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Bi-layer stiffness identification of soft tissues by aspiration.

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Abstract The aim of this work was to develop both experimental and inverse analyses methods of aspiration/suction measurement onto bilayered soft tissues structures.

An original aspiration system is described. The main features are volumic measurements (no camera or mirror constraining the aperture design), cyclic partial vacuum (reproducibility, airtightness checking), low deformation loading (elasticity domain, damageless for *in vivo* and *in situ* applications) and the use of disposable aspiration cups of various aperture diameters ranging between 4 and 30 mm (easy to sterilize, adaptable to accessibility constants).

The aspiration system was tested *in silico* on controlled silicone bilayer specimens (reference layers' Young Moduli characterized by tensile test, reference layers' thickness destructively measured *a posteriori*); the apparent stiffness of the bilayer structure is measured for 9 different aperture sizes.

Using the experimental apparent stiffness changes with diameters, (i) the upper and lower layer Young moduli are identified in almost real-time using

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a Finite Element database interpolated with a Principle Component Analysis. Optionally, the optimal upper layer thickness is also identified. (ii) the possible ill-posedness of the problem is analysed in terms of parameter identifiability/indifference region (prediction of experimental standard deviation).

For an upper layer thickness of about 3 *mm*, the Young moduli identified by aspiration onto a bilayer sample presented a relative error lower than 10% compared to reference values. The layer thickness was also identified with an error lower than 2%.

These validations give confidence to apply this method *in vivo* and *in situ* to soft tissues such as human skin if considered as bilayered structures.

Keywords Suction/Aspiration Method · Soft tissues Characterization · *in vivo* Measurement · Validation on silicone specimens · Inverse Finite Element Analysis · Experimental Mechanics.

1 Introduction

Since the pioneer book of Yuan-Cheng Fung published in 1981 [1], many research groups in the world have designed biomechanical models of human soft tissues for applications such as Computer Assisted Medical Interventions [2]. To be relevant, these computational models must be adapted in terms of geometrical and mechanical behavior which are unfortunately subject-specific, age dependent and which can differ significantly between *in vivo* and *ex vivo* conditions (*e.g.* vascularization of the tissue [3–5], preservation process [6], *etc.*). Moreover, biological tissues usually exhibit nonlinear, time dependent, inhomogeneous, and anisotropic behaviors. It is also well-known nowadays that such tissues develop, grow, remodel, and adapt so as to maintain particular mechanical target metrics (*e.g.*, stress).

In practice, the diversity of encountered phenomena for soft tissue modeling is simplified by choosing constitutive models and parameter identification methods. As such, most complex nonlinear stress-strain curves of human soft tissues can be identified *ex vivo* through experimental tests such as uni or bi-axial tensile tests [6–9], pure shear [9, 10], plain strain compression [9], bulge tests [11, 12], indentation [13, 14] or aspiration [15]. If such tests proved invaluable, they can usually not simply be performed *in vivo*.

These observations highlight the extensive work to be dealt with for *in vivo* characterization. A first step toward this direction is the estimation of subject-specific tissue stiffness, which is even in itself a challenge. This property is usually the prerequisite to clinical use of any model in the context of Computer Assisted Medical Interventions.

A possibility largely studied method is to use aspiration techniques [16], consisting in putting a sterile chamber with an aperture in contact with the investigated tissue and in decreasing the pressure inside the chamber. The amount of tissue aspirated is related to tissue stiffness. The aspirated tissue height is generally estimated either using ultrasound methods [17, 18], mechanical stops [19], optical coherence tomography [18] or cameras usually associated with mirrors or prisms [20–28].

To widen the use of such method, it would make sense to design the system so that the head is inexpensive, disposable, highly customizable (aperture size, shape, material) and able to meet any required severe sterilization process. Our group has thus recently proposed to replace the measurement of the tissue height with the measurement of the aspirated tissue volume [29]. Such a change in the method enables the elimination of camera, mirror, prism, and all the electronic parts from the system head that was basically reduced to a simple tube with an aperture. The corresponding device, called VLASTIC, enable to evaluate silicones stiffnesses with a maximal error of about 10% compared to

uniaxial tensile tests [30]. It was also used in a clinical study to evaluate the tongue stiffness in ten patients for two conditions: at rest and under general anesthesia [31]. Yet, in these studies, the underlying tissues were assumed homogeneous. As most human tissues are inhomogeneous, the method must be further developed.

The homogeneity hypothesis while using aspiration was challenged in literature. When extracting only the superficial layer mechanical properties, Zhao *et al.* [15] demonstrated *in silico* that an aspiration diameter smaller or equal to the upper layer thickness should be used. Other works [25, 32, 33] use data from tests with two different diameters (2 and 8 mm) to estimate the mechanical response of the most superficial layers of the human skin, and sometimes complete the data using *ex vivo* tensile test [33]. In [18], 3 diameters (1, 2 and 6 mm) and Optical Coherence Tomography were used to evaluate the mechanical behavior of the softer 130 μm upper skin layer.

This work aims at going beyond the state of the art (i) by using a single, easy to use and adaptable aspiration system, switching only aperture heads to perform all the measurements, (ii) by proposing identification of Young moduli of each constituent of bi-layered structures using an off-line Finite Element database (almost real time identification), (iii) by evaluating the parameter identifiability of the tested situation

The whole method has been evaluated experimentally to test the ability of VLASTIC to estimate the stiffnesses of bi-layers silicone phantoms compared with classical experimental tensile tests.

To the author's knowledge, no previous work implemented these last points. The hope is that the VLASTIC system and method will be used to estimate mechanical properties of most accessible soft tissues, such as for example the skin stiffness for face [34, 35], breast [36], sacrum [37] or foot [38] modeling.

2 Material and Methods

As an overview, the paper structure is graphically summarized in figure 1.

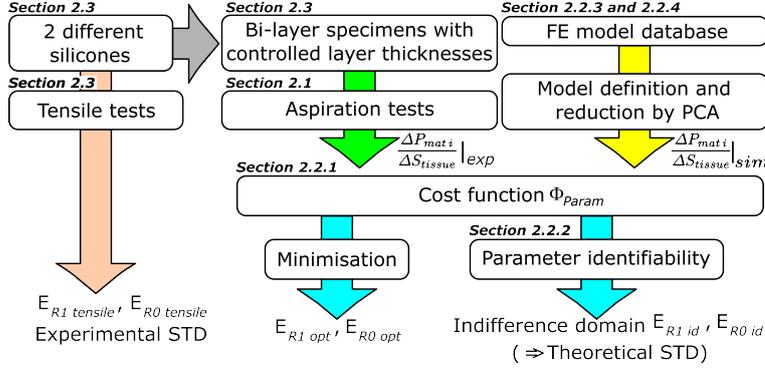


Fig. 1 Paper structure and main output notations: $E_{R1\ tensile}$ and $E_{R0\ tensile}$ stand for both layers' Young moduli measured through uniaxial tensile test on specimens. $E_{R1\ opt}$ and $E_{R0\ opt}$ are Young moduli identified with aspiration data and minimizing the cost function Φ_{Param} .

2.1 Volume based aspiration tests

2.1.1 Cyclic testing device

The testing system is composed of two air-filled parallel circuits defined as "Reference line" and "Material line", respectively, both connected at a valve, a manometer^a and a syringe^b (Figure 2 a). The syringes stroke ΔL is simultaneous and cyclic, inducing a volume variation ΔV in each line.

The "Reference line" is simply a tube closed at its end. It converts the volume variation ΔV (that may not be perfectly controlled during movement inversions due to the syringe piston deformation) into a pressure variation ΔP_{ref} . The tube length is chosen so that the pressure variation ΔP_{ref} in

^a AMS-5812-0015-D-B, Analog Microelectronics GmbH

^b CODAN 1mL Luer TBC

the "Reference line" sweeps the whole sensor pressure range given the input volume variation ΔV .

The "Material line" is composed of a tube linked to a 3D printed resin cup of aspiration diameter D_i (Figure 2 d) applied on the tested tissue. All cup geometrical features in contact with the tested tissue (wall thickness, fillet radius, *etc.*) are proportional to the aperture diameter D_i . This line converts the input volume variation ΔV into a pressure variation ΔP_{mat} .

The general idea is that the tested tissue mechanical behavior can be deduced by comparing the pressures variations in the two lines (ΔP_{ref} and ΔP_{mat}) while testing the tissue or an undeformable material (calibration test); the ratio of pressure variation in both lines $\frac{\Delta P_{ref}}{\Delta P_{mat}}$ will change between these two cases and contains the sought properties.

2.1.2 Protocol and elastic model

Practically, every test starts with the same protocol:

- ✓ The cup D_i is placed on the tested material. An ultrasound gel cord fills an external groove to ensure air tightness. The syringes are set in their empty reference position using the homing sensor (Figure 2 b). The valves are then closed, the air volume enclosed in the system being thus reproducible and minimum at the starting point ($n = 0$).
- ✓ A volume ΔV is withdrawn simultaneously and cyclically from both the material and reference lines (Figures 2 a and b). The pressure variations $(\Delta P_{ref}, \Delta P_{mat})_n = (P_{ref n} - P_{ref 0}, P_{mat n} - P_{mat 0})$ are measured in the reference and material lines, respectively. The underscript n represents the pressure index.
- ✓ Pressure variations during the test $(\Delta P_{ref}, \Delta P_{mat})_n$ are supposed proportional to the volume variation ΔV using elastic stiffnesses in a spring

model (Figure 2 c) such as:

$$k_{ref} = \frac{\Delta P_{ref}}{\Delta V} \quad (1)$$

$$k_{cup\ i} = \frac{\Delta P_{mat\ i}}{\Delta V_{cup\ i}} \quad (2)$$

$$k_{tissue\ i} = \frac{\Delta P_{mat\ i}}{\Delta V_{tissue\ i}} \quad (3)$$

$$\text{where } \Delta V = \Delta V_{tissue\ i} + \Delta V_{cup\ i} \quad (4)$$

To simplify the notations, expressions are articulated in the next equations using the ratio $Q_{i\ X}$ defined as:

$$Q_{i\ X} = \frac{\Delta P_{ref}}{\Delta P_{mat\ i}} \quad (5)$$

where underscript i refers to the aperture diameter D_i and X indicates whether $Q_{i\ X}$ is obtained during a calibration step ($X = cal$) or by testing a tissue ($X = exp$).

In practice, the ratios $Q_{i\ exp}$ and $Q_{i\ cal}$ are slopes of linear polynomial identified on the $(\Delta P_{ref}, \Delta P_{mat\ i})_n$ curve. It can be obtained by minimizing the linear function Φ_Q defined in the least square sense:

$$\Phi_Q = \sum_{n=1}^N \left(\Delta P_{ref} - (Q_{i\ X} \Delta P_{mat\ i} + A) \right)_n^2 \quad (6)$$

where N is the number of used pressure measurements and A is a constant accounting for any pressure offset difference between the material and reference lines due to potential thermal drifts.

2.1.3 Calibration and measurement

The system reference and material lines must be calibrated before testing deformable tissues:

- **Calibration of the "Reference line" (stiffness k_{ref}):**

Different cyclic volume amplitudes ΔV are applied to the reference line by increasing step by step the syringe courses ΔL with an adjustable $500 \mu\text{m}$ thread pitch screw-driven eccentric mechanism (Figure 2 b). The slope of the pressure variation amplitude ΔP_{ref} versus the volume amplitude $\Delta V = \Delta L S_{syringe}$ is the sought reference line stiffness $k_{ref} = 0.992 \text{ mbar.mm}^{-3}$ (equation 1).

- **Calibration of the "Material line" for each cups (ratio $Q_{i cal}$):**

The material line is calibrated for each cup D_i applied on an undeformable material ($k_{tissue} = \infty, \Delta V_{tissue i} = 0$). Equation 5 simply provides the material line ratio $Q_{i cal}$ during calibration:

$$Q_{i cal} = \frac{\Delta P_{ref cal}}{\Delta P_{mat i cal}} \quad (7)$$

- **Test on deformable tissue $k_{tissue i}$:**

When testing a soft tissue, the tissue apparent stiffness $k_{tissue i}$ is computed combining equations 1 to 5 and 7 so that:

$$k_{tissue i} = \frac{k_{ref}}{Q_{i exp} - Q_{i cal}} \quad (8)$$

Note that the information about the tissue apparent stiffness $k_{tissue i}$ is mainly contained in a difference of ratios $Q_{i X}$ measured during the calibration and the test on a soft tissue while using the same aperture diameter D_i (see Figure 8b in the results section).

2.1.4 Apparent stiffness normalization

The tissue apparent stiffness $k_{tissue i}$ contains information about the constituent tissue stiffnesses integrated over the loaded material volume beneath the aperture diameter D_i (Figure 3). Unfortunately, even when testing an

homogeneous material, this stiffness $k_{tissue\ i}$ (in $Pa.m^{-3}$) depends on the aspiration aperture size.

To compare the results obtained with different aperture size, the notion of tissue shape S_{tissue} is defined by normalizing the aspirated tissue volume $V_{tissue\ i}$ by the volume of a half sphere $V_{ref\ i}$ of diameter D_i [31]^c:

$$S_{tissue} = \frac{V_{tissue\ i}}{V_{ref\ i}} \quad (9)$$

$$\text{with } V_{ref\ i} = \frac{4}{6}\pi \left(\frac{D_i}{2}\right)^3 \quad (10)$$

A shape $S_{tissue} = 1$ means a volume of half a sphere has been aspirated into the cup.

Using this shape definition and equation 3, it comes:

$$\frac{\Delta P_{mat\ i}}{\Delta S_{tissue}} = V_{ref\ i} k_{tissue\ i} \quad (11)$$

where $\frac{\Delta P_{mat\ i}}{\Delta S_{tissue}}$ (in Pa) represents the normalized constituent material apparent stiffness:

On a homogeneous specimen, a change of aperture diameter D_i is equivalent to a change of test scale (same shape obtained for the same pressure variation); the constituent material apparent stiffness $\frac{\Delta P_{mat\ i}}{\Delta S_{tissue}}$ is independent of aperture-diameter D_i .

On bi-layered specimens, a change of aperture diameter D_i modifies the upper layer relative contribution to the shape S_{tissue} (Figure 3, illustration for $S_{tissue} = 1$). In particular, a cup of diameter smaller or equal to the thickness L_{R_1} of the upper layer of material labeled R_1 mainly extracts the upper layer stiffness [15] as will be further developed in section 3.

^c Similar normalization of the apex height is also found in [15].

Combining equations 8 and 11, the normalized constituent material apparent stiffness $\frac{\Delta P_{mat i}}{\Delta S_{tissue}}$ is thus simply obtained by:

$$\frac{\Delta P_{mat i}}{\Delta S_{tissue}} = V_{ref i} \frac{k_{ref}}{Q_{i exp} - Q_{i cal}} \quad (12)$$

2.2 Bi-layer mechanical properties inverse identification

The inverse identification consists in estimating both layer Young's moduli (E_{R1} and E_{R0}), and optionnally, the thickness L_{R1} of the upper layer, minimizing a cost function Φ_{Param} (section 2.2.1). A Finite Element (FE) model has been created to simulate the aspiration procedure and predict each parameter effect.

2.2.1 Cost Function

The cost function Φ_{Param} is defined in the least square sense on the experimentally measured material apparent stiffness $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$:

$$\Phi_{Param} = \sum_{i=1}^I \frac{1}{\sigma_i^2} \sum_{j=1}^{J_i} \left(\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim} (\theta, \beta) - \left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp} \right)_j^2 \quad (13)$$

where $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim}$ is the simulated material apparent stiffness for a cup diameter D_i (section 2.2.3 and 2.2.4), θ is a P -dimensional vector representing the sought unknowns and referred to as "parameter vector", and β represents the other model parameters (aperture diameter, friction, compressibility coefficient, *etc.*). I is the number of aperture diameter D_i used and J_i is the number of cycles performed for each aperture of diameter D_i . The number of measurements used to compute Φ_{Param} is thus $N_m = \sum_{i=1}^I J_i$. The weighing denominator σ_i^2 is the experimental unbiased variance of the experimental apparent stiffness $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ for aperture D_i and is used so that the cost

function Φ_{Param} is not dominated by the experimental data provided by a specific aperture diameter D_i [39].

Unknowns and model parameters θ and β are composed of four main characteristics (Figure 3), namely the aperture diameter D_i , the upper layer thickness L_{R1} and associated Young's modulus E_{R1} and the lower layer Young's modulus E_{R0} . The optimal parameter vector θ_{opt} minimizing the cost function Φ_{Param} is estimated using the Levenberg-Marquardt method [40] under different situations referred to by the number of sought parameters P .

$P = 3$ (**bi-layer**, $\theta = \langle E_{R1}, E_{R0}, L_{R1} \rangle^T$): when the specimen is a bi-layer specimen, the upper layer thickness L_{R1} , Young's modulus E_{R1} and the lower layer Young's modulus E_{R0} can all be estimated.

$P = 2$ (**bi-layer**, $\theta = \langle E_{R1}, E_{R0} \rangle^T$): when the specimen is a bi-layer specimen, the upper layer thickness shall be provided in β by an annex measurement. In such a case, only the upper and lower layer Young's moduli E_{R1} and E_{R0} are estimated in θ .

$P = 1$ (**homogeneous**, $\theta = \langle E_{R1} \rangle$): when the specimen is considered homogeneous, only the averaged material Young's modulus is estimated ($E_{R1} = E_{R0}$). In such a case, the theoretical constituent material apparent stiffness $\frac{\Delta P_{mat i}}{\Delta S_{tissue}} \Big|_{sim}(\theta, \beta)$ is independent on the upper layer thickness value L_{R1} .

2.2.2 Parameters' identifiability

The question of parameters' identifiability has been analysed following the principles detailed in [39]. The main hypotheses of such an analysis are:

- H1** : The model describing the phenomeon is non-linear.
- H2** : There is no mismatch between model and the experimental data: all important predictor variables are included in the parameter vector θ and no unimportant predictor variables are included in the parameter vector θ .

H3 : The disturbance model is additive, normally distributed, of zero mean (no bias), with variances σ_i^2 known for each diameter D_i (so that all weighted data value are equally unreliable, see equation 13), and independent on each other.

The parameters identifiability is expressed as indifference region computed with a confidence level of 95%, meaning that:

- For a $P = 1$ -parameter model, the indifference region at 95% is equivalent to predicting the value of twice the STandard Deviation (STD) of the results $\theta_{opt} = \langle E_{R1 id} \rangle$ if the same measurements were performed with a new disturbance.
- For a $P = 2$ or $P = 3$ -parameter model, the indifference region at 95% is the region where another optimal solution θ_{opt} would have 95% of chance to be if the same measurements were performed with a new disturbance.

This region is defined as the P -dimensional domain over which the parameter vector θ verifies:

$$\Phi_{Param}(\theta, \beta) - \Phi_0 < \delta \quad (14)$$

where Φ_0 is the minimum value of the cost function Φ_{Param} obtained for the optimal value θ_{opt} . If the function $\left. \frac{\Delta P_{mati}}{\Delta S_{tissue}} \right|_{sim}(\theta, \beta)$ was linear, such a domain would be an hyperelipsoïd of dimension P .

The threshold δ is defined using a statistical approach so that:

$$\delta = P\sigma^2 F(P, N_m - P, \alpha) \quad (15)$$

where $F(P, N_m - P, \alpha)$ is the upper α quantile for Fisher's F distribution with P and $(N_m - P)$ degrees of freedom, respectively. σ^2 is the variance of the normalized error vector in Φ_0 . In practice, given the definition of the function Φ_{Param} (equation 13) and hypotheses **H2** and **H3**, Φ_0 should follow a chi-

squared distribution with $(N_m - P)$ degree of freedom. The unbiased variance is can thus be evaluated for each fitting as:

$$\sigma^2 = \left(\frac{\Phi_0}{N_m - P} \right) \quad (16)$$

where the unbiased variance σ^2 is computed over a normalized (reduced) error vector and has a theoretical expectation of 1. If hypotheses **H2** or **H3** were not met (model mismatch or bias in $Q_{i \text{ exp}}$, $Q_{i \text{ cal}}$ or k_{ref} , equation 12), the values of variance σ^2 will become greater than 1. The associated threshold δ (equation 15) and the indifference region would be overestimated.

During the optimization process, the theoretical constituent material apparent stiffness function $\frac{\Delta P_{mat i}}{\Delta S_{tissue}} \Big|_{sim}$ (equation 13) is evaluated using the model reduction provided by a Principal Component Analysis (PCA) applied to a pre-calculated Finite Element (FE) database. The following section presents the FE database construction. For detailed description of model reduction using the PCA method and interpolation, the reader is kindly referred to [41].

2.2.3 Database definition

To compute the minimum of the cost function Φ_{Param} (equation 13), the theoretical constituent material apparent stiffness $\frac{\Delta P_{mat i}}{\Delta S_{tissue}} \Big|_{sim}(\theta, \beta)$ must be estimated (equation 13). At each step of the minimizing process, evaluating the cost function Φ_{Param} thus require to create I different models, each with a specific combination of the four main parameters D_i , L_{R1} , E_{R1} and E_{R0} . To reduce the required CPU time, a database is created and interpolated. These 4 parameters can be combined to reduce the required FE database dimension from 4 to 2 as also proposed with a different philosophy in [15].

Material stiffness contrast: considering a material stiffness contrast ratio $\frac{E_{R1}}{E_{R0}}$, the constituent material apparent stiffness function $\frac{\Delta P_{mat i}}{\Delta S_{tissue}} \Big|_{sim}$ can be seen as proportional to the bottom layer stiffness E_{R0} (equation 17).

Scale effect: assuming the lower layer thickness is infinite (in practice, the total layer thickness is much greater than the aperture diameter D_i), the upper layer relative contribution to the shape S_{tissue} is governed only by the ratio $\frac{D_i}{L_{R1}}$ between the aperture diameter D_i and upper layer thickness L_{R1} ; redundant $\frac{D_i}{L_{R1}}$ ratio would provide redundant information in the FE database. This hypothesis has been confirmed numerically.

The actual FE database is thus reduced to two parameters so that:

$$\frac{\Delta P_{mat i}}{\Delta S_{tissue}} \Big|_{sim} (\theta, \beta) = E_{R0} f_{sim} \left(\frac{E_{R1}}{E_{R0}}, \frac{D_i}{L_{R1}} \right) \quad (17)$$

where f_{sim} is an adimensional function depending on the layer stiffness contrast ratio $\frac{E_{R1}}{E_{R0}}$ and on the size ratio $\frac{D_i}{L_{R1}}$.

2.2.4 FE model

Applying these considerations, an axisymmetrical model taking in account large displacements has been defined (ANSYS APDL) with an aspiration cup diameter of $D_i = 10 \text{ mm}$ (Figure 4). To ensure the use of a unique converged mesh for all simulations in the database, $M = 20$ pre-meshed layers have been prepared at different depths $L_{layer m}$. The ratio $\frac{D_i}{L_{R1}}$ is modified between simulations by attributing a Young's Modulus of E_{R1} to the first $[1, m]$ pre-meshed upper-layers and a Young's modulus of E_{R0ref} to the other layers in $[m + 1, M]$.

The following additional parameters in vector β have been chosen for the FE simulation:

- An **Incompressible Neo-Hookean model** simulates the material behavior of each layer. The constituent material apparent stiffness $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim}$ is evaluated at shapes equal to 0.1; for such a small deformation state, the material incompressibility (Poisson coefficient $\nu \in [0.45 \ 0.5]$) has no influence onto the results (numerically tested).
- **Aspiration cup geometry** in contact with the sample is representative of the reality (wall thickness, fillet radius). 3D printed cup parts in contact with the tissue are proportional to the cup aperture D_i .
- **Boundary conditions:** the cup itself is immobilized in all directions on line CD (Figure 4). Contact elements are created between the line AB on the tissue and the cup (zoom-in in Figure 4). Partial vacuum (pressure noted $-P_{mat}$) is applied on line AB including the line part beneath the cup contact. With these boundary conditions, the tissue sample is free to move up or down relatively to the cup according to the applied pressure $-P_{mat}$. The line AB displacement versus pressure $-P_{mat}$ is post-processed to compute the aspirated shape S_{tissue} (equation 9). The slope $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim}$ is computed at shape $S_{tissue} = 0.1$, which provides in turn the adimensional value f_{sim} (equation 17).
- The **friction coefficient** between tissue and cup is chosen of $f = 0.2$. During the experiment, this parameter is actually an unknown and is affected by the ultrasound gel cord. The friction coefficient influence has been tested numerically (no friction to glued boundary conditions). Its effect was considered negligible (as also reported in [42]) when the upper layer is stiffer than the lower layer.
- **External loads applied on the cup** are as small as possible during the experiments (Figure 6, illustration on sample A). No additional external loads are considered in the simulation. This hypothesis will be discussed while analyzing the results.

- The **tissue sample size** in the model is chosen so that rigid or free boundary conditions applied on the tissue outer boundaries have negligible influence onto the sought function (numerically tested).
- **Mesh convergence** : The layers are meshed using 8 nodes elements (*Q8*, *Plane183*, ANSYS). The mesh convergence has been checked on the tissue shape prior to the database simulation, the mesh being the same for all simulations. A zoom-in of the meshing size is reported in Figure 4.

2.2.5 Database range and interpolation

The FE database has been computed on stiffnesses ratios ranges: $\frac{E_{R_1}}{E_{R_0}} \in [0.1 \ 120]$ and $\frac{D_i}{L_{R_1}} \in [0.33 \ 133]$ (Figures 4 and 5).

A PCA is applied to the database to extract eigenvectors and weighting functions. Only the 3 first eigen-vectors and associated weighting functions were kept, representing more than 99.99% of the database information. Eigen vectors and weighting functions were both interpolated with splines to compute f_{sim} (equation 17, Figure 5).

2.3 Controlled bi-layer silicone specimens

To validate the method, bi-layered specimens made of two mechanically characterized R_0 (soft) and R_1 (stiffer) silicones were tested.

These silicones were obtained mixing equal mass of component A and B^d and adding silicone softener^e (14.6% of $(A + B)$ mass for R_1 , 30% of $(A + B)$ mass for R_0). The mixed silicones were vacuumed during 5 *min* to remove air bubbles before pouring.

Three types of samples were made:

^d Skin FX10 110019

^e Deadner Skin FX10 110020

- **Homogenous aspiration specimens:** simple cylinders of $\varnothing 96 \text{ mm} \times 70 \text{ mm}$ used as references and labelled R_0 and R_1 (Figure 6a).
- **Bi-layered aspiration specimens:** cylinders of $\varnothing 96 \text{ mm}$, with thick R_0 bottom layer (soft), and thin upper R_1 layer (stiffer) of thickness L_{R_1} (A to E , Figure 6a). The samples were molded upside-down: the R_1 stiffer layer was molded first controlling the poured volume with a syringe, followed one hour later by the softer layer of R_0 .

The upper layer thicknesses $L_{R_1 \text{ pic}}$ were measured after all aspiration tests by cutting the samples in half and taking magnified scale controlled pictures. Thicknesses were evaluated in 8 different locations

- **Flat tensile specimens:** 5 to 10 flat specimens ($40 \times 160 \times 3 \text{ mm}^3$ molds) were molded from the same mixes as the aspiration samples. The averaged section A_0 of these samples are estimated by weighting each specimen mass m_{tens} so that $m_{\text{tens}} = \rho b A_0$ with ρ the silicone volumetric mass and b the mold length.

The tensile specimens Young's Moduli $E_{R_1 \text{ tens}}$ and $E_{R_0 \text{ tens}}$ were evaluated during quasi-static uniaxial tensile tests on a MTS tensile machine.

3 Results

3.1 Reference values

The tensile results on the R_0 and R_1 flat specimens are presented in Figure 6b. Fitting a Neo-Hookean incompressible model onto the tensile curves for $\lambda_1 = \frac{L}{L_0} \in [1, 1.1]$ provides Young's moduli of $E_{R_1 \text{ tensile}} = 74.7 \pm 2.3 \text{ kPa}$ and $E_{R_0 \text{ tensile}} = 8.97 \pm 0.64 \text{ kPa}$ where the tolerance interval is given as twice the experimental STD (Table 1). The stiffness ratio $\frac{E_{R_1 \text{ tensile}}}{E_{R_0 \text{ tensile}}}$ is equal to 8.3.

The optically measured thicknesses $L_{R_1 pic}$ of reference samples are presented in Table 2. Errors intervals are given as twice the experimental STD.

Flat tensile specimens	$\mathbf{E}_{tensile}$ (kPa)
R_0	9.0 [8.3, 9.6]
R_1	74.7 [72.4, 77.0]

Table 1 Reference values: identified Young Moduli from tensile test on flat specimens.

Aspiration specimens	R_1 layer thickness $L_{R_1 pic}$
R_0	0 mm
A	1.08 ± 0.064 mm
B	3.27 ± 0.06 mm
C	6.22 ± 0.055 mm
D	9.16 ± 0.076 mm
E	11.75 ± 0.05 mm
R_1	69 mm

Table 2 Reference values: layer thicknesses $L_{R_1 pic}$ evaluated by an annex destructive measurement.

3.2 Aspiration settings

The cup was held into place while minimizing external loads applied to the cup thanks to a special 3D printed holder^f (Figure 6a, applied to specimen A).

The volumes of air withdrawn from the reference and material lines ΔV was of 0.1 mL and kept identical for all the cups diameters D_i . A complete cycle (withdrawing and injecting ΔV) was of about 10 seconds. A total of 5 cycles were performed during each acquisition (intra-test reproducibility). Each test has been performed 2 to 3 times (inter-test reproducibility).

^f 3D printer Prusa MK3S+

The pressure-pressure curve $\Delta P_{mat} - \Delta P_{ref}$ was monitored during all the tests; tests with leakage were identified when pressure P_{mat} drifted cycle after cycle. Such tests were immediately discarded.

Only pressure signals obtained during ΔV withdrawing and for $\Delta V_{syringe} > 0.01 \text{ mL}$ were used to evaluate $Q_{i \text{ exp}}$ (equation 6) to avoid impact of possible syringe piston asymmetrical behavior during movement inversion in the reference and material lines.

3.3 Constituent material apparent stiffnesses: $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$

The experimental constituent material apparent stiffnesses $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ (equation 12) for each cycle are presented versus the aperture diameter D_i in Figure 7 a. Taking advantage that the thicknesses $L_{R1 \text{ pic}}$ are measured during an annex measurement (Table 2), the experimental constituent material apparent stiffness $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ are also plotted versus the ratio $\frac{D_i}{L_{R1 \text{ pic}}}$ in Figure 7 b.

3.4 Optimal identified parameter θ and indifference regions

For illustration purpose, details of fitted curves and indifference regions are presented on sample B (Figure 8).

Aspiration results on specimens R_0 and R_1 for $P = 1$ are summarized in table 3 with direct comparisons to the tensile reference values.

		Aspiration test
$\mathbf{P} = 1$	σ^2	\mathbf{E}_{opt} (kPa)
R_0	367	11.7 (+29.9%) [11.5, 11.9]
R_1	7.9	78.9 (+5.6%) [78.7, 79.1]

Table 3 Identified Young Moduli from aspiration data on homogeneous specimens for the $P = 1$ -parameter model are in **bold**. The Relative Error (RE) between optimal aspiration and reference value is in (*italic*). Values in bracket are the minimum and maximum values of the indifference region at 95% for each parameter identified with aspiration.

Aspiration results on specimens A to E are summarized in table 4 for $P = 2$ and 3 with direct comparisons to the reference values.

$P = 2$	σ^2	$L_{R_1 \text{ pic}}$ (mm)	$E_{R_1 \text{ opt}}$ (kPa)	$E_{R_0 \text{ opt}}$ (kPa)
A	71	1.08 \pm 0.064	96.4 (+29.1%) [81.3, 113.2.0]	8.8 (-2.5%) [8.4, 9.1]
B	32	3.27 \pm 0.060	80.7 (+8.0%) [78.3, 83.3]	8.9 (-1.0%) [8.5, 9.3]
C	7	6.22 \pm 0.042	80.0 (+7.1%) [79.6, 80.9]	12.0 (33.8%) [11.7, 12.8]
D	15	9.16 \pm 0.076	79.7 (+6.7%) [79.3, 80.3]	12.5 (39.3%) [11.6, 13.7]
E	10	11.75 \pm 0.050	79.3 (+6.1%) [79.0, 79.6]	11.6 (28.6%) [10.3, 13.2]
$P = 3$	σ^2	$L_{R_1 \text{ opt}}$ (mm)	$E_{R_1 \text{ opt}}$ (kPa)	$E_{R_0 \text{ opt}}$ (kPa)
A	62	1.51 (+39.5%) [1.51, 1.76]	57.5 (-23.1%) [46.82, 76.2]	8.4 (-6.8%) [7.9, 8.9]
B	31	3.26 (-0.33%) [3.09, 3.44]	80.9 (+8.4%) [77.0, 85.5]	8.9 (-0.6%) [8.3, 9.5]
C	3	5.52 (-11.2%) [5.39, 5.67]	83.2 (+11.4%) [82.4, 84.0]	15.3 (+70.2%) [14.5, 16.04]
D	13	8.37 (-8.5%) [7.55, 9.08]	80.7 (+8.1%) [79.7, 82.1]	16.5 (+83.8%) [12.8, 20.9]
E	6	8.85 (-24.7%) [7.91, 9.66]	80.9 (+8.3%) [80.2, 81.8]	27.8 (+209.3%) [23.4, 32.7]

Table 4 Identification results for the $P = 2$ and $P = 3$ -parameter models: the optimal value using aspiration data is in **bold**. The Relative Error (RE) between optimal aspiration and reference value is in (*italic*). Values in bracket are the minimum and maximum values of the indifference region at 95% for each parameter identified with aspiration. Color code: green if $|RE| < 15\%$, orange if $15\% < |RE| < 30\%$, red if $|RE| > 30\%$.

A more global summary is graphically represented in Figure 9 to highlight the indifference regions variations depending on the tested sample (A to E) and value of parameter P . Results obtained on homogeneous samples ($P = 1$) and by tensile tests are reported as horizontal red, blue and black bands of twice the experimental STD (indifference regions at 95%).

4 Discussion

Given that only two silicone mixes R_1 and R_0 were used, the stiffness ratio $\frac{E_{R_1}}{E_{R_0}} \approx 8.3$ is identical for aspiration specimens A to E .

- This corresponds to a unique curve of the FE database (equation 17). The database stiffnesses ratios range of $\frac{E_{R_1}}{E_{R_0}} \in [0.1 \ 120]$ is thus overkill but was chosen to allow computation of large indifference regions and to anticipate

application to human skin, with hypodermis tissues that are much softer than the dermis/epidermis layer.

- The experimental curves overlap in Figure 7b confirms this stiffness ratio uniqueness is also observed experimentally with the aspiration tests; this is a qualitative assessment of the experimental measurement quality of both $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ and $L_{R1 pic}$. Depending on the layer thickness L_{R1} , the aperture diameters from 4 to 30 mm extract different parts of the curve.

4.1 Homogeneous aspiration samples, $P = 1$ -parameter model:

Stiffer R_1 silicone: the measurement $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ is almost independent on the aperture diameter D_i (Figure 7a, red curve) which is in accordance with theory for a upper layer thickness greater or equal to the maximal aperture diameter [15]. The aspiration test over-estimates the R_1 Young Modulus compared to the tensile result $E_{R1 tensile}$ by 5.6% (Table 3), which is the same order of magnitude as in [30].

Softer R_0 silicone: The aspiration test with the $P = 1$ -parameter model over-estimates the R_0 Young Modulus compared to the tensile one $E_{R0 tensile}$ by 29.9%. The fitting score of $\sigma^2 = 367$ is far from the expected value of 1 (Table 3). This poor fitting score is due to the fact that the measurement $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$ increases for small D_i (Figure 7a, blue lowest curve). This behavior was not expected for an homogeneous sample. A possible explanation is that soft materials are very sensitive to normal loading applied to small cups [43]. Such an initial load makes the material surface to be curvaceous, which replaces some air into the cup by material before closing the system valve. The used calibration value $Q_{i cal}$, measured on a flat undeformable surface, is thus larger than reality. This bias induces an over-

estimation of the measurement $\frac{\Delta P_{mat.i}}{\Delta S_{tissue}}|_{exp}$ (equation 8). This phenomena should yet be limited by the presence of the holding system (Figure 6a).

4.2 Bi-layer aspiration sample, $P = 2$ or 3-parameter models:

The computed indifference region sizes are in accordance with "ill-posedness" aspect of the tested case:

- Sample *A* has a very thin upper layer of 1.08 mm. Aperture diameters from 4 to 30 mm are thus well adapted to extract information about the lower layer stiffness E_{R_0} . Relative Errors with tensile test (-2.5 and -6.8% for $P = 2$ or $P = 3$ -parameter models, respectively, table 4) and indifference regions lower than 1 kPa on E_{R_0} confirm this observation.

The smallest aperture diameter being 4 times larger than the upper layer thickness, the upper modulus E_{R_1} is the least well identified among the tested samples (relative error of $+29.1$ and -23.1% for $P = 2$ or $P = 3$ -parameter models, respectively, table 4), which is pointed out by aspiration indifference region greater than 32 kPa on E_{R_1} .

- Sample *B*, with an upper layer of 3.27 mm is the most adapted among the made samples to provide both proper upper and lower layer moduli given the used aperture diameters range ($|RE| < 15\%$ for all optimal values and for both $P = 2$ and 3-parameter models, table 4). The computed indifference regions are of about 5 kPa for the upper modulus E_{R_1} and 1 kPa for the lower modulus E_{R_0} . This highlights the identification robustness even though the residual error vector shows that hypotheses **H2** and **H3** are not experimentally met and $\sigma^2 = 31$ is far from the expected value of 1 (Figure 8c). The knowledge of the upper layer thickness is thus not mandatory when the layer thickness is well adapted to the used aperture diameter range.

- Samples C to E , with layers thicker than 6 mm, provide proper upper layer modulus E_{R1} identification and indifference regions of the order of 1 kPa for $P = 2$ or $P = 3$ -parameter models (RE lower than 15%); the upper layer thickness knowledge is not mandatory in this case.

As expected, the lower layer modulus E_{R0} identifiability decreases as the upper layer thickness increases ($RE > 30\%$ and increasing with the layer thickness $L_{R1\ pic}$), which is pointed out by indifference region increasing from 1 to 10 kPa . Knowing the upper layer thickness improves the identification but the RE remains around 30%.

From a practical point of view, the upper layer thickness knowledge is thus not mandatory: when this value is known ($P = 2$ -parameter model), the obtained RE is stabilized but not fully satisfactory for the lower layer modulus E_{R0} identification; identifications results are considered similar with both $P = 2$ and 3-parameter models.

5 Limitation/Perspectives

The current system hardware, experimental and identification method provide satisfactory results. Some limitations and associated perspectives should be yet be mentioned in anticipation of application to skin measurements:

5.1 Hardware limitation

The same volume ΔV is cyclically withdrawn from the "Material line" for all aperture diameter D_i . The maximal shape reached for small cups is thus larger than for large diameters. This has no impact while analysing linear materials but should be taken into account while applying the method to non-linear materials (stiffening for example). Being able to adjust the withdrawn volume ΔV with the diameter size would be advisable.

5.2 Protocole limitation

The aperture diameter is applied onto a flat surface and held in place with the lowest possible initial load. In any other case, the tissue sample will be initially curved into the aspiration chamber. This would modify the reference air quantity into the system and associated calibration curve; a bias would be added to the experimental result. Moreover, the surface curvature shall impact airtightness for large aperture diameter cups, preventing their use. Eventually, performing measurements *in vivo* will provide noisier data (breathing, muscle activation, etc) averaged over the cycles. These phenomena impact onto the whole identification process should be evaluated.

5.3 Inverse analyse limitation

Identification is performed using a linear model for both the system lines (including the tested tissue, equations 1 to 3), reducing the extracted mechanical parameters to the layers' Young Moduli. More complex behavior should be considered to extract richer model parameters such as stiffening of the upper layer for example. Second, the two layer thickness is considered much bigger than the aperture diameter D_i . Such a parameter should be satisfied experimentally. If not, a FE database adapted to this situation should be evaluated.

6 Conclusion

A clinically friendly aspiration system has been developed. Cyclic partial vacuum is applied to the tested tissue to evaluate its apparent mechanical properties at very low deformation. The system aspiration cup can easily be switched for aperture diameters D_i between 4 and 30 mm. The developed identification method enables, almost in real-time, to

- identify mechanical Young moduli and the upper layer thickness of bilayered structures using an off-line Finite Element database,
- evaluate indifference regions (prediction of standard deviation) for each identified parameter given the experimental results,

The system was tested *in silico* on controlled bilayer specimens for upper layer thicknesses from 1 to 12 mm. The bilayer sample with an upper layer of 3 mm presented the best parameter identifiability for both Young's moduli (relative errors lower than 10% compared to reference values obtained during tensile tests). The upper layer thickness was also identified with an error lower than 2%. On other upper layer thicknesses, identified results were of the proper order of magnitude. The obtained indifference regions in each case were representative of the identification quality and "ill-posedness" of the experimental situation.

These results provide hope to apply the method to human skin for which the L_{layer} range is compatible (Bmode US scanner used to identify visually the dermis and hypodermis interface, 1.36 to 3.5 mm on head skin [16]).

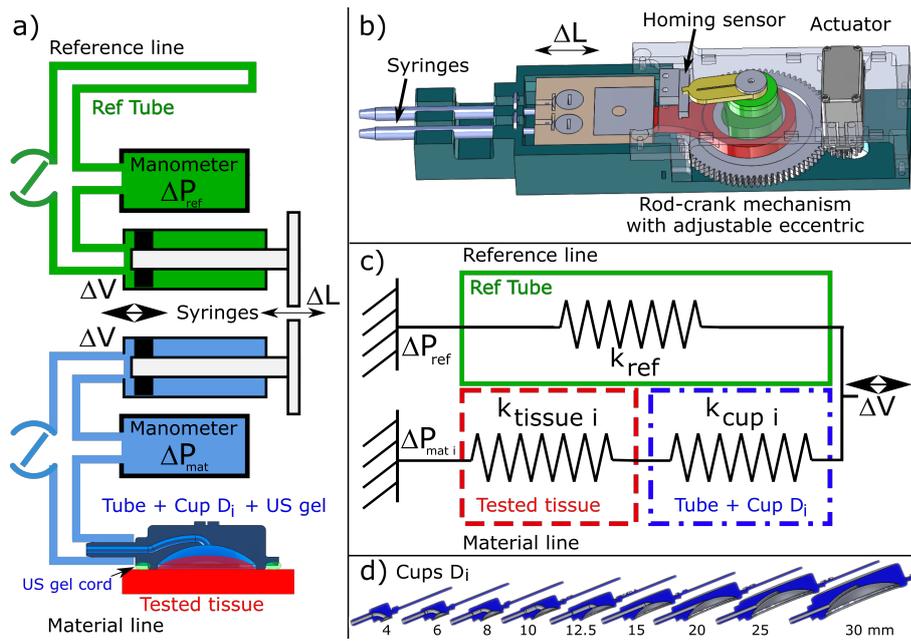


Fig. 2 Subplot a) Principle of the two lines system to evaluate the material mechanical answer of soft tissues during cyclic aspiration.

Subplot b) Syringes cyclic actuator with adjustable screw-driven eccentric and homing sensor.

Subplot c) Equivalent spring model of the whole system.

Subplot d) Aspiration cups with aperture diameters ranging from 4 to 30 mm.

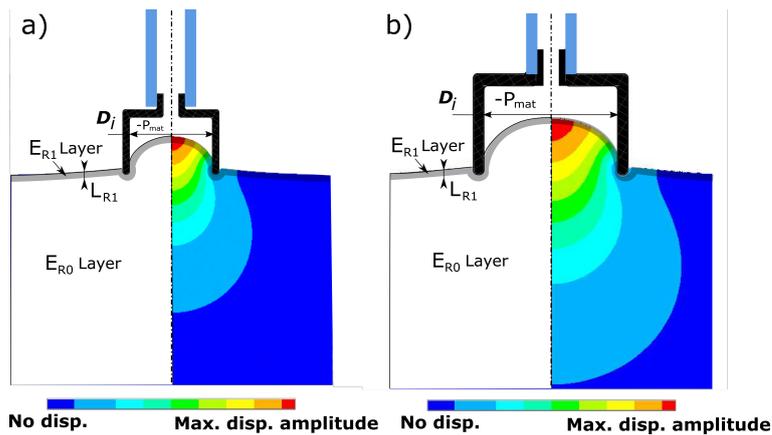


Fig. 3 Illustration of aspiration on a bi-layer sample using different aperture diameters for $S_{tissue} = 1$. The colors under the cups schematically represent the material volume over which the material stiffness information is extracted. Changing the aspiration diameter D_i modifies the relative contribution of the upper layer to the final shape S_{tissue} .

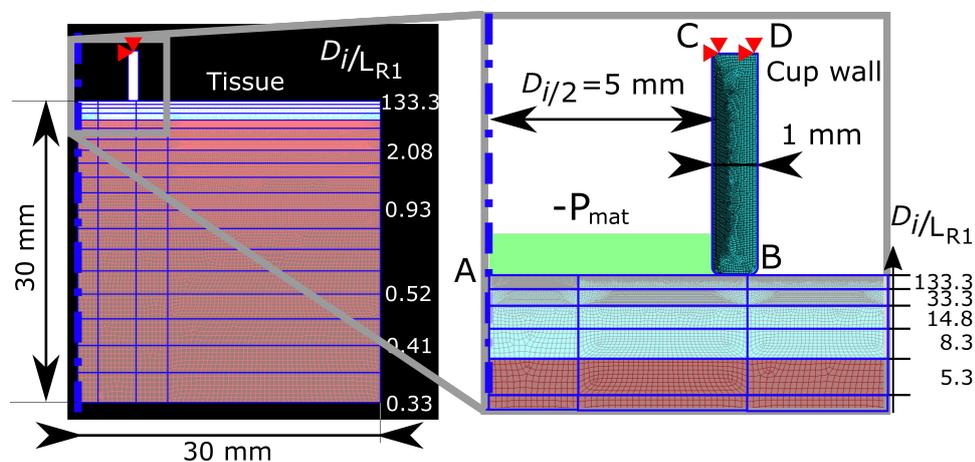


Fig. 4 Axisymmetrical FE model geometry. Zero displacements are presented as red triangles. Partial vacuum homogeneous pressure is represented by the green rectangle in the zoom in subplot. Pre-meshed layers are defined at different depths to use the same converged mesh for all calculations. Material mechanical properties E_1 is applied to elements of the upper pre-meshed layers (illustration for a ratio $\frac{D_i}{L_{R1}} = 8.3, m = 4$).

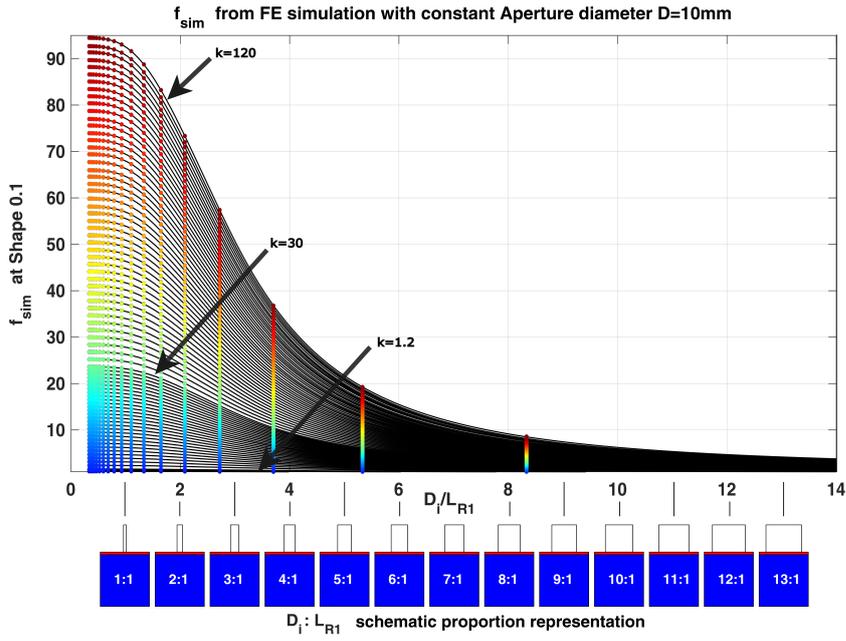


Fig. 5 The FE normalized results stored in the database are represented as points (computed at shape $S_{issue} = 0.1$) versus depths ratio $\frac{D_i}{L_{R1}}$. The stiffnesses ratios range is $k = \frac{E_{R1}}{E_{R0}} \in [1.2 \ 120]$. The PCA interpolation enables to interpolate the database, as presented with the black curves. The meaning of the depths ratio $\frac{D_i}{L_{R1}}$ is visually represented for integer values under the abscisse axis.

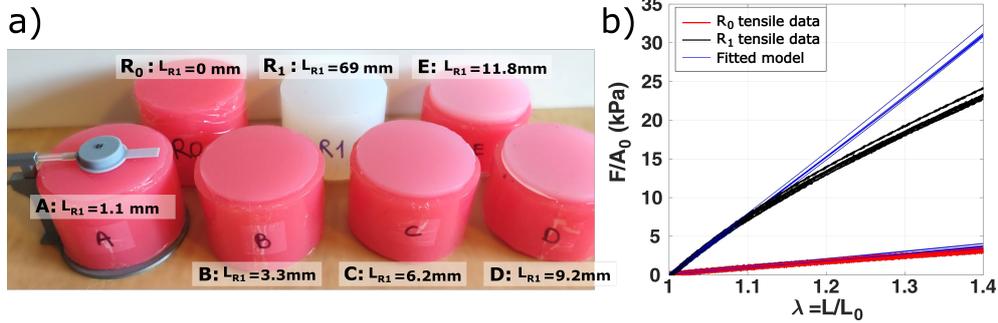


Fig. 6 Subplot a) Homogeneous (R_0 and R_1) and bi-layered aspiration samples (A to E) made with stiffer R_1 silicone as upper layer (white) and softer R_0 silicone as bottom layer (pink).

Subplot b) Tensile test results on flat rectangular specimens ($40 \times 160 \times 3 \text{ mm}^3$). Softer R_0 silicone (red curve) and stiffer R_1 silicone (black curve). Associated Neo Hookean curve fitting using data over the domain for $\lambda_1 = \frac{L}{L_0} \in [1, 1.1]$.

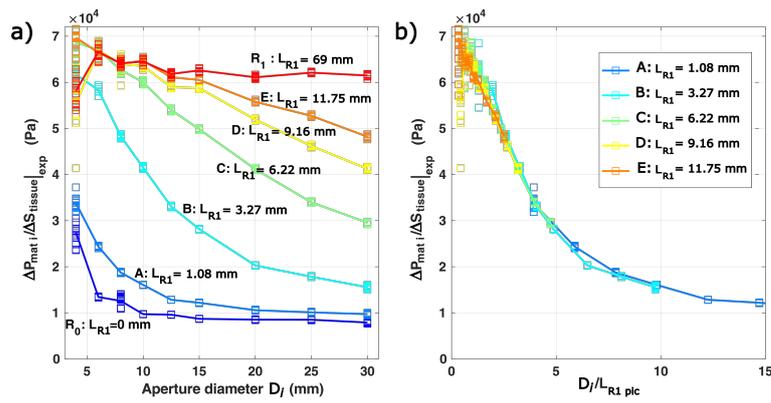


Fig. 7 Experimental results $\left. \frac{\Delta P_{mat}}{\Delta S_{tissue}} \right|_{exp}$ on bi-layered and homogenous specimens (Table 2): **subplot a)** Plot versus aperture diameter D_i , **subplot b)** Plot versus ratio $\frac{D_i}{L_{R1 pic}}$ where $L_{R1 pic}$ is evaluated during an annex destructive measurement.

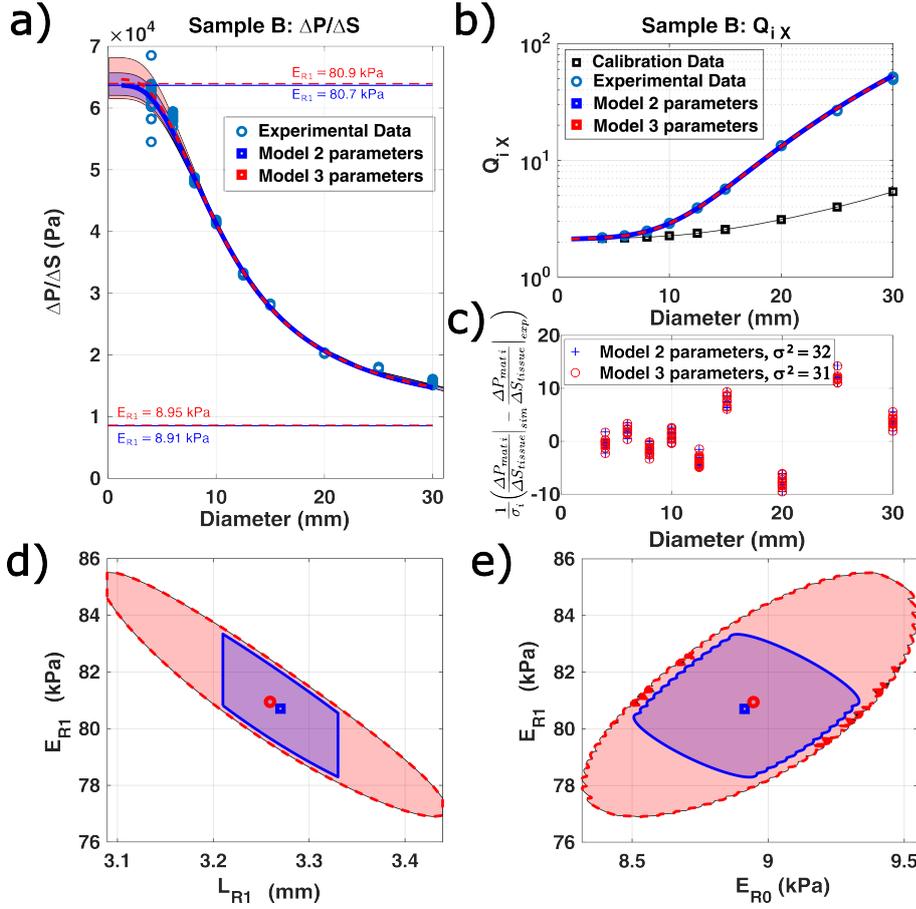


Fig. 8 Experimental data and inverse identification on sample *B*.

Subplot a) Experimental material apparent stiffnesses $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp}$, best fitted curves and swept area for parameters in the P -dimensional indifference region. For homogeneous samples, values of $\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim}$ are independent on the aperture diameter. Such cases are represented as dashed horizontal lines corresponding to the optimal identified upper and lower material Young Moduli ($E_{R1 opt}$ and $E_{R0 opt}$).

Subplot b) Calibration Q_i and experimental Q_i values (equation 5) versus aperture diameter D_i . The calibration curve represents the aspiration results obtained onto an infinitely stiff material. Note that the ordinate is in log scale.

Subplot c) Optimized reduced error vectors $\frac{1}{\sigma_i} \left(\left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{exp} - \left. \frac{\Delta P_{mat i}}{\Delta S_{tissue}} \right|_{sim} \right)$ to compute Φ_0 (equation 13) for both $P = 2$ and $P = 3$ -parameter models. Note that the error spread is similar for each diameter D_i thanks to the use of the weighting factor $\frac{1}{\sigma_i}$. The hypothesis of a disturbance with no bias is not experimentally met here, explaining the "unsatisfactory" value $\sigma^2 = 32$ for both $P = 2$ and $P = 3$ -parameter models.

Subplots d) The markers and areas represent optimal identified Young moduli E_{R1} versus layer thickness L_{R1} and associated indifference regions in the cases $P = 2$ (assuming a layer thickness $L_{R1 pic} = 3.27 \pm 0.05$ mm, Table 2) and $P = 3$, respectively.

Subplots e) The markers and areas represent optimal identified Young moduli E_{R1} and E_{R0} and associated indifference regions in the cases $P = 2$ (assuming a layer thickness $L_{R1 pic} = 3.27 \pm 0.05$ mm, Table 2) and $P = 3$, respectively. Such good areas overlapping is not met for all specimens and depends on the closeness between the optimal layer thickness L_{R1} and actual layer thickness $L_{R1 pic}$.

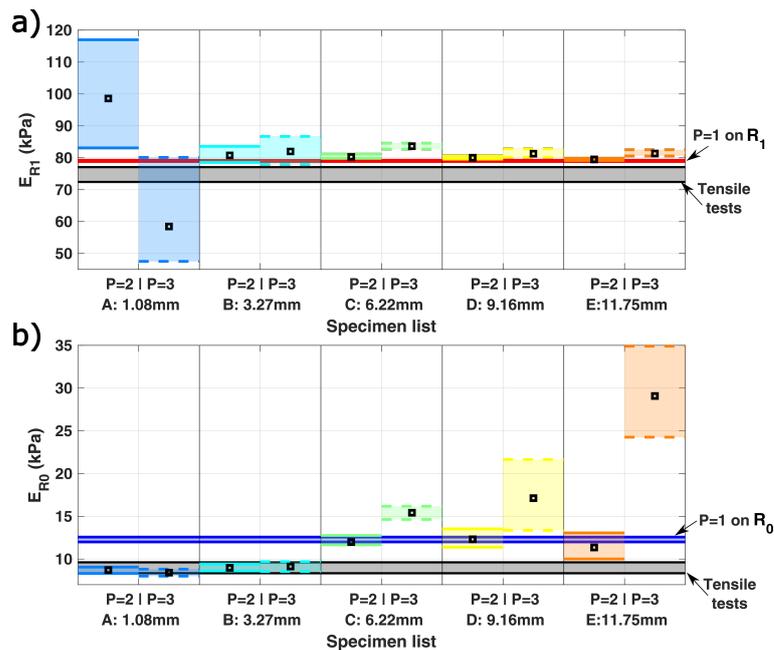


Fig. 9 Experimental indifference ranges on samples *A* to *E*:

Subplot a) Identification ranges for the upper layer R_1 using a $P = 2$ or $P = 3$ -parameters model. The horizontal black band represents the tensile test indifference region at 95% (average ± 2 Std) on silicones R_1 . The horizontal red band represents the 95% indifference region on aspiration using a $P = 1$ -parameter model on the homogeneous sample R_1 .

Subplot b) Identification ranges for the lower layer R_0 using a $P = 2$ or $P = 3$ -parameters model. The horizontal black band represents the tensile test indifference region at 95% (average ± 2 Std) on silicones R_0 . The horizontal blue band represents the 95% indifference region on aspiration using a $P = 1$ -parameter model on homogeneous samples R_0 .

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