

A NANOMECHANICAL ANALYSIS OF 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: 1) a human lung sample from a healthy donor, obtained from research-consented organ donors in the framework of the prospective clinical study PROMole; 2) a porcine lung sample, obtained from a slaughterhouse; 3) a gelatin-methacryloyl (GelMa) hydrogel sample, designed for in vitro 3D modelling lung tissue. Stress-relaxation nanoindentation tests were performed (probe stiffness = 0.024 N/m; probe radius $R = 25.5 \mu\text{m}$; Piuma Nanoindenter, Optics11) in wet conditions at 37°C , setting the indentation mode (max indentation depth $d_{\text{max}} = 1\text{-}5 \mu\text{m}$). The imposed indentation depth was reached between 0.1 s and 0.2 s, it was then held constant during load recording, and after 5 s the tip was retracted.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. In detail, the Prony-series that describes the loading history during the holding phase [2] was adopted:

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

Where p_K and τ_k (the characteristic time constant) are the unknown parameters. The Eq. 2 (where β_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:

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

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.

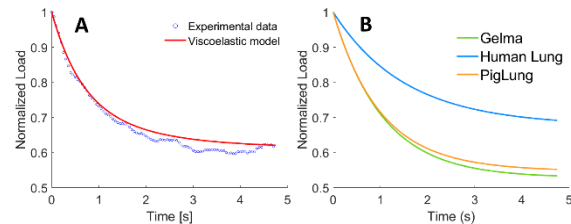


Figure 2: A) Representative experimental and fitted curves for human lung sample; B) Curves from mean parameters set for all samples.

| Sample | $G(0)$ (Pa) | $G(\infty)$ (Pa) |
|----------|------------------|------------------|
| HUMAN | $29,4 \pm 23,8$ | $22,2 \pm 17,3$ |
| Pig Lung | $35,6 \pm 32,1$ | $25,0 \pm 24,1$ |
| GelMa | $117,0 \pm 58,4$ | $69,1 \pm 39,7$ |

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

Discussion

The proposed approach allowed comparing the time-dependent behavior of the tested samples. Although the high variability of the results reported in Table 1 due to the heterogeneous nature of the samples, a quantitative measurement of the time-dependent behavior of each sample was provided in terms of instantaneous and equilibrium response, by calculating the relaxation modulus at the onset and at the end of the holding phase. Furthermore, the analysis pointed out the tremendous effect of the time-dependent phenomena on the evaluation of the mechanical properties of the samples.

References

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