

Mechanical characterization of sublingual mucosal tissues by uniaxial tensile tests – A protocol definition

Nouha Souiki^a, Pierre Frémondier^b, Emilie Clady^b,
Maxime Calka^c, Pascal Perrier^c, Mohammad Ali Nazari^c,
Yohan Payan^c, Morgane Evin^{a*}

^a Laboratoire de Biomécanique Appliquée, Univ. Gustave Eiffel, Marseille, France

^b ADES UMR 7268 Aix Marseille Univ, Marseille, France

^c Univ. Grenoble-Alpes, CNRS, Grenoble INP, GIPSA-Lab, F-38000 Grenoble, France.

* Corresponding author: morgane.evin@univ-eiffel.fr

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1. Introduction

Ankyloglossia is a condition where the lingual frenulum is shorter than normal, which restricts tongue movement. This condition affects between 0.1 % to 13.4 % of infants and can cause breastfeeding difficulties and speech disorders (Sawai et al., 2024). It can have an impact on both the infant's health and the mother's breastfeeding experience. Several studies have shown an increase in the systematic use of frenotomy (an incision is made in the frenulum to release it and improve tongue mobility) for treating ankyloglossia without a consensus on a comprehensive diagnosis (Rowan-Legg et al., 2015), which makes such frenotomy controversial.

Biomechanical features of the tongue, particularly regarding the use of models for surgical predictions, have been previously highlighted using numerical simulation (Buchaillard et al., 2007). However, the existing literature has notable limitations, primarily the lack of modeling for the restrictive sublingual frenulum (ankyloglossia). There is a need for more accurate material properties of the oral structures of children and sublingual tissue in general. This research aims at quantifying the biomechanical properties of frenulum as part of sublingual mucosal tissues and analyzing their behavior under various strain rates. The tongue's superior longitudinal muscle (SLM) was also considered in the study to be compared with the mucous membranes.

2. Methods

2.1 Sample preparation

The specimens were collected from two mammal species: swine (5 animals approximately 4 months, 32 to 45 Kg) and lamb (4 animals ranging between 5 to 12 months, approximately 59.9 ± 2.4 Kg). The animals were euthanized for reasons unrelated to this study and not impacting the lingual tissues. Muscle samples from lamb have a thickness of 3.73 mm in average compared to 3.54 mm for swine samples. Likewise, the thicknesses for mucous membrane samples were: 1.56 mm in lamb and 1.25 mm for swine.

2.2 Mechanical test

Uniaxial tensile tests were performed using an ACUMEN 3 MTS electromechanical actuator, operated with the MTS TestSuite™ Multipurpose Elite Software, with a force cell of 50 N. Tests were carried out at ambient temperature of 20°C and all samples were tightened to the self-tightening mini jaws with horizontal grooves (Fig.1.C). Tested samples were humidified along the test and the room temperature was maintained to 20°C. Mechanical properties of the superior longitudinal muscle and sublingual mucosal tissues were obtained by combining the evaluation of strain and stress at different loading speeds (1.46 %·s⁻¹, 2%·s⁻¹, 2.5 %·s⁻¹, 5 %·s⁻¹ and 10 %·s⁻¹). The testing protocol includes a pre-conditioning of 10 traction cycles (ramp shaped) with a strain rate of 2.5 %·s⁻¹ and to a displacement

	Sample type	unit	LAMB					SWINE				
			SLMa	SLMp	TTC	TTL	TTR	SLMa	SLMp	TTC	TTL	TTR
Nb of sample			6	4	3	6	5	5	5	5	5	4
Thickness	mm		3.4±0.2	4.1±0.8	1.3±0.1	1.8±0.6	1.6±0.4	3.3±0.8	3.8±0.9	1.1±0.3	1.4±0.2	1.3±0.2
1.45%/s	Elastic modulus	kPa	317.5±116.9	294.8±167.9	184.2±142.4	515.9±376.8	1773.9±975.2	500.6±404.4	337.4±141.2	1077.5±838.6	1138.3±972.1	1058.5±971.8
	RMSE	kPa	17±1.2	1.6±1.3	0.5±0.1	2.3±2.1	6.9±2.0	3.3±3.2	1.2±0.6	3.8±5.4	6.4±5.5	3.3±2.6
	Elastic modulus	kPa	477.5±162.9	462.6±248.1	182.0±66.4	736.6±551.1	2265.9±1234.9	865.5±826.8	494±200.2	1043.1±1064.9	1503.8±1248.1	1337.5±1017.9
2.5%/s	RMSE	kPa	12±0.6	0.9±0.6	1.1±0.7	0.9±0.6	3.2±1.5	1.2±1	0.8±0.4	1.31±1.31	1.7±1.8	1.5±1.2
	Elastic modulus	kPa	431.5±142.2	406.3±218.7	215.5±77.3	598.8±442.5	2005.3±1131	715.1±657.8	420.3±165.4	872.9±895.0	1087.8±1041.5	1073.5±792.7
	RMSE	kPa	0.8±0.3	0.7±0.4	1.3±0.9	0.8±0.6	2.6±1.5	1.1±1	0.6±0.3	1.1±1.1	1.8±1.6	1.4±0.9
10%/s	Elastic modulus	kPa	298.6±90.5	276.3±139.4	192.8±55.9	383±283.6	1267.9±703.6	438.2±387.9	265.5±109.6	530.3±526.2	709.9±614.6	649.3±466.4
	RMSE	kPa	0.9±0.3	0.9±0.4	0.6±0.2	1.1±0.8	3.5±1.1	1.1±0.9	0.7±0.3	1.4±1.1	1.7±1.5	1.3±0.8
	Elastic modulus	kPa	608±283.3	580.3±425	773.1±534.1	1562.6±1340.3	4122.5±2225.3	1006.8±715.1	718±281.9	2043.8±2023.1	3573.2±2288.4	1790.6±1231.4
	RMSE	kPa	3.4±1.3	2.4±1.8	5.5±3.4	8.9±6.3	17.3±12.3	9.4±7.6	6.8±7.2	12.4±10.7	20.4±14.3	12.3±6.3

Table 1. Experimental data from samples tested during protocol 1; SLMa and SLMp means “anterior” and “posterior superior longitudinal muscle”, TTC, TTL & TTR means “Tongue frenulum Center, Left and Right (location of the sublingual mucosa tissue with animal reference).

threshold of 10 % of the initial length. Next, 4 loadings are applied consisting of an increase to a displacement threshold of 40 %, a relaxation lasting 60s, and a discharge with a return to the initial state. Finally, a last load up to rupture was added (Fig.1.A). No-slipping was insured by marking the sample before the test at the jaw and visually insuring no change of the mark after testing.

2.3 Post-process

The data were post-processed using a passband filter from signal processing with Matlab software (v.R2018b, Matlab® MathWorks). Assuming a bilinear behavior, The Cauchy strain and stress together with the elastic moduli were calculated and evaluated with a root mean squared error (RMSE) and r^2 performance measure.

2.4 Statistical analysis

The Shapiro-Wilk normality test and the Wilcoxon significance test were carried out using RStudio (v2024.04.2+764 by Posit Software, PBC) for the different features considered: population (or specimen), type of sample (tissue) and strain rate.

3. Results and discussion

Overall, the elastic moduli of porcine tongue tissues appear to be higher than those of ovine tissues for most strain rates (Fig.1.B and Table.1, r^2 value higher than 0.99 in all case). This difference is more pronounced at higher strain rates (10 %.s⁻¹), suggesting that porcine tissues are stiffer, and that a notable difference exists in mechanical behavior between the two species. The first tensile pre-conditioning test was found to be systematically different from the following ones questioning the effect of loading history. A first loading effect was

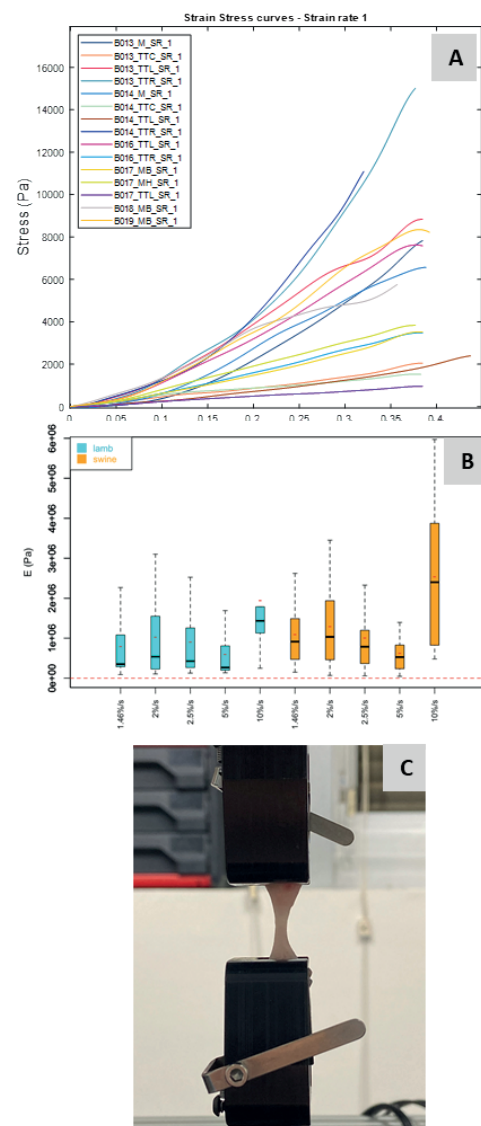


Figure 1. (A) Force (red) and Displacement (blue) curves versus time (B) Influence of displacement rates on the elastic moduli of mucous membranes for the 2 species (cyan: Lamb; Orange : Swine (C) Mechanical tests setup.

shown using a specific cyclic test procedure. A final protocol was proposed to overcome this effect of first load consisting in a preconditioning and the four other rates tests until a constant threshold of 40% except the last rupture test for the highest strain rate. This protocol suffers from several limitations. The long testing time could alter the quality of the samples and the precision of the tests. There is also a lack of uniformization in sample dissection, that leads to uncertainties related to the area measured during the test. Integrating a digital image correlation measurement could mitigate such limitations.

4. Conclusions

The preliminary results showed an increase in the elastic moduli with the strain rate increasing. The presence of a first load effect has been depicted which was overcome thanks to the preconditioning (same threshold and strain rate than first test). Further work could lead to the identification of a constitutive law toward the numerical modelling of the infant tongue as well as an attempt for the characterization of the viscoelasticity of the tissues.

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