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B. Confavreux

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Stéphane Nicolle, Jean-François Palierne, David Mitton, Hélène Follet, Cyrille B. Confavreux. Multi-frequency shear modulus measurements discriminate tumorous from healthy tissues. *Journal of the mechanical behavior of biomedical materials*, 2023, 140, pp.105721. 10.1016/j.jmbbm.2023.105721 . hal-03999379

**HAL Id: hal-03999379**

**<https://hal.science/hal-03999379v1>**

Submitted on 21 Feb 2023

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# Journal Pre-proof

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PII: S1751-6161(23)00074-7

DOI: <https://doi.org/10.1016/j.jmbbm.2023.105721>

Reference: JMBBM 105721

To appear in: *Journal of the Mechanical Behavior of Biomedical Materials*

Received Date: 19 July 2022

Revised Date: 7 February 2023

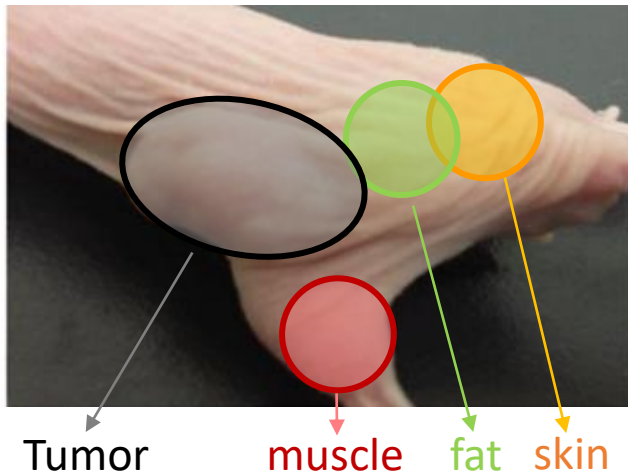
Accepted Date: 8 February 2023

Please cite this article as: Nicolle, S., Paliarne, J.-F., Mitton, D., Follet, H., Confavreux, C.B., Multi-frequency shear modulus measurements discriminate tumorous from healthy tissues, *Journal of the Mechanical Behavior of Biomedical Materials* (2023), doi: <https://doi.org/10.1016/j.jmbbm.2023.105721>.

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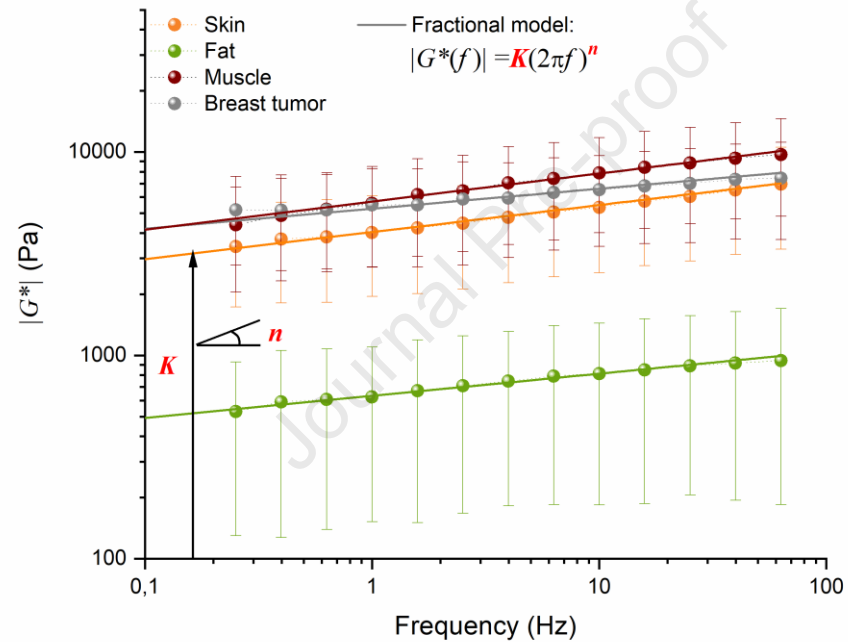
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## Mouse samples



Subcutaneously injected with  
human breast cancer cells

## Experiment and Modelling



## Analysis and Conclusions

Mann-Whitney test	$p$ -value for $K$	$p$ -value for $n$
Fat – Tumor	<b>0.00025</b>	0.43
Muscle – Tumor	0.84	<b>0.00041</b>
Skin – Tumor	0.14	<b>0.000019</b>

**Bold:** significant difference at level  $\alpha = 0.05$

$$K_{\text{fat}} \ll K_{\text{tumor}} \approx K_{\text{muscle}} \approx K_{\text{skin}}$$

→ Tumors are distinguished from fat by the **modulus magnitude**

$$n_{\text{tumor}} \approx n_{\text{fat}} < n_{\text{muscle}} \approx n_{\text{skin}}$$

→ Tumors are distinguished from muscle and skin by the **modulus frequency dependence**

1 **Multi-frequency shear modulus measurements discriminate tumorous from**  
2 **healthy tissues**

3  
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## 1 Abstract

2 As far as their mechanical properties are concerned, cancerous lesions can be confused  
3 with healthy surrounding tissues in elastography protocols if only the magnitude of moduli is  
4 considered. We show that the frequency dependence of the tissue's mechanical properties allows  
5 for discriminating the tumor from other tissues, obtaining a good contrast even when healthy and  
6 tumor tissues have shear moduli of comparable magnitude. We measured the shear modulus  
7  $G^*(\omega)$  of xenograft subcutaneous tumors developed in mice using breast human cancer cells,  
8 compared with that of fat, skin and muscle harvested from the same mice. As the absolute shear  
9 modulus  $|G^*(\omega)|$  of tumors increases by 42% (from 5.2 to 7.4 kPa) between 0.25 and 63 Hz, it  
10 varies over the same frequency range by 77% (from 0.53 to 0.94 kPa) for the fat, by 103% (from  
11 3.4 to 6.9 kPa) for the skin and by 120% (from 4.4 to 9.7 kPa) for the muscle. These measurements  
12 fit well to the fractional model  $G^*(\omega) = K(i\omega)^n$ , yielding a coefficient  $K$  and a power-law  
13 exponent  $n$  for each sample. Tumor, skin and muscle have comparable  $K$  parameter values, that of  
14 fat being significantly lower; the  $p$ -values given by a Mann-Whitney test are above 0.14 when  
15 comparing tumor, skin and muscle between themselves, but below 0.001 when comparing fat with  
16 tumor, skin or muscle. With regards the  $n$  parameter, tumor and fat are comparable, with  $p$ -values  
17 above 0.43, whereas tumor differs from both skin and muscle, with  $p$ -values below 0.001. Tumor  
18 tissues thus significantly differs from fat, skin and muscle on account of either the  $K$  or the  $n$   
19 parameter, *i.e.* of either the magnitude or the frequency-dependence of the shear modulus.

20

21 *Keywords:* shear viscoelastic properties, tumorous tissues, soft tissues, MR elastography

22

## 1 **1. Introduction**

2 Emerging imaging and computational tools in medicine require a solid knowledge of the  
3 mechanical properties of pathological and healthy tissues, key ingredients of the computational  
4 models used in clinical imaging and computer-assisted surgical techniques.

5 As a first example, finite element models are increasingly used for surgeons' training, for  
6 predicting the organ deformation during a needle biopsy or for developing non-rigid registration  
7 algorithms transforming images of an organ, taken at the time of diagnosis, to images projected  
8 during a conventional operation, robot-guided operation, or during radiotherapy (Tanner *et al.*,  
9 2002; Samani *et al.*, 2004; Griesenauer *et al.*, 2017). Approximating the tissues in these models  
10 by simple linear elastic materials is often a first approach. However, the ability of FE models to  
11 correctly deform, provide the right haptic feel, or realistically distort preoperative images into  
12 intraoperative ones critically depends on the accuracy of mechanical models of tissues going  
13 beyond linear elasticity (Samani *et al.*, 2007; Pathmanathan *et al.*, 2008).

14 As a second example, the tissue elasticity measured using ultrasound or magnetic  
15 resonance elastography holds promise as a biomarker for cancer diagnostic in deep organs, the  
16 tumor usually being stiffer than the surrounding tissues (Hoyt *et al.*, 2008a; Glaser *et al.*, 2012;  
17 Ramião *et al.*, 2016; Samani *et al.*, 2007; Wells et Liang, 2011). First thought of as the virtual  
18 counterpart of the time-honored manual palpation, elastography initially used the linear elasticity  
19 theory to reconstruct the elasticity images of soft tissues from information on propagating sound  
20 waves (Krouskop *et al.*, 1998; Sarvazyan *et al.*, 2011; Wells et Liang, 2011; Gennisson *et al.*,  
21 2013). Taking advantage of both the direct elastogram interpretation and the simpler algorithmic  
22 methods allowed by the linear elastic approach, a number of experimental works assess the Young  
23 modulus or the shear modulus (two linear elastic parameters) of soft tissues from *ex vivo* and *in*  
24 *vivo* protocols (Krouskop *et al.*, 1998; Lawrence *et al.*, 1998; McKnight *et al.*, 2002 ; Gennisson  
25 *et al.*, 2013).

26 In many situations, soft tissues are thus reduced to their isotropic linear elastic properties  
27 for computational reasons when they exhibit anisotropy and nonlinear viscoelasticity. However,

1 these last characteristics cannot always be ignored and can even be used in particular to improve  
2 the imaging tools and the interpretation of the resulting data to identify tumors.

3 For instance, in a preliminary work, Sinkus *et al.* (2000) surmised that tissue anisotropy  
4 could be used to distinguish benign from malignant tumors, the first ones being isotropic and the  
5 second ones anisotropic. However, Sinkus *et al.*, (2005) found later that some regions of healthy  
6 breast tissue can also be strongly anisotropic, making the tumor indistinguishable.

7 The large strain properties of tissues have been extensively studied as a most crucial feature  
8 to discriminate soft tissues. Generally, soft tissues stiffen as the deformation increases.  
9 Misinterpretation of results at different compression levels can thus occur when two neighboring  
10 tissues exhibit different nonlinear behaviors. In this regard, some studies provide contradictory  
11 results and opposite recommendations for the elastographic protocol: Krouskop *et al.* (1998) and  
12 Wellman *et al.* (1999) observed from compression tests on cancerous breast samples excised from  
13 patients that tumors exhibit stronger strain-nonlinearity than surrounding tissues, to the point that  
14 the tumors can be distinguished from healthy tissues at high compressive strain while they are  
15 indistinguishable from normal tissues at low compressive strain. The recommendation is then to  
16 increase the strain level during elastography to better discriminate the malignant tissues from the  
17 surrounding tissues. On the other hand, Barr and Zhang (2012), using elastography on patient's  
18 breast tissues in clinical situation, found that the contrast between Young's modulus of tumorous  
19 and normal tissues decreases with increasing strain, normal tissue stiffening more than cancerous  
20 tissue. Umemoto *et al.* (2014) reach the same conclusion through indentation tests on surgical  
21 samples of breast with cancerous lesions. These last two works then recommend that the  
22 practitioner minimizes the precompression to better distinguish tumorous tissues from the  
23 surrounding ones.

24 Viscoelastic properties of the tissues can also help discriminate benign from malignant  
25 tumors according to Sinkus *et al.* (2007) and Siegmann *et al.* (2010), the malignant ones having a  
26 more fluid behavior characterized by a higher loss (viscous) modulus than the benign ones. This

1 conclusion is however moderated by Xydeas *et al.* (2005), who stated that shear viscosity alone  
2 does not represent a good parameter for lesion classification.

3 Another feature of biological soft tissues seen as viscoelastic materials is that their modulus  
4 depends on the measurement frequency. First, together with the aforementioned large strain  
5 behavior, this could contribute to the disparity of reported ‘elastic’ moduli in the literature using  
6 elasticity instead of viscoelasticity when experiments are carried out at different frequencies.  
7 Second, the linear viscoelastic modulus of soft biological tissues generally increases with  
8 frequency, the increase rate depending on the tissue (Kiss *et al.*, 2004; Nicolle *et al.*, 2013; Yeung  
9 *et al.*, 2019). In this work, we use this frequency dependence to distinguish breast tumors from  
10 normal tissues. To this purpose, we compared the frequency-dependent behavior of tumor tissue  
11 based on human breast cancer cells developed subcutaneously in a murine model (Peyruchaud *et*  
12 *al.*, 2001; Schmidt *et al.*, 2016) together with surrounding normal tissues such as fat, skin and  
13 muscle taken from the same mice. In order to avoid the abovementioned nonlinear stiffening, the  
14 viscoelastic behavior of each tissue is probed at very small strain.

## 16 **2. Materials and Methods**

### 17 *2.1 Sample Origin*

18 Using a xenograft model (Schmidt *et al.*, 2016), tumors were resected from  
19 immunocompromised six-week-old BALB/c nude female mice (Janvier Laboratories®) following  
20 a protocol approved by the Claude Bernard Lyon I University Ethical Committee for Animal  
21 Experimentation (DR2015-39).

22 Seven mice were anesthetized using an induction box with a 1 L air/min stream, 3% isoflurane  
23 concentration, and maintained with a mask at 2% isoflurane. The mice were then subcutaneously  
24 injected with MDA-B02 human breast cancer cells (Peyruchaud *et al.*, 2001) - *B02 being cells*  
25 *isolated from human mammary carcinoma* - ( $2 \cdot 10^6$  cells in 100  $\mu$ L Phosphate Buffered Saline  
26 (PBS) solution using a 1 mL syringe, Myjector 070151, Terumo Japan). After injection, tumor  
27 development was monitored through palpation and caliper, and as soon as its diameter was close



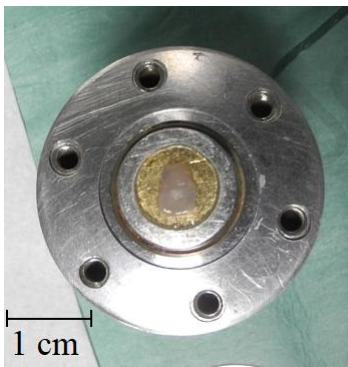
1 to 1 cm (more suitable for the subsequent mechanical test) or the surrounding tissues showed the  
 2 first signs of necrosis, the tumor was resected after having anesthetized and euthanized the mouse  
 3 via cervical dislocation. The resected tumor was then immersed in phosphate-buffered saline  
 4 (PBS) until preparation for the mechanical test performed on the same day.

5 For further comparison of mechanical properties with healthy tissues, skin, muscle and fat  
 6 tissues were removed from each mouse in addition to the tumor. Skin was harvested from the  
 7 abdomen and the legs, muscle was removed from legs and fat was removed from the abdomen.

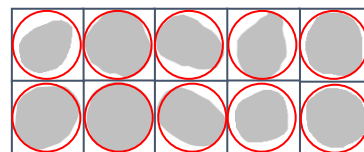
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## 9 2.2 Sample Preparation

10 Due to two tumors in an advanced state of necrosis, only five tumors (B02) out of seven  
 11 planned were usable. Slices of an average thickness of  $790 \pm 2 \mu\text{m}$  (two slices by tumor) were cut  
 12 by using a custom double-bladed scalpel as described in Nicolle *et al.* (2013). As tumors became  
 13 necrotic before reaching a size large enough to cover the 10 mm diameter plates of the rheometer  
 14 (Fig. 1), we adapted the measurement method to accommodate smaller and irregularly-shaped  
 15 samples, as described in a forthcoming section.



(a)



(b)

16 **Figure 1:** Typical sample too small to match the glass plate of the rheometer (a). Size of tumor  
 17 B02 samples relative to the size of the glass plate (b).

19

20 The muscle samples were taken from the thighs with no particular attention paid to the  
 21 direction of the fibers, although we are aware of the proven anisotropy of the muscle (Green *et al.*,  
 22 2013). The muscle slices were cut longitudinally along the femur, thus the fibers' direction lies

1 presumably in the slice plane. These samples covered the 10 mm diameter plates of the rheometer  
2 and were slightly compressed between the rheometer plates to make them flat. The final average  
3 thickness of muscle samples was  $980 \pm 160 \mu\text{m}$ .

4 Each piece of skin was first stripped from the underlying connective and adipose layers,  
5 and then cut using a punch to obtain a dermis sample of 10 mm diameter and of average  
6 thickness  $590 \pm 195 \mu\text{m}$ .

7 The fat samples were ‘fluid’ enough to fill the 10 mm diameter plates of the rheometer with  
8 about  $500 \mu\text{m}$  thickness.

9 Once prepared, each sample was immediately immersed in a saline solution and preserved  
10 at  $4^\circ\text{C}$  until the test. The number of samples tested for each type of tissue is given in Table 1.

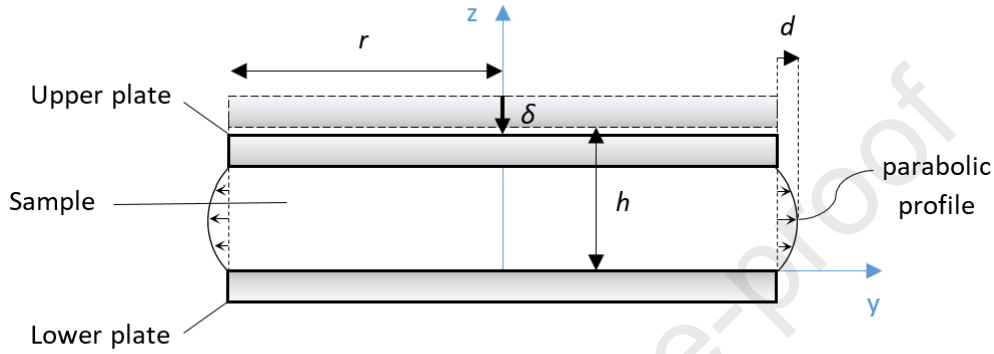
11

### 12 *2.3 Mechanical Tests*

13 The mechanical device used in this study is a custom squeezing deformation rheometer  
14 designed to determine the linear viscoelastic behavior of materials at acoustic frequencies.  
15 Samples are placed between parallel circular plates of  $r = 5 \text{ mm}$  radius separated by an adjustable  
16 gap  $h$ . In order to prevent slip, the samples were glued to the plates using cyanoacrylate adhesive  
17 (Nicolle and Paliarne, 2012). They were then surrounded with a saline solution in order to prevent  
18 dehydration of the tissue, according to Nicolle and Paliarne (2010). The entire device was finally  
19 brought to body temperature ( $37^\circ\text{C}$ ) in a hermetic enclosure to prevent thermal gradients and water  
20 losses.

21 The upper plate of the rheometer is vertically driven by a piezoelectric actuator through an  
22 oscillatory motion  $\delta^* = \delta_0 e^{i\omega t}$ , of amplitude  $\delta_0 = 2 \text{ nm}$  and angular frequency  $\omega = 2\pi f$  [our use  
23 of complex quantities follows standard practice, see Appendix]. An incompressible sample with  
24 no-slip boundary condition then undergoes a squeezing deformation with a local Poiseuille  
25 parabolic displacement profile (Fig. 2). The typical shear applied to the sample is given by the  
26 following order of magnitude considerations. The volume displaced by the motion of the plate is

1  $\propto \delta_0 r^2$  causing an outwards bulge of amplitude  $d$  and volume  $\propto hrd$  at the edge (Fig. 2) where  
 2  $r$  and  $r^2$  are the order of magnitude of the plate perimeter and area, respectively. Equating these  
 3 volumes gives  $d \sim \delta_0 r/h$ , therefore the strain magnitude is of the order of  $d/h \sim \delta_0 r/h^2$ . With  
 4 typical gap  $h = 800 \mu\text{m}$ , the strain order of magnitude is  $d/h \sim \delta_0 r/h^2 \sim 2 \times 10^{-5}$ , well within the  
 5 linear regime, ensuring that the sample is not altered by the measurement.



6  
 7 **Figure 2:** The upper plate of radius  $r$  is initially separated from the lower plate by a height  $h$ , then  
 8 it is subjected to a vertical displacement  $\delta$  which produces a parabolic-shaped bulge on the free  
 9 edge of the sample whose the maximum displacement is  $d$ .

10  
 11 The lower plate registers the oscillatory transmitted force

$$12 \quad F^* = (F' + i F'') e^{i\omega t} \quad (1)$$

13 where  $F'$  and  $F''$  are the in-phase and quadrature component of the complex force  $F^*$ ,  
 14 respectively. As derived in the Appendix, this force can be written as the product

$$15 \quad F^* = \beta G^* \delta^* \quad (2)$$

16 being separately proportional to upper plate motion  $\delta^*$ , to a shape factor  $\beta$  depending on the  
 17 sample geometry, and to the sample's frequency-dependent complex shear modulus  
 18  $G^*(\omega) = G'(\omega) + i G''(\omega)$ . The real and imaginary parts  $G'$  and  $G''$  are real numbers named the  
 19 storage and the loss modulus, respectively. The fact that  $G^*$  is complex is responsible for a phase

1 lag between the oscillating quantities  $F^*$  and  $\delta^*$ , which is a characteristic of viscoelasticity. For  
 2 circular plates of radius  $r$  separated by gap  $h \ll r$ , the shape factor assumes the analytic form  
 3  $\beta = 3\pi r^4 / 2h^3$  (Reynolds 1886, our equation (A.11) with  $A = B = r$ ). The tumor samples were  
 4 too small to fill the rheometer plates; their shapes were thus recorded and the shape factor  $\beta$  was  
 5 numerically computed for each of them according to equations (A.9)-(A.10) of Appendix, the  
 6 Poisson equation (A.9) being numerically solved using Mathematica™ (Wolfram Research, Inc.,  
 7 100 Trade Center Drive, Champaign, IL 61820-7237, USA, version 13.0). Precise shape  
 8 determination however proved not to be critical since applying formula (A.11) to the best-fitting  
 9 ellipse (having the same area and second order moments ratio as the sample shape), as given by  
 10 ImageJ (National Institute of Health, Bethesda, USA, Rasband 1997-2012), produces  $\beta$  values  
 11 within 4% of those exactly computed.

12 In short, the material viscoelastic modulus  $G^* = F^* / \beta \delta^*$  is obtained from imposing the oscillatory  
 13 displacement  $\delta^*$ , determining the form factor  $\beta$  and measuring the transmitted force  $F^*$ .

14 We fit our measurements of  $G^*(\omega)$  to the linear part of the model proposed in Kiss *et al.*  
 15 (2004) and Nicolle *et al.* (2013) for biological soft tissues, drawing from Bagley and Torvik  
 16 (1986). The frequency-dependent complex modulus  $G^*(\omega)$  is written

$$17 \quad G^* = K(i\omega)^n = G' + iG'', \quad (3)$$

$$\quad \text{with } G' = K\omega^n \cos \frac{n\pi}{2} \quad \text{and} \quad G'' = K\omega^n \sin \frac{n\pi}{2}$$

18 in terms of two parameters: the coefficient  $K$  ( $\text{Pa}\cdot\text{s}^n$ ) and the power-law exponent  $n$   
 19 (dimensionless). The absolute modulus is the positive real quantity

$$20 \quad |G^*| = \sqrt{G'^2 + G''^2} = K\omega^n \quad (4)$$

21 The dimensionless parameter  $n$  characterizes the elastic/viscous balance, with  $n=0$  for elastic  
 22 materials and  $n=1$  for Newtonian fluids. Graphically,  $n$  is the slope of  $|G^*|$  vs  $\omega$  in a log-log plot  
 23 showing as a straight line, and  $K$  is the intercept at  $\omega = 1 \text{ rad s}^{-1}$ , *i.e.*  $f = 1/2\pi \text{ Hz}$ .

1 A condition of physical admissibility is that  $n$  satisfies  $0 \leq n \leq 1$  in order to prevent divergence of  
 2 the relaxation modulus (Tschoegel 1989, Chap. 6). A characteristic of the power-law frequency  
 3 dependence is that the loss ratio

$$4 \quad \frac{G''}{G'} = \tan \frac{n\pi}{2} \quad (5)$$

5 is a function of  $n$  only, independent of frequency. This constant ratio is a strong property of power-  
 6 law models, it follows from either  $G' \propto \omega^n$  or  $G'' \propto \omega^n$ , as a consequence of causality (Tschoegl  
 7 1989, Chap. 8).

8 The material parameters ( $K, n$ ) are obtained for each sample by fitting the power-law model (3)  
 9 to the measured  $G'(\omega)$  and  $G''(\omega)$ . We used the Levenberg-Marquardt algorithm provided by  
 10 OriginPro2020™ (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA  
 11 01060, United States, version 2020) to fit simultaneously  $G'$  and  $G''$  with the same ( $K, n$ )  
 12 parameters in “multi-data fit mode” by using “statistical” weighting, inversely proportional to the  
 13 data value, thus minimizing the quantity

$$14 \quad \chi^2 = \sum_i \frac{(G'_i - K\omega_i^n \cos \frac{n\pi}{2})^2}{G'_i} + \frac{(G''_i - K\omega_i^n \sin \frac{n\pi}{2})^2}{G''_i} \quad (6)$$

15 where the sum extends to the 13 measurement frequencies  $\omega_i = 2\pi f_i$ ,  $f_i = 10^{(i-4)/5}$  Hz  
 16  $= 0.251 \dots 63.1$  Hz,  $i = 1 \dots 13$ , spanning  $2\frac{1}{2}$  decades. Such weighting increases the fit sensitivity  
 17 to  $G''$ , of comparatively smaller magnitude than  $G'$ .

18 We performed two-by-two statistical comparisons of the  $n$  and  $K$  parameters distributions  
 19 of the different tissues using a nonparametric Mann-Whitney test (provided by OriginPro2020™,  
 20 with a 0.05 significance level). The inter-sample variability of experimental data was also  
 21 examined in relation to the variability of the model parameters.

22

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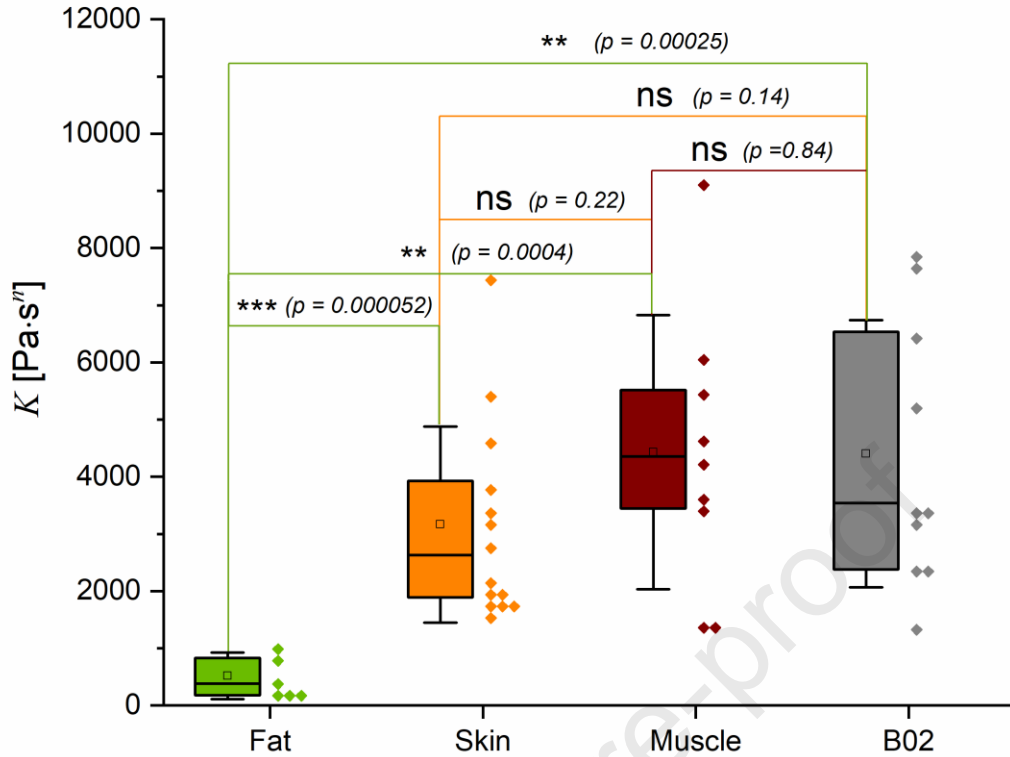
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### 1 3. Results

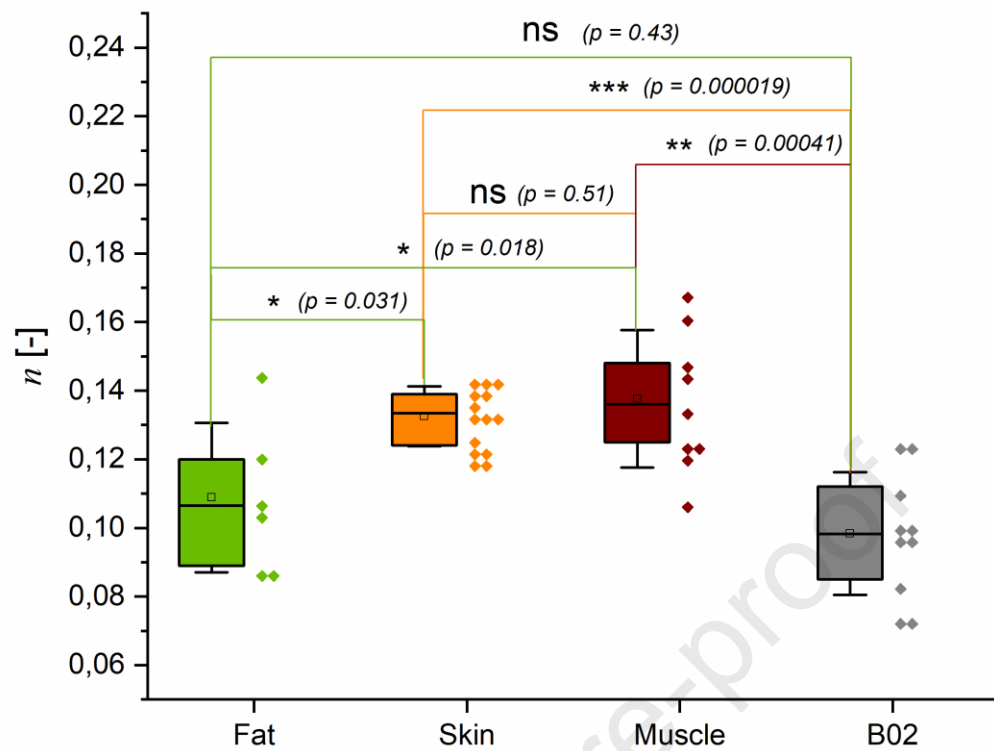
2           The power-law model (3) is fit to the measured  $G'(\omega)$  and  $G''(\omega)$  for each one of the 39  
3 samples. For the four different tissues under investigation, namely B02 tumor, muscle, skin and  
4 fat, the distribution of the fitted  $K$  and  $n$  parameters is given in Figure 3 and 4, and the average  $K$   
5 and  $n$  are reported in Table 1. The standard deviations given therein are inter-sample variabilities,  
6 whereas the fitting uncertainty for the  $K$  and  $n$  value associated with each given sample is much  
7 lower, about one tenth of the inter-sample variability. The relative inter-sample variability of  $K$  is  
8 80% for fat and 50-55% for the other tissues; these values are typical of material constants of  
9 biological tissues (Cook *et al.*, 2014). The relative inter-sample variability of the index  $n$  is  
10 comparatively smaller, being about 7% to 24%. [Strictly speaking, since the physical dimension  
11 of  $K$  is  $\text{Pa}\cdot\text{s}^n$ , in comparing different  $K$  we compare values of  $K/(1\text{Pa}\cdot\text{s}^n)$ , *i.e.* the numerical value  
12 of  $K$  in the MKS unit system.]. In addition, it can also be noted from Figure 5 that the intra-  
13 individual variability ( $K = 3600 \pm 2600$ ;  $n = 0.13 \pm 0.01$ ) represents a major contribution to the  
14 total population variability ( $K = 3160 \pm 1710$ ;  $n = 0.133 \pm 0.009$  in Table 1) for skin.

15



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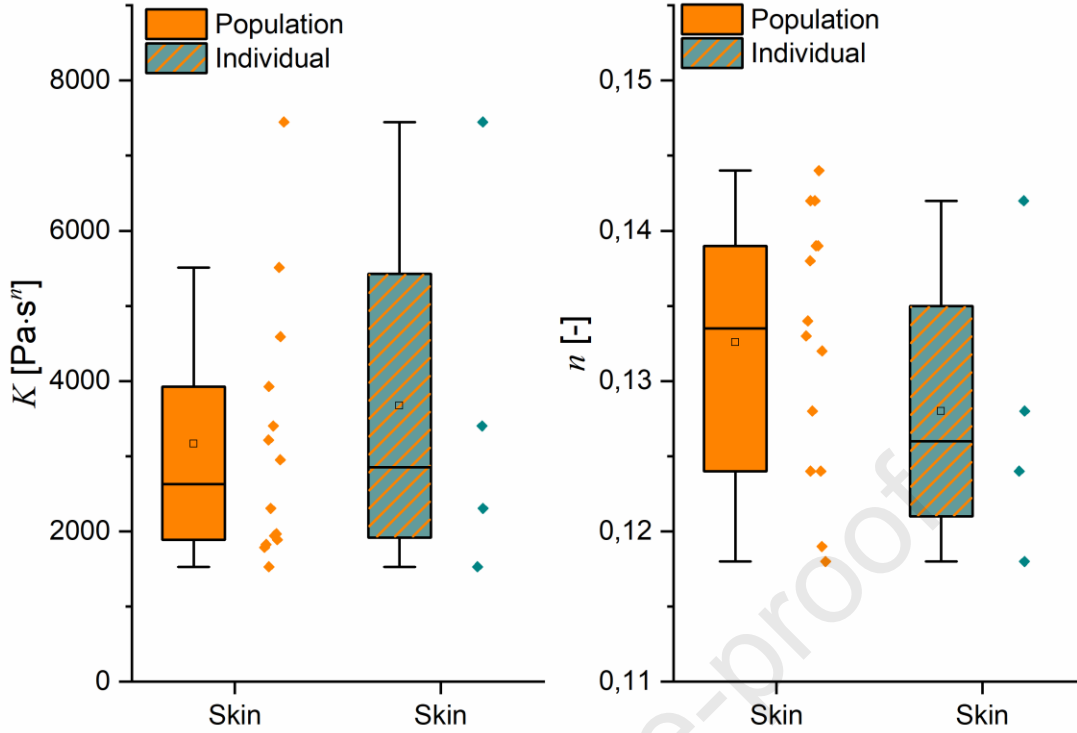
2 **Figure 3:** Distribution of the parameter  $K$  (dynamic modulus at  $\omega = 1$  rad/s, coming from the fit  
 3 of all results obtained for each tissue. The set of data (diamonds) is represented for each  
 4 distribution. Comparison of these distributions by using a nonparametric Mann-Whitney test: (ns)  
 5 non-significant, (\*) significant ( $p$ -value  $< 0.05$ ), (\*\*) very significant ( $p$ -value  $< 0.001$ ),  
 6 (\*\*\*) extremely significant ( $p$ -value  $< 0.0001$ ). B02: subcutaneous tumor (human breast cancer  
 7 cells (B02))



1

2 **Figure 4:** Distribution of the parameter  $n$  (frequency-dependence of the dynamic modulus), for all  
 3 tissue samples. Comparison of these distributions by using a nonparametric Mann-Whitney test:  
 4 (ns) nonsignificant, (\*) significant ( $p$ -value  $< 0.05$ ), (\*\*) very significant ( $p$ -value  $< 0.001$ ),  
 5 (\*\*\*) extremely significant ( $p$ -value  $< 0.0001$ ). B02: subcutaneous tumor (human breast cancer  
 6 cells (B02))





1  
2 **Figure 5:** Distribution of the model parameters  $K$  and  $n$  for the entire population of skin samples  
3 (14 samples), and for skin samples from a single individual (4 samples).

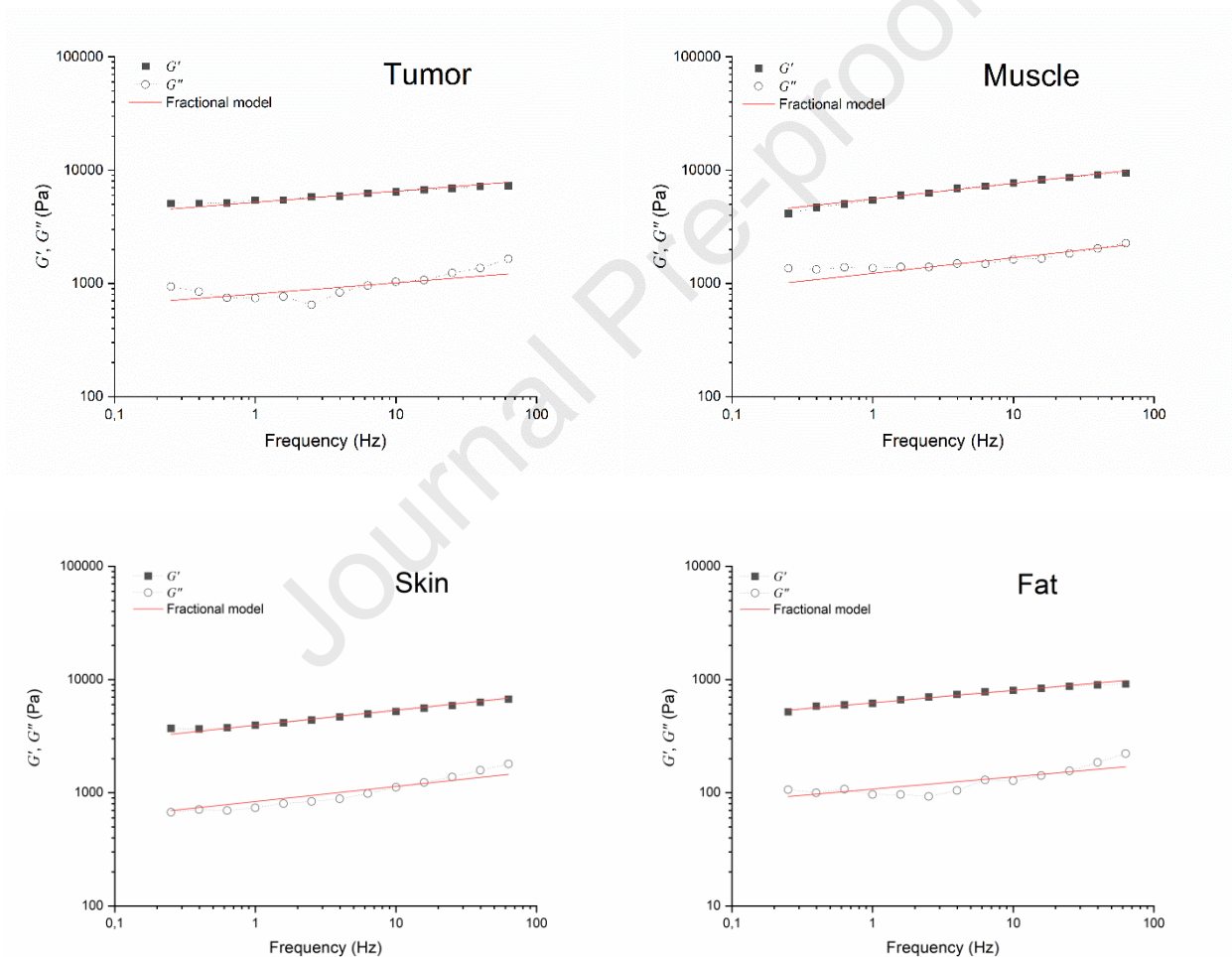
4

	#	$K$ (Pa·s <sup>n</sup> )	$n$ (-)
5 <b>B02</b>	10	4400 ± 2340	0.098 ± 0.018
6 <b>Muscle</b>	9	4430 ± 2400	0.138 ± 0.020
7 <b>Skin</b>	14	3160 ± 1710	0.133 ± 0.009
8 <b>Fat</b>	6	520 ± 400	0.109 ± 0.022

9  
10 **Table 1:** Average material parameters of the fractional model (3) for mice tissues: subcutaneous  
11 inter-sample variabilities, which are about ten times the parameters fitting precision for each given  
12 sample.

13  
14 The average moduli  $G'$  and  $G''$  of tumor, muscle, skin and fat tissue are plotted versus  
15 frequency on Figure 6, together with the model response according to equation (3) using the  
16 average material parameters given in Table 1; the coefficient of determination  $R^2$  averages to 0.95

1 for  $G'$  and 0.68 for  $G''$  [in calculating  $R^2$ ,  $G'$  and  $G''$  values are separately referred to their  
 2 respective average. Referring them to their common average would result in an unnaturally high  
 3  $R^2$ , about 0.99]. For any given sample, the measured  $G'$  and  $G''$  and their fit by equation (3) look  
 4 very similar to the averages reported in Figure 6. Although not warranted in general for non-linear  
 5 models (Robertson and Cook, 2014), the fact that our model (3), provided average  $K$  and  $n$   
 6 parameters, gives such a good fit to the average  $G'$  and  $G''$  is due to the fact that it is linear in  $K$   
 7 and that the only non-linear parameter,  $n$ , has a quite low variability, less than 2.2% of its (0...1)  
 8 admissible range, thus making the fit an almost linear fit over  $K$  only.



9

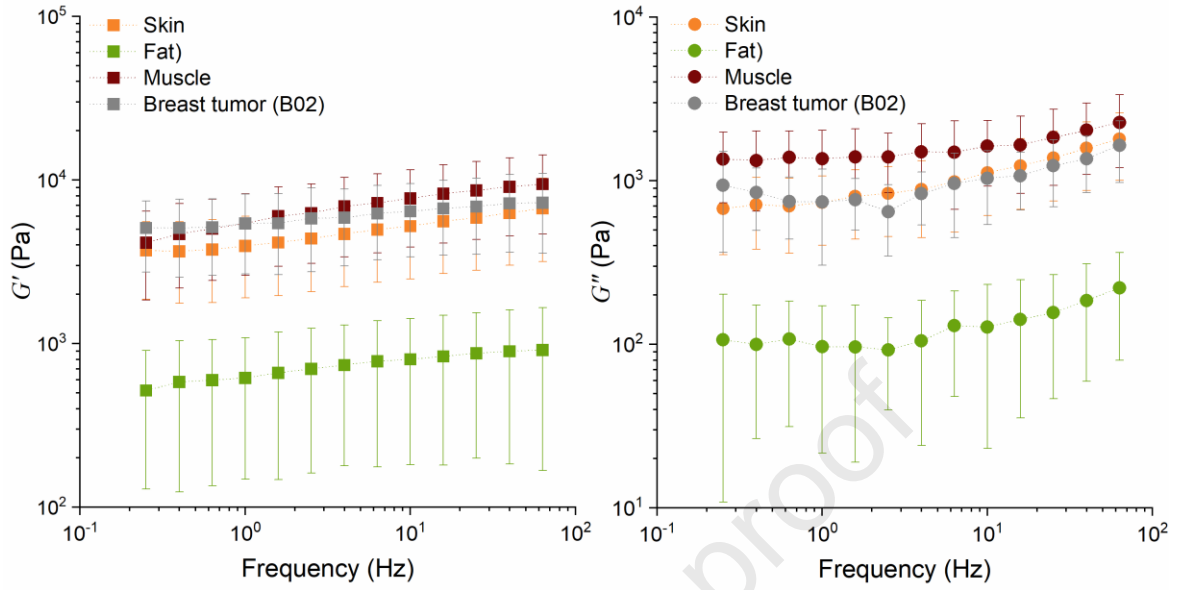
10

11 **Figure 6:** Symbols: average storage and loss modulus vs frequency of B02 tumor muscle, skin and  
 12 fat. Continuous lines: fit by model (3) using the parameters of Table 1. B02 tumor:  $R^2 = 0.864$  for  
 13  $G'$  and 0.617 for  $G''$ , Muscle:  $R^2 = 0.982$  for  $G'$  and 0.602 for  $G''$ , Skin:  $R^2 = 0.971$  for  $G'$  and  
 14 0.873 for  $G''$ , Fat:  $R^2 = 0.979$  for  $G'$  and 0.636 for  $G''$ .

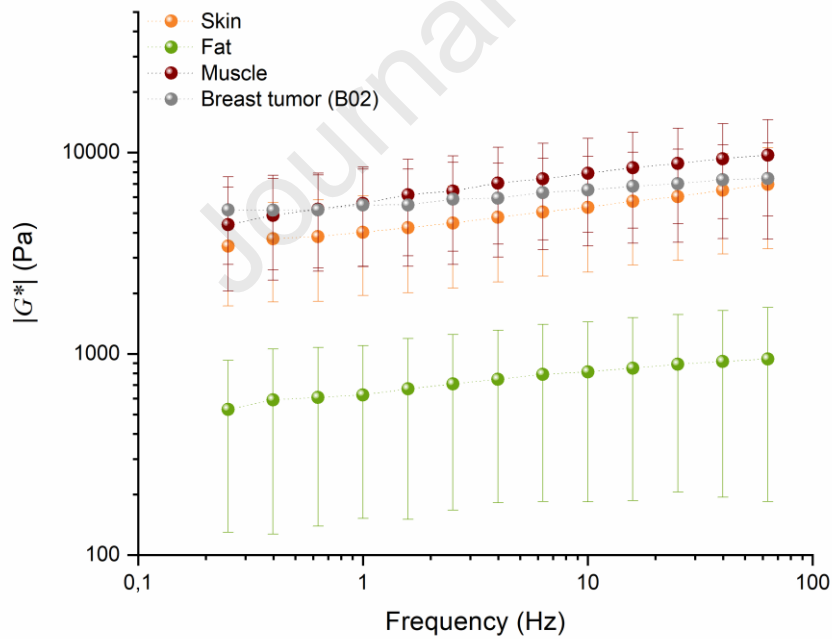
15

1 Figure 3 and Table 1 show that the parameter  $K$  is quite similarly distributed for B02 tumor,  
2 muscle and skin tissues, the only tissue with a recognizably different distribution of  $K$  being fat.  
3 A Mann-Whitney test finds significant differences only between fat and the other tested tissues, as  
4 reported on Figure 3. By contrast, as shown in Figure 4 and Table 1, the distributions of parameter  
5  $n$  reveal two different groups: the low- $n$  fat and B02 tumor tissues, with  $n$  about 0.10, and the  
6 (relatively) high- $n$  muscle and skin, with  $n$  about 0.13. The Mann-Whitney test reveals no  
7 significant difference between (high- $n$ ) muscle and skin, on the one hand, and between (low- $n$ ) fat  
8 and B02 tumor, on the other hand, whereas significant differences appear between low- $n$  and high-  
9  $n$  tissues (Figure 4).

10 The aim of this study, differentiating B02 tumorous tissue from healthy tissues, cannot be  
11 realized by just comparing the measured moduli. Figure 7a displays the average storage and loss  
12 moduli of all tested tissues: it appears clearly that, within the entire explored frequency range,  
13 tumor, skin and muscle are bunched together and can only be set apart from fat. Indeed, the inter-  
14 sample variability of  $G'$  and  $G''$  is dominated by the variability of the  $K$  parameter, of 50% to  
15 80% relative value, comparable with the spread of the average  $G'$  or  $G''$  for tumor, skin and  
16 muscle. The same issue arises when considering the absolute modulus  $|G^*|$ , which is the only  
17 quantity reported in some studies: Figure 7b shows a similar confusion of  $|G^*|$  for tumor, skin and  
18 muscle, the only tissue standing out being fat.



1 (a)



2 (b)

3 **Figure 7:** Average experimental values and respective standard deviation of (a): storage ( $G'$ ) and  
 4 loss ( $G''$ ) moduli, (b): absolute shear modulus ( $|G^*|$ )

5

1 Examining the model parameters  $K$  and  $n$  however brings a clearer perspective: the fat  
2 being singled out due to its very low  $K$ , the tumor's lower  $n$  (0.10) can be distinguished from skin  
3 and muscle's higher  $n$  (0.13). Tumor B02 ( $n = 0.098$ ) thus appears as the most elastic tissue  
4 compared to fat ( $n = 0.104$ ), skin ( $n = 0.133$ ) and muscle ( $n = 0.134$ ), its lower  $n$  value making its  
5 modulus less frequency-dependent. Over the 0.25–63 Hz frequency range we explored, the  
6 modulus of the tumor increases by 42%, from 5.2 kPa to 7.4 kPa, whereas that of fat increases by  
7 77%, from 0.53 kPa to 0.94 kPa, that of skin increases by 103%, from 3.4 kPa to 6.9 kPa, and that  
8 of muscle increases by 120%, from 4.4 kPa to 9.7 kPa.

#### 10 4. Discussion

11 New clinical tools such as elastography and computational patient-specific models require  
12 a solid knowledge of the mechanical properties of pathological and healthy soft tissues, a task  
13 made difficult by the anisotropy, the nonlinearity and the viscoelasticity of soft tissues. This  
14 explains in part the disparity of the properties reported in the literature under various experimental  
15 protocols.

16 Because soft tissues are viscoelastic, we propose that healthy and pathological tissues be  
17 distinguished not only according to their moduli but also by considering the frequency dependence  
18 of these moduli. Tissue's viscoelasticity is related to their microstructure, roughly speaking to the  
19 density and arrangement of cells, as well as the network of fibers such as collagen. Cross-links in  
20 the fiber network contribute to elasticity, while loose and dangling parts of the network add to the  
21 viscosity (Sack *et al.*, 2013). In particular, due to excessive and uncontrolled remodeling of the  
22 extracellular fiber matrix, tumor tissues are known to be stiffer and more elastic (i.e. less  
23 frequency-dependent) than normal tissues (Cox and Epler, 2011; Pepin *et al.*, 2015; Li *et al.*, 2019).

24 In this study, we observe that the shear modulus of the tumor tissue clearly differs from  
25 that of the fat, by almost an order of magnitude. It is however very close to that of the skin and  
26 muscle, making the tumor indistinguishable from such fibrous tissues. However, the modulus'  
27 frequency dependence, characterized by the fractional exponent  $n$ , is lower for the tumor ( $n =$

1 0.098) than for the skin ( $n = 0.133$ ) and the muscle ( $n = 0.138$ ), although statistically  
2 indistinguishable from that of fat ( $n = 0.109$ ). This means that the tumors appear more elastic than  
3 surrounding tissues in accordance with the abovementioned remodeling of their extracellular  
4 matrix.

5 The primary importance of the moduli's frequency dependence warrants reliable  
6 experimental determinations. The model (3) predicts that log-log plots of  $G'$  and  $G''$  versus  $\omega$   
7 are straight lines, the gap between them, ruled by (5), being univocally related to their common  
8 slope  $n$ . Mechanical measurements perform properly in this respect (our results, Kiss *et al.* (2004),  
9 Holt *et al.* (2008)). By contrast, MRE (magnetic resonance elastography) seems inclined to  
10 produce moduli  $G' \propto \omega^n$  and  $G'' \propto \omega^n$ , according to (3), with however an exponent  $n$  greater than  
11 the value  $2/\pi \arctan G''/G'$  prescribed by relation (5) (Sack *et al.*, 2009, Riek *et al.*, 2011); even  
12 greater than 1, the limit of physically possible exponents (Sinkus *et al.*, 2007, Bohte *et al.*, 2017).  
13 Papazoglou *et al.* (2012) present both mechanical and MRE data on agar-based gels mimicking  
14 biological tissues: whereas their mechanical data agree with our equations (3) and (5), their MRE  
15 data show the aforementioned tendency to higher exponents  $n$ . MRE is thus a promising technique,  
16 as it allows *in vivo* mechanical measurements, but not yet wholly reliable in the assessment of  
17 frequency dependence.

18 That the tumor is more elastic than the healthy tissues is corroborated by Sack *et al.* (2013)  
19 who observed that the exponent  $n$  slightly decreases with the fibrosis grade of liver, decreasing  
20 from 0.266 for a normal liver to 0.238 for a fibrotic liver of grade F4, in relation with the  
21 densification of the extracellular matrix occurring in tumorous tissue. This conclusion is confirmed  
22 by Sauer *et al.* (2019) who proved that the power law exponent decreases with an additional  
23 crosslinking of the collagen network composing the stromal tissue.

24 The mechanical properties found in this study are compared with those reported in the  
25 literature in Table 2. Despite differences in origin of the samples, in techniques and protocols used,  
26 the current data are found to be consistent with the values presented in this Table, which reports  
27 both *in vitro* and *in vivo* studies. The comparison *in vivo/in vitro* is never straightforward in the

1 mechanical studies since the deformation geometry is controlled *in vitro* but the physiological  
2 conditions are not strictly satisfied, causing postmortem effects (Chatelin *et al.*, 2011), while the  
3 *in vivo* conditions preclude an exact control of the deformation geometry. Nevertheless, our  
4 rheometric data converge in magnitude with those coming from MRE, which shows that the *in*  
5 *vitro* conditions applied in the current study (saline solution at body temperature for hydrating the  
6 tissue during the test, according to Nicolle and Paliarne (2010)) satisfactorily reproduce the *in vivo*  
7 conditions of MRE. This is supported by Kerdok *et al.* (2006), who found similar moduli in  
8 mechanical measurements on *in vivo* and *ex vivo* liver, perfused with a saline solution at body  
9 temperature. Contrary to their protocol, however, our tissue samples are hydrated but not  
10 pressurized. How pressurization affects the tissue viscoelasticity remains to be determined;  
11 moreover, as discussed by Kerdok *et al.*, these effects are likely organ-dependent.

12 At this stage, it can be stated that when the tumor tissues and the surrounding tissues have  
13 comparable stiffness, a multi-frequency assessment of the small-strain shear modulus is in order,  
14 to facilitate the distinction of a tumor from a healthy tissue environment, in particular in the cases  
15 of benign tumors and suspicious lesions sought using MRE protocols.

16 A concern of this study is whether the tested tumors are representative of breast cancers.  
17 Although human in origin, the tumor cells were developed subcutaneously in mice instead of  
18 human breasts. The comparison of the viscoelastic properties was made between the tumor, the  
19 skin and the muscle coming from different anatomical regions in mice, not with the surrounding  
20 tissues present in the breast, such as the adipose and the fibroglandular tissues. It would be  
21 worthwhile to validate the present observations and conclusions on pathological breast tissues  
22 resected from patients.

23

## 24 **5. Conclusions**

25 The methods developed in this study allow measuring irregularly shaped samples of  
26 centimeter size to assess their viscoelastic properties in *in vitro* conditions. By comparing  
27 subcutaneous tumor with fat, muscle and skin in a murine model, we show that the frequency

1 dependence of the tissues' viscoelastic properties allows distinguishing tumors from other soft  
2 tissues, even of comparable moduli, the tumor's modulus being less frequency-dependent than that  
3 of normal tissues. The present results thus call for a deeper investigation of the viscoelastic  
4 properties of healthy (adipose and fibroglandular tissues) and pathological breast tissues at  
5 different grades (fibroadenoma and carcinoma tissues).

6

Journal Pre-proof



<b>Skin</b>	<b>Species</b>	<b>Exp. Techniques</b>	<b><math> G^* </math> at 1 Hz</b>	<b>Exponent <math>n</math></b>		
Current Study	mouse	rheometer	4 kPa	0.133		
Nicolle <i>et al.</i> (2017)	rat	rheometer	7.3 kPa	0.137		
Lamers <i>et al.</i> (2013)	human	rheometer	2.7 kPa	-		
Rieger and Deem (1974)	human	tensile tests	-	0.173		
Holt <i>et al.</i> (2008)	human	rheometer	-	0.16		
Corr <i>et al.</i> (2009)	porcine	tensile tests	-	0.17 – 0.22		
<b>Muscle</b>						
	<b>Species</b>	<b>Exp. Techniques</b>	<b><math> G^* </math> at 1 Hz</b>	<b>Exponent <math>n</math></b>	<b><math> G^* </math> at 140 Hz/<math> G^* </math> between 200 Hz and 800 Hz</b>	
Current Study	mouse	rheometer	5.6 kPa	0.138	11.3 kPa <sup>†</sup>	11.9 to 14.5 kPa <sup>†</sup> ; $ G^*  = 4430\omega^{0.138}$
Tan <i>et al.</i> (2015)	bovine	rheometer	5.7 kPa	-	-	-
Hoyt <i>et al.</i> (2008b)	human	sonoelastography	-	-	9.95 kPa	-
Riek <i>et al.</i> (2011)	bovine	MR elastography	-	0.25	-	12.25 to 35.9 kPa <sup>†</sup> ; $ G^*  = 3640\omega^{0.25}$
<b>Fat</b>						
	<b>Species</b>	<b>Exp. Techniques</b>	<b><math> G^* </math> at 0.1 Hz</b>	<b><math> G^* </math> at 40 Hz</b>	<b><math> G^* </math> at 63 Hz</b>	
Current Study	mouse	rheometer	0.49 kPa <sup>†</sup>	0.92 kPa	0.94 kPa	
Samani <i>et al.</i> (2007)	human	indentation tests	1.1 kPa <sup>‡</sup>	-	-	
Chen <i>et al.</i> (2013)	human	MR elastography	-	0.33 kPa	-	
Lawrence <i>et al.</i> (1998)	human	MR elastography	-	-	0.43 kPa between 50 and 100 Hz	
Lorenzen <i>et al.</i> (2002)	human	MR elastography	-	-	0.5 to 4 kPa (median = 2 kPa)	

<sup>†</sup> Using our Eq. (3) with material parameters from Table (1).

<sup>‡</sup> Young's modulus / 3 (owing to incompressibility)

**Table 2:** Comparison of the current data to the literature ( $|G^*|$  is the absolute shear modulus, the exponent  $n$  characterizes the frequency-dependence of the shear modulus through the power law  $|G^*| = K\omega^n$ )

1  
2 **Appendix: deformation of a sample of arbitrary shape in the rheometer**

3 Reynolds (1886) considered the squeeze flow of a Newtonian fluid as a special case of lubricating  
4 flow; here we extend his equations (9-29) to linear viscoelasticity in harmonic regime and to  
5 arbitrary geometry. [We follow the IUPAC recommendations pertaining to linear viscoelasticity  
6 (Kaye *et al.* 2008, §5): Asterisks designate complex quantities, being either oscillating quantities,  
7 here  $\delta^*$ ,  $\mathbf{d}^*$ ,  $p^*$ ,  $\boldsymbol{\sigma}^*$  or  $F^*$ , whose real part corresponds to the physical quantity, or (non-oscillating)  
8 ratios of oscillating quantities such as  $G^*$ , assuming non-real values when there is a phase lag  
9 between these quantities.]

10 The sample is a slab of thickness  $h$  and arbitrary section  $S$ , placed between two plates, its upper  
11 and lower faces following the corresponding plate's motion without slip; the upper face thus  
12 moving by  $\delta^* = \delta_0 e^{i\omega t}$  in the perpendicular direction whereas the lower face remains fixed. Let  
13 the upper and lower plates be in the  $(x-y)$ -plane at  $z = h + \delta^*$  and  $z = 0$ , respectively. Assuming  
14  $\delta_0 \ll h$ , calculations will be carried out at linear order in  $\delta_0/h$ , within the frame of linear  
15 viscoelasticity, and, in the lubrication approximation (Reynolds 1886), at the lowest significant  
16 order in  $h/r \ll 1$  ( $r$  being a typical linear extension of surface  $S$ , its radius if  $S$  is circular).

17 The no-slip boundary conditions for the space- and time-dependent displacement field  $\mathbf{d}^*(x, y, z, t)$   
18 read

$$19 \quad \begin{cases} \mathbf{d}^* = \mathbf{0}, & z = 0 \\ \mathbf{d}^* = d_z^* \mathbf{e}_z = \delta^* \mathbf{e}_z, & z = h \end{cases} \quad (\text{A.1})$$

20 with  $\mathbf{e}_z$  the unit vector in the  $z$ -direction. The material obeys the incompressibility condition

$$21 \quad \nabla \cdot \mathbf{d}^* = 0 \quad (\text{A.2})$$

22 where  $\nabla$  is the three-dimensional gradient operator, and the dynamics equation reduces to

$$23 \quad \nabla p^* = G^* \Delta \mathbf{d}^* \quad (\text{A.3})$$

1 where  $\Delta = \nabla^2$  is the Laplacian operator, when the inertial term  $\rho \partial^2 \mathbf{d}^* / \partial t^2 = -\rho \omega^2 \mathbf{d}^*$  is  
 2 negligible, *i.e.* when  $|\rho \omega^2 \mathbf{d}^* / G^* \Delta \mathbf{d}^*| \ll \omega^2 \tau^2 \ll 1$ , where  $\tau = h \sqrt{\rho / |G^*|}$  is the characteristic time  
 3 of transverse waves [in our measurements,  $|G^*| \sim 10^3$  Pa,  $\rho \sim 10^3$  kg m<sup>-3</sup> and  $h \sim 10^{-3}$  m,  
 4 therefore  $\tau \sim 10^{-3}$  s; this sets an upper frequency limit  $f = \omega / 2\pi < 1 / 2\pi\tau = 160$  Hz ].

5 The displacement is written as the sum of a Poiseuille parabolic profile  $\mathbf{d}_\square^*$  parallel to the plates,  
 6 driven by the pressure gradient, and a  $z$ -component  $d_z^* \mathbf{e}_z$  obeying boundary conditions (A.1):

$$7 \quad \mathbf{d}^* = \mathbf{d}_\square^* + d_z^* \mathbf{e}_z, \quad \mathbf{d}_\square^* = -\frac{z(h-z)}{2G^*} \nabla_\square p^* \quad (\text{A.4})$$

8 with  $\nabla_\square = \mathbf{e}_x \partial / \partial x + \mathbf{e}_y \partial / \partial y$ . Rewriting the divergence of the displacement as  
 9  $\nabla \cdot \mathbf{d}^* = \nabla_\square \cdot \mathbf{d}_\square^* + \partial d_z^* / \partial z$ , integrating incompressibility condition (A.2) over  $z$  from 0 to  $h$  and  
 10 using boundary conditions (A.1) results in

$$11 \quad \Delta_\square p^* = \frac{12}{h^3} G^* \delta^* \quad (\text{A.5})$$

12 where  $\Delta_\square = \nabla_\square^2 = \partial^2 / \partial x^2 + \partial^2 / \partial y^2$  is the two-dimensional Laplacian. The boundary condition for  
 13 the pressure is that it vanishes at the edge of the sample, *i.e.* on the boundary  $\partial S$  of  $S$ :

$$14 \quad p^*(x, y) = 0 \quad \text{for } (x, y) \in \partial S \quad (\text{A.6})$$

15 A fundamental feature of squeezing deformations is that the typical magnitude of  $\mathbf{d}_\square^*$  is much  
 16 greater than that of  $d_z^*$ : Because the sample volume is constant, outward (resp. inward) bulging of  
 17 the sample edge occurs when the plates get closer (resp. farther away). The perimeter length is  
 18  $\sim r$ , the area of the free surface at the sample edge is thus  $\sim hr$  and the bulge volume is  $\sim hr d_\square^*$   
 19 , equal to the volume  $S \delta^*$  swept by the upper plate motion, so we have  $d_\square^* \sim \delta^* r / h$  with  $r \ll h$ .

20 Since the  $z$ -component magnitude is  $d_z^* \sim \delta^*$ , one has

$$21 \quad d_\square^* \sim \frac{r}{h} d_z^* \ll d_z^* \sim \delta^* \quad (\text{A.7})$$

1 The force on the plates obtains by integrating the surface force  
 2  $\boldsymbol{\sigma}^* \cdot \mathbf{e}_z = G^*(\nabla \mathbf{d}^* + (\nabla \mathbf{d}^*)^T) \cdot \mathbf{e}_z - p^* \mathbf{e}_z$  over  $S$  at  $z = h$ . According to (A.5), the pressure  
 3 magnitude is  $p^* \sim G^* \delta^* S / h^3$ , whereas due to condition (A.1) the only non-zero component of  
 4  $G^*(\nabla \mathbf{d}^* + (\nabla \mathbf{d}^*)^T)$  at  $z = h$  is  $G^* \partial \mathbf{d}^* / \partial z = h \nabla_{\square} p^* / 2 + G^* \mathbf{e}_z \partial d_z / \partial z$ , negligible with respect to  $p^*$   
 5 since  $h \nabla_{\square} p^* \sim h p^* / r \sim G^* \delta^* r / h^2$  and  $G^* \partial d_z / \partial z \sim G^* \delta^* / h$ . The force the material exerts on the  
 6 upper plate is then in the  $z$ -direction and reduces to

$$7 \quad F^* = - \int_S p^* dS \quad (\text{A.8})$$

8 The solution to the problem then follows: Let the function  $\phi(x, y) \geq 0$  be defined within  $S$  and on  
 9 the boundary  $\partial S$ , such that

$$10 \quad \begin{cases} \Delta \phi = -1 & \text{within } S \\ \phi = 0 & \text{on } \partial S \end{cases} \quad (\text{A.9})$$

11 then the pressure (A.5) reads  $p^* = -\frac{12}{h^3} G^* \delta^* \phi$  and the force  $F^*$  can be written  $F^* = \beta G^* \delta^*$ , where

$$12 \quad \beta = \frac{12}{h^3} \int_S \phi dS \quad (\text{A.10})$$

13 As an example, when  $S$  is an ellipse of semi-axes  $A$  and  $B$  along the  $x$ - and  $y$ -axes, one has  
 14  $\phi = (1 - x^2/A^2 - y^2/B^2) / (2/A^2 + 2/B^2)$ , therefore

$$15 \quad \beta = \frac{3\pi}{h^3} \frac{A^3 B^3}{A^2 + B^2} \quad (\text{A.11})$$

## 18 Acknowledgements

19 The authors are deeply grateful to Lamia Bouazza and Sandra Geraci for their technical supports  
 20 on cells development and animals' experiment, and with the use of the ALECS platform. This  
 21 work was partly funded by LabEx Primes (ANR-11-LABX-0063) and MSD AVENIR Research  
 22 Grant (CBC), and Philippe Clézardin (Labex DevWeCan).

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### Highlights

- The frequency dependence of moduli discriminates tumors from healthy tissues
- Tumors are more elastic than healthy tissues
- The spring-pot model describes the linear viscoelastic behavior of soft tissues
- Multi-frequency measurements could improve diagnosis by MR elastography

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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