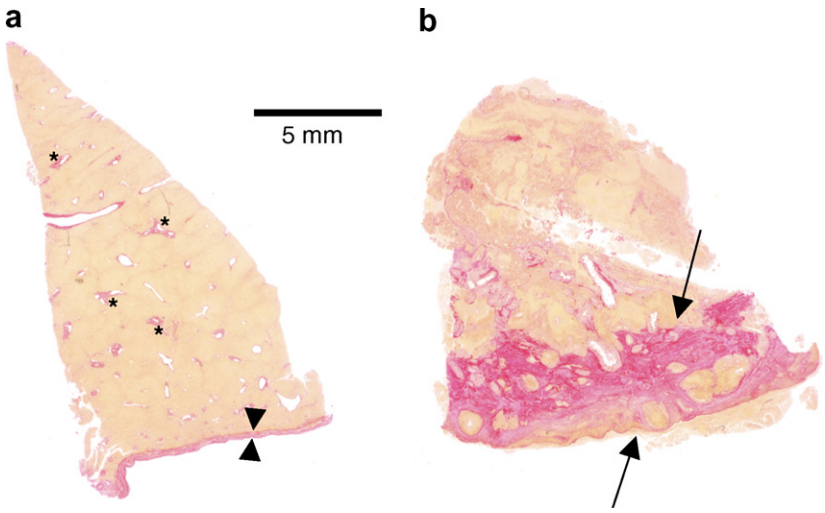

Fig. 8. Histological sections of samples L1 (a) and F2 (b) stained with Sirius Red. Collagen is stained red.

changes. Blood content and pressure are lower for the ex vivo measurements.

Comparison is made by evaluation of the mechanical parameters η, δ and τ normalized with respect to the corresponding mean in vivo value, in order to eliminate the influence of the large organ to organ variability. Only the 12 samples that were tested both in vivo and ex vivo are considered in this comparison. For each parameter the average of the normalized in vivo values is 1 (per definition).

Fig. 9 shows the Gaussian distribution of the normalized value of the stiffness parameter η for in vivo and ex vivo tests: an increase of 17% is observed from in vivo to ex vivo for the average value, with a relatively low relative standard deviation (11% and 22% for the normalized in vivo and ex vivo data, respectively).

A reduction of stiffness might be expected in the absence of blood pressure for the ex vivo tests. However, lower content of liquid phase and surface dehydration effects seem to have had a stronger influence leading to increased stiffness ex vivo. The increase of stiffness in post mortem experiments is in general agreement with the findings of other studies (Brown et al., 2003 and Kerdok et al., 2006).

In a similar clinical study with aspiration tests on the uterine cervix of post menopausal woman (Mazza et al., 2006) the relative standard deviation of the normalized stiffness parameter were larger, 19% and 27% (in vivo and ex vivo, respectively). Lower standard deviation in the present experiments might be due to the easier accessibility (smaller experimental errors) and the higher homogeneity of the liver.

Modest differences were observed between in vivo and ex vivo measurements for the rising time and the creep parameter (see Figs. 6 and 7). The corresponding evaluation for the uterine cervix showed negligible changes for the stiffness and detectable difference for creep and rising time. This is an indication of the different response of a tissue with relatively low perfusion (uterine cervix) with respect to a highly vascularized organ (liver).

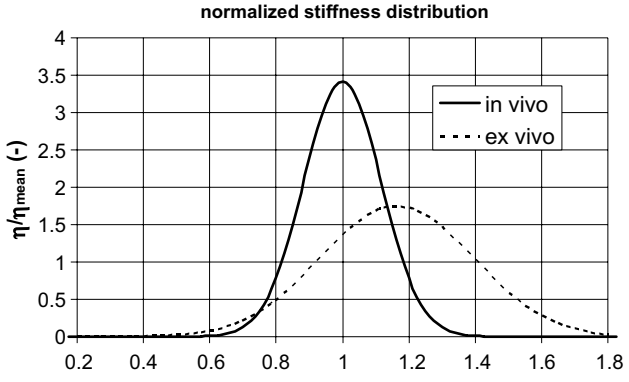

Fig. 9. Comparison of distribution of the normalized stiffness parameter from in vivo and ex vivo measurements.

4.2. Stiffness and pathology

The correlation between stiffness index and histological findings was evaluated considering in vivo and ex vivo values, thus calculating the corresponding mean values and standard deviation for each sample. Fig. 10 reports the corresponding data for all 17 samples with indication of the pathological classification with a capital letter. Specifically, according to Table 2, the following categories are indicated: N, normal parenchyma; S, steatosis; F, fibrosis; C, carcinoma; H, cavernous hemangioma (vascular tumor composed of large dilated blood vessels and containing large blood-filled spaces).

Values for steatotic and normal healthy tissue are all in the range from 0.07 to 0.18 bar/mm. The two fibrotic samples (B2 and C2) show the largest stiffness values (0.3 and 0.44 bar/mm), thus up to three times larger than normal tissue. The six biopsies with carcinoma tissue also show values larger than normal liver tissue (ranging from 0.29

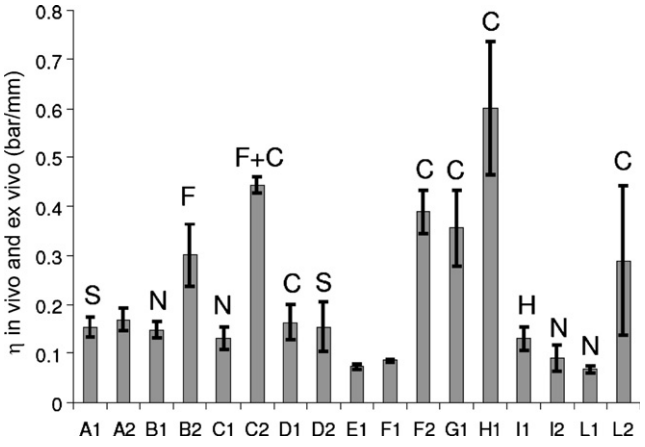

Fig. 10. Stiffness and pathology. Capital letters refer to pathologies listed in Table 2: N, normal parenchyma; S, steatosis; F, fibrosis; C, carcinoma; H, cavernous hemangioma.

to 0.6 bar/mm), except for sample D1 (0.16 bar/mm) which corresponds to the carcinoma with lowest content of connective tissue (see Table 3).

Pathologies are associated with microstructural changes that affect the parenchyma to larger extent than Glisson's capsule, and the latter significantly contributes in determining the mechanical resistance to deformation in the aspiration experiment. Increased stiffness of the parenchyma (such as in case of pronounced fibrosis) leads to a detectable increase of the stiffness index, whereas decreased stiffness of the parenchyma might be masked by the resistance of the capsule. It should be pointed out that the same problem applies for digital palpation of liver's surface.

Rising time and creep parameter are similar for normal and steatotic liver tissue and do not allow identifying sample D1 with a carcinoma free of fibrosis. Kerdok et al. (2006), reported time constants of 1.8 and 51 s for describing the time dependent response of liver in large deformation creep tests. The duration of their experiments (300 s) is much longer than for the present tests. Aspiration experiments with longer loading phase might be required in order to detect differences in the rising time parameter for different pathologies.

On the basis of the present data, no conclusions can be drawn on the detection of specific pathologies using the mechanical parameters. In fact, the number of samples is generally low and, specifically, the collection is heterogeneous (e.g. only two samples with steatosis, one single hemangioma), so that evaluation of sensitivity and specificity is omitted.

Increased stiffness might be detected by digital palpation (often referred to as increased "consistency") and is typically considered as an indication of the presence of a relevant pathology, e.g. carcinoma. The present observations suggest however that stiffness increase cannot be attributed to the tumoral tissue itself, but rather to the fibrotic stroma within or adjacent to the tumor.

The stiffness changes measured in the present study provide first quantitative data on the stiffness increase associated with the different pathologies found in the liver. A comparison can be made with the observations from dynamic elastography of the liver reported by Huwart et al. (2006) for in vivo evaluation of liver fibrosis. Good agreement is found with the reported increase of the elastic shear modulus by a factor of up to 2.5 (mean values) with respect to normal liver tissue. The reported variability of dynamic shear modulus for normal liver is in the range of ±10%, which is considerably lower when compared with the scatter of the stiffness index η among normal liver samples. Larger scatter in the present study might be attributed to three main reasons: (i) experimental uncertainties associated with the aspiration device, (ii) larger intrinsic variability of tissue response when subjected to large deformations (aspiration test) in comparison with small deformations (dynamic elastography), (iii) lower number of samples in the present study.

4.3. Stiffness and connective tissue content

Analysis of the data in Table 3 and the mechanical parameters from Figs. 5–7 suggests a correlation between connective tissue content and stiffness increase, rising time decrease and creep parameter increase.

A representation of the relationship between stiffness index and connective tissue content is shown in Fig. 11 for a sample selection. It is namely proposed to consider only samples for which in vivo measurements were performed and that correspond, according to the classification of Table 2, to either normal liver tissue (open triangles) or to samples affected by carcinoma/fibrosis (filled squares). In this way, the influence of connective tissue content can be evaluated among consistent sample families. The disadvantage, of course, is the relatively low number of data points.

Bearing this limitation in mind, the following observations can be proposed: (i) normal liver tissue contains up to about 4% connective tissue but (ii) its stiffness does not seem to be significantly dependent on connective tissue

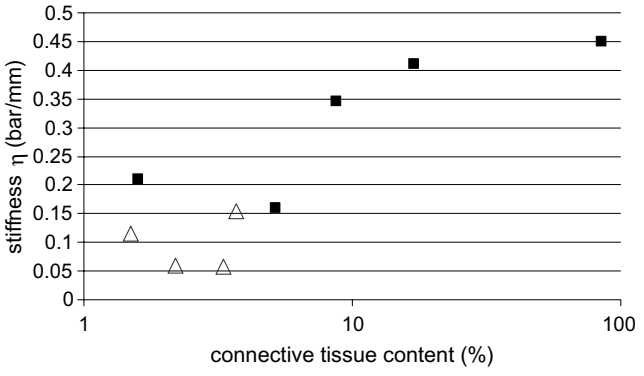

Fig. 11. Stiffness and histology. Open triangles: normal liver tissue; Filled squares: carcinoma/fibrosis.

content; (iii) connective tissue content of carcinoma/fibrosis samples varies considerably, from 2% to 85%; (iv) the corresponding stiffness values are in the range (at the upper bound) of normal samples in case of low connective tissue content; (v) the increase in stiffness is strong for connective tissue percentage, up to a factor 3 for 85% connective tissue.

The histological evaluation did not consider the inhomogeneity of the deformation in the aspiration test: the experiment generates larger deformations at the liver surface so that tissue at increasing depth contributes to a lesser extent to the mechanical response. In this sense, concentration of connective tissue at the surface is expected to lead to larger stiffness values as compared with the same amount homogeneously distributed allover the sample area. In Hollenstein et al. (2006), aspiration experiments on bovine liver with and without capsule have demonstrated the important role played by this connective tissue layer on the mechanical response measured with the aspiration experiment. These conclusions are in line with the findings of Stingl et al. (2002) and Kerdok et al. (2006).

5. Conclusions

Preliminary results of a clinical study on the mechanical response of liver tissue in vivo and its correlation with tissue histology have been presented. Measurements were performed during open surgery and the first general conclusion is that the feasibility of this approach is confirmed: (i) no problems were encountered with the sterilization or in general with the experimental procedures, (ii) the patients volunteered to participate, (iii) no damage occurred to the tissue as a consequence of the mechanical tests, (iv) the surgical procedure was not prolonged or disturbed by the aspiration experiments, (v) the scatter of the intra-operative data is in a similar range as for bench-top application of the same technique, (iv) identification of the test location and extraction of corresponding biopsies for histological examination did not pose any difficulties.

The change of the mechanical parameters from in vivo to ex vivo has been determined, with an increase of 17% of the proposed stiffness index. The dependence of mechanical parameters on different pathologies affecting the tissue samples has been quantified, with fibrosis leading to a response up to three times stiffer as compared with normal tissue. Variation of the mechanical parameters as a function of connective tissue content has been evaluated based on the histological examinations and the results confirm the expectation of a direct proportionality between stiffness index and connective tissue percentage.

Ten organs were tested, with 17 locations subjected to multiple tests, leading to 72 measurements. The data collection however is non-homogeneous, with only few samples for each type of pathology. The total number of tests is however sufficient for general considerations and preliminary observations (as proposed in the present article). An evaluation of the diagnostic relevance of the mechanical parameters as well as conclusions on the interdependence of mechanical response of the liver and its micro-structure require a larger number of samples.

Further experiments are currently ongoing so to increase the number of data points. In particular, samples that belong to the same category (e.g. steatosis, fibrosis, or carcinomas) will be evaluated and the dependence of their mechanical index on histological parameters (e.g. percentage of fat, connective tissue, or tumoral tissue) will be studied. The distribution of tissue components within the biopsies will also be assessed, according to the non-homogeneous deformation field induced by the aspiration experiment. The diagnostic relevance of the mechanical characterization with the aspiration device will be evaluated also through a comparison between the measured mechanical parameters and the response classification from digital palpation by experienced doctors.

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