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### First identification of Wistar rat parietal bone Young's modulus through punching tests combined to finite element simulation

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#### 1. Introduction

The development of innovative medical treatments for bone regeneration requires the assessment of the *de novo* bone's mechanical properties that cannot be easily obtained from classical tests (Laurent *et al.*, 2022; Mariolani *et al.*, 2021). Animal models are often used for testing new medical device creating a critical bone defect in parietal bone (>5mm in diameter) and

monitoring the bone regeneration by *in vivo* imaging (Scomazzon *et al.*, 2024). The quality of the regenerated tissue is usually assessed through x-ray computed tomography that gives insights on the mineralized part of the bone defect. Even though the bone gap is filled with a new tissue, its quality in terms of mechanical properties remains underexplored area. Therefore, this preliminary work is binding experimental and numerical punching tests to identify healthy rat parietal bone mechanical characteristics.

#### 2. Methods

#### 2.1 Samples and mechanical test

Thanks to the approval of the Committee on Animal Care of Reims University (n°2018111612178592), parietal bone samples were harvested with a 5mm diameter trephine drill at 1500rpm under saline irrigation (n=6). A dedicated punching setup has been developed and utilized to conduct tests on whose dimensions are gathered in Table 1. Mounted on a universal tensile machine (ZWICKY0.5) equipped with a 500N load cell, samples were punched with a 2mm diameter cylindrical tool at 0.01mm/s speed. As boundary condition, the samples were maintained on the punching matrix thanks to a side clamp, shown in Figure 1A.

	Mean Value	Standard Deviation
Diameter	4.788 mm	0.0851 mm
Thickness	0.574 mm	0.0495 mm
Mass	17.777 mg	1.695 mg

**Table 1.** Characteristic dimensions and mass of the samples. Credit: Mouret V.

The samples were punched through the application of a prescribed displacement up to complete fracture while recording their force response.

#### 2.3 Finite element analysis

Finite element analyzes of the punching test were conducted with the software Abaqus/Standard 2022\* considering a 2D axisymmetric representation of the parietal bone sample as well as its close environment, including the punch, the side clamp, and the matrix. The simulation was conducted considering frictionless contacts between the sample and the other parts. Besides, the overall compliance of the setup was quantified from previous dedicated experiments and accounted for via spring elements. An isotropic linear elastic constitutive law was chosen to describe the homogeneous behavior of the bone tissue using a Young's modulus of 3GPa and a 0.3 Poisson ratio as initial guess values (Laurent *et al.*, 2022).

#### 2.4 Optimization

Due to the linear behavior of the chosen material, an optimization was made by comparison of the maximal forces to get a correction coefficient for the Young's modulus leading to an optimal correlation.

#### 3. Results and discussion

Figures 1B emphasizes a repeatable elastic response of the 6 parietal bones followed by a drop in the force indicating the samples fracture. The finite element simulation results point out that a linear elastic behavior is sufficient at first for enabling an indirect characterization of the Young's modulus for the bone tissue.

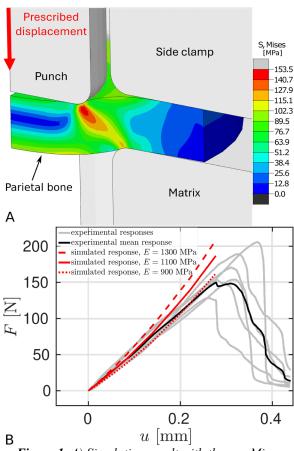


Figure 1. A) Simulation result with the von Mises stress field over the structure for a 0.27mm prescribed displacement. B) Force displacement responses of 6 rats parietal bone undergoing punching test up to fracture. Finite element simulation results are superimposed. Credit: Praud F.

Ultimately, it can be estimated to: E=1100+/-200MPa, which is consistent with the values obtained by Laurent

et al., 2022 for murine tissues. However, it is worth pointing out that this value might be underestimated since the parietal bone's porous microstructure (housing bone marrow) is not accounted for in the present description. In fact, the identified Young's modulus corresponds to the apparent stiffness of the parietal bone structure, including porosities rather than the bone material itself.

The Figure 1A shows the von Mises stress distribution suggesting a conical fracture path for the punched samples, which is in tune with the experimental observations. Indeed, x-ray tomography allowed capturing a conical shape of the punched sample. This confirms the identification procedure and the interest of this combined analysis providing intrinsic mechanical properties for regenerative treatment quality assessment.

#### 4. Conclusions

This inverse procedure for investigating the linear elastic response of the parietal bone is a first step for improving the quantitative evaluation of bone healing/remodeling on rat animal models. The use of finite element simulation allowed overcoming the size limitation while enabling indirect characterization of the bone tissues. The use of x-ray scan will help take into account a better description of the microstructure morphology (Laurent *et al.* 2022) while using the phase field fracture approach (Praud *et al.* 2021) to simulate the fracture behavior of bone tissues. Ultimately, anisotropy can be considered according to second harmonic generation microscopy observations.

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#### **Conflict of Interest Statement**

None

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