

# A METHODOLOGICAL APPROACH TO INTERPRET AND COMPARE THE VISCOELASTIC BEHAVIOR OF BIOLOGICAL TISSUES AND HYDROGELS

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## Introduction

Cell behavior is strongly influenced by the physical properties of the microenvironment and complex mechanotransduction mechanisms are involved in cell and tissue development, homeostasis and even pathologies [1]. Thus, when developing materials mimicking the extracellular matrix of healthy or pathological tissues their mechanical features should be closely considered.

In this context, nanoindentation is a powerful technique for mechanically characterizing biomaterials and hydrogels at the cell-length scale, however, standardized experimental protocols and data analysis techniques are lacking. Here, we propose a methodological approach for quantitatively analyzing and comparing the time-dependent mechanical responses of different samples. As an explanatory study, stress-relaxation nanoindentation tests were performed on human and pig lung samples and on hydrogels in order to quantify and compare their viscoelastic properties.

## Materials and Methods

Three different samples were mechanically characterized by nanoindentation: a human lung sample from a healthy donor, produced from research-consented organ donors in the framework of the prospective clinical study PROMole, a porcine lung sample from a slaughterhouse, and a gelatin-methacryloyl (GelMa) hydrogel sample, designed for in vitro 3D modelling lung tissue. Stress-relaxation tests were performed by using the Piuma nanoindenter (Optics11) in wet conditions at 37°C (probe stiffness = 0.024 N/m; probe radius  $R = 25.5 \mu\text{m}$ ) and, setting the indentation mode (max indentation depth  $d_{max} = 1-5 \mu\text{m}$ ). To perform stress-relaxation tests the set indentation depth was reached between 0.1 s and 0.2 s. The indentation depth was then held constant while recording the load, and after 5 s the tip was retracted. For each sample, 10 indentations were performed. The experimental curves were then fitted through a genetic algorithm, imposing 15 sets of initial random parameters and a value of the cost function equal to  $10^{-4}$  as stopping criteria, and using the Prony-series reported in Eq. 1, which describes the loading history during the holding phase [2]:

$$P(t) = P(\infty) + \sum_1^N p_K \cdot e^{-\frac{t}{\tau_k}} \quad N = 2 \quad (1)$$

Where  $p_K$  and  $\tau_k$  (the characteristic time constant) are the unknown parameters. The Eq. 2 (where  $\beta_k$  is a parameter used to take into account the non-ideality of

the ramp time [3]), based on the  $p_K$  parameters obtained from the fitting procedure and defined as:

$$g_k = \frac{p_k}{16/\sqrt{3} \cdot \sqrt{R} \cdot \sqrt[3]{d_{max} \beta_k}} \quad (2)$$

permits to define the relaxation modulus:

$$G(t) = G(\infty) + \sum_1^N g_K \cdot e^{-\frac{t}{\tau_k}} \quad (3)$$

where  $G(t=0)$  is the instantaneous and  $G(t=\infty)$  is the equilibrium relaxation modulus. Then, for each sample, a set of mean  $p_K$ -parameters was obtained and consequently the mean instantaneous and equilibrium relaxation modulus were calculated, according to Eq. 3.

## Results

Representative experimental and fitted curves for the human lung sample show the accuracy of the fitting method (Fig. 2A). Figure 2B shows the curves obtained using the set of mean parameters for all the samples. Average values of the instantaneous and equilibrium relaxation modulus are reported in Table 1.

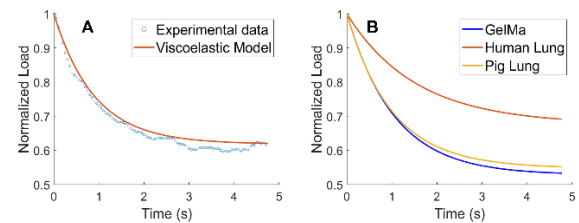


Figure 2: A) Representative experimental and fitted curves for human lung sample; B) Curves from mean parameters set for all samples.

Sample	G(0) (Pa)	G(∞) (Pa)
HUMAN LUNG	29,4 ± 23,8	22,2 ± 17,3
PIG LUNG	35,6 ± 32,1	25,0 ± 24,1
GELMA	117,0 ± 58,4	69,1 ± 39,7

Table 1: Average values of instantaneous and equilibrium relaxation modulus for all samples.

## Discussion

The proposed approach allowed comparing the time-dependent behavior of the analyzed samples. In detail, nevertheless, the high variability of the results reported in Table 1 due to the heterogeneous nature of the tested samples, a quantitative measurement of the time dependent behavior of each sample was provided in terms of instantaneous and the equilibrium response, calculating the relaxation modulus at the onset and at the end of the holding phase.

## References

1. Handorf et al, Organogenesis, 11(1):1-15, 2015.
2. Mattice et al, J. Mater. Res., 21(8): 2003-2010, 2006.
3. Qiang et al, IEEE, 58(7), 1418-1429, 2011

