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# Exploring the Correlation between Pressure and Bone Volume Fraction: Introduction to a Novel Experimental Device

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## 1. Introduction

Bone mechanobiology is known to be associated with fluid movements within a multiscale porous network. During loading, the pressure gradients developed in this porosity promote fluid flow across bone matrix that is sensed by bone cells through the fluid flow-induced shear stress. It is generally assumed that pores pressure gradients arise from bone matrix deformation. However, recent research has reviewed that the intramedullary pressure (ImP) can also promote fluid flow without bone matrix deformation (Fernandes Da Costa et al., 2024). This ImP is known to be associated with blood pressure, and increases during muscle stimulation or external loading of the bone. In the current study, the ImP will be considered as an essential boundary condition to induce fluid flow within bone porous network.

In that context, a new *ex vivo* experimental device has been developed in order to monitor the pressure developed within a bone explant subjected to an applied hydrostatic pressure. In the current study, preliminary results on the relationships between the pressure developed within the bone's and the bone vascular porous network are introduced.

## 2. Methods

### 2.1. The experimental set up

The device is a "Hassler" cell made of polycaprolactone, designed to allow the perfusion of a cylindrical bone sample while preventing leakage from the sample's surface. To that end, the sample is wrapped into a cylindrical soft sleeve onto which a constant hydrostatic pressure is applied (~5 bar) that is higher than the inlet pressure within the sample. The bone sample will thus be investigated in a confined configuration. The set up allows the application of a fluid flow or pressure through the top of the cylindrical samples using a pressure controller (OB1 MK4, Elveflow, France), while the samples outlet pressure is measured using a pressure sensor with sensitivity of 4 mbar (MPS 3, Elveflow, France).

### 2.2. Bone samples

Bovine bones from different anatomical regions were collected from the butcher. Cylindrical samples, 10 mm in diameter and height, were prepared from epiphyses, composed of trabecular bone, or metaphyses, with a gradient from trabecular to cortical bone, in order to evaluate samples with different vascular pores morphologies (n = 6). The samples were immersed in acetone and subjected to a 20-minute ultrasonic bath to remove any remaining bone marrow. Bone architecture was analysed through micro-computed tomography (V|Tome|x, Phoenix X-ray), with an isotropic voxel size of 20 µm. Data processing involved Otsu thresholding to segment the bone part. Subsequently, bone volume fraction over the tissue volume (BV/TV (%)) was then calculated.

### 2.3. Samples pressurization

The samples were infiltrated with a saline solution. A step loading pressure (ranging from 0 to 1000 mbar) was swiftly applied at the top of the sample. The pressure was maintained at 1000 mbar until equilibrium was reached, as indicated by stable pressure measurements. The resulting pressure relaxation curves were processed by fitting a stretched exponential function (Figure 1):

$$p(t) = A \left( 1 - e^{-\left(\frac{t}{\tau}\right)^\beta} \right) + B, \quad \text{Eq.1}$$

where p (mbar) is the measured pressure, t (s) is the time, τ (s) is the time constant, β is a constant used to stretch (β < 1) or compress (β > 1) the exponential and adjust the fitting (Qin et al., 2002), A and B are two constants associated with the pressure magnitude.

The relationships between the BV/TV and time constant was then analysed.

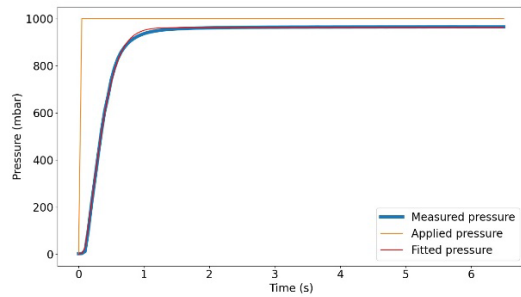


Figure 1: Example of the pressure vs time curve showing the applied, the measured, and the fitted pressure.

Sample B2:  $BV/TV = 35\%$ ,  $\tau = 0.31\text{ s}$

### 3. Results and discussion

The samples  $BV/TV$  ranged from 24 to 89%. Conversely, the time constants ranged from 0.31 to 23.51 s (Table 1). By analysing the relationships between these two parameters, a significant Pearson negative correlation was observed between  $BV/TV$  and  $1/\tau$  (Table 1).

Table 1: Summary of the median value for  $BV/TV$ , the time constant and the time constant, its inverse for each sample and results from the Pearson correlation analysis. SEE: standard error of the estimate of the resulting linear fit

Samples	B1	B2	B3	B4	B5	B6
$BV/TV$ (%)	24	35	55	70	81	89
$\tau$ (s)	0.35	0.31	0.41	3.19	7.84	23.51
$1/\tau$ ( $s^{-1}$ )	1.17	1.29	1.10	0.31	0.13	0.04
$r^{\text{Pearson}}$	<b>p-value</b>		<b>SEE</b>			
<b>-0.94</b>	<b>0.005</b>		<b>0.56</b>			

These preliminary results suggest that bone vascular network pressurization is not immediate but rather requires more time as the bone density increases. This challenges the hypothesis used in a previous study that states that fluid relaxation in the vascular pores induced by an increase in the ImP could be neglected. The assumption that the pressure relaxation curve solely characterizes the lacunocanalicular network (LCN) (Gardinier et al., 2010) may require reconsideration. Characterizing the LCN is of great interest as the osteocytes trapped within it are assumed to be the orchestrators of bone remodelling. Hence, understanding how fluids flow within the LCN is of major importance to better handle bone mechanobiology.

While only six samples have been investigated in the current study, these preliminary results show that it is relevant to investigate bone explants with different vascular morphology to enhance our understanding of bone fluid flow behaviour induced by ImP. Consequently, more samples will be examined with this new experimental device in order to validate the relationship between bone vascular network and the pressurization time constant. Additionally, the streaming potentials within the explant will also be evaluated. Such potentials

arise from bone when fluid flows within the LCN. Therefore, evaluating streaming potentials during the application of a pressure is relevant to distinguish the induced fluid flow within the lacuno-canalicular networks.

### 4. Conclusions

The present study introduces a new experimental device allowing to investigate bone fluid relaxation within a bone explant subjected to a simulated ImP. The preliminary results suggested that the fluid relaxation time within bone vascular network cannot be neglected.

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### Conflict of Interest Statement

The authors declare no conflict of interest

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