Ex vivo analysis of pressure and streaming potential in bone explants during loading: development of a new experimental protocol

Elisa Bertrand[1,2], Guilhem Rival[2], Benoît Ter-Ovanessian[1], Nina Attik[3], and Rémy Gauthier*[1]

[1]Matériaux, ingénierie et science [Villeurbanne] – Université Claude Bernard Lyon 1, Institut National des Sciences Appliquées de Lyon, Centre National de la Recherche Scientifique, Centre National de la Recherche Scientifique : UMR5510 – France

[2]Laboratoire de Génie Electrique et Ferroélectricité – Institut National des Sciences Appliquées de Lyon – France

[3]Laboratoire des Multimatériaux et Interfaces – Université Claude Bernard Lyon 1, Institut de Chimie - CNRS Chimie, Centre National de la Recherche Scientifique – France

Abstract

Introduction
Bone remodelling is known to be driven through biomechanics. Mechanobiological mechanisms operating in bone architecture are based on the circulation of fluids within bone multiscale porous network. During loading, pressure gradients arise in bone porosity due to matrix deformation. It is assumed that the osteocytes, bone cells orchestrating the biology of bone remodelling, are able to sense the shift in fluids velocity and pressure induced by such poroelastic behaviour1. Fluid hence acts as the intermediate transmitting the mechanical information from the organ to the cell's scale. Understanding how fluid behaves during loading thus appears essential to better handle bone remodelling and further develop suitable bone regenerative strategies.

To that end, it is important to focus on bone porous network that represents the fluids pathway. In the current study, we will focus on two different pores scales: the vascular and the lacuno-canalicular networks. The vascular porosity is the largest porosity within bone tissue. It corresponds to the 100 μm cylindrical pores observed within cortical bone2 and to the large pores of spongy bone3. This porosity is filled with the blood vessels, the bone marrow, and different cells involved in bone resorption and formation. At a lower scale, the submicrometric lacuno-canalicular porosity tightly trapped within bone mineralized matrix hosts the interconnected osteocytes network4, bone mechanosensitive cells. These two porous networks are involved in fluid behaviour during loading. But due to their different size, their influence on osteocytes mechanical stimulation is different. Distinguishing these two types of porosity during load-induced fluid behaviour is hence of great interest.

While fluid relaxation during loading is associated with both the vascular and lacuno-canalicular porosities, it is assumed that the development of streaming potential is mainly

*Speaker

due to fluid circulation within the lacuno-canalicular network5. Because bone surface is negatively charged, an outer counter-ions diffuse layer is formed at the pores wall. During load-induced fluid flow, this charged layer is advected inducing the generation of streaming potentials. Due to a high specific surface (> 20,000 mm2.mm-3) compared to the vascular network (< 100 mm2.mm-3 in cortical bone), these potentials are generally attributed to the fluid circulation within the lacuno-canalicular network. Measuring these potentials will provide insights on fluid flow within this lacuno-canalicular porosity. The objective of the current study is thus to develop an experimental protocol allowing to measure both the fluid relaxation and streaming potentials within bone explant under loading.

Materials & methods

Bone samples: Cylindrical bone samples (10 mm in diameter and height) were harvested from bovine femoral condyle, femoral head, and tibial plateau obtained from the butcher. Samples (n=14) were scanned through micro-computed tomography (voxel size = 10 μm) in order to calculated their bone volume fraction (BVTV in %). An Otsu automatic threshold was applied in order to segment the bone from the vascular porosities. BVTV was calculated as the ratio between the volume of segmented bone over the total volume of the sample.

Experimental set up: In parallel, an experimental device was developed allowing to apply a fluid pressure within a bone explant while measuring both the outlet relaxation pressure and the potential difference developed within the sample. Briefly, the device is made of a polycarbonate Hassler cell, designed to prevent leakage from the bone sample surface during pressurization. To that end, the cylindrical bone sample is wrapped into a polymeric soft sleeve subjected to a hydrostatic pressure (5 bar) to hold it against the sample and prevent fluid to flow out from bone porosity. The bone sample is then in a confined configuration. A fluid pressure will be applied using a pressure controller (OB1 MK3, Elveflow, France) while the outlet pressure is measured using a pressure sensor with a 4-mbar sensitivity (MPS 3, Elveflow, France). The potential difference was measured using home-made Ag/AgCl electrodes. Silver wires were coated with AgCl for 2 min in a FeCl3 bath (50 mM) and placed within a 3D printed electrode filled with KCl (3.5 M) that can be integrated within the hydraulic circuit. The potential developed during loading was measured using a potentiostat (Interface 1000, Gamry, USA) in open circuit potential configuration.

Samples pressurization: The samples were immerged in a saline solution at least the night before the experimentation. A step loading pressure (ranging from 0 to 1000 mbar) was applied at the top of the cylindric sample. A 1000 mbar pressure was maintained for 15 s and then released for 15 s. The resulting pressure and potential relaxation curves were processed by fitting a stretched exponential.

Results:

The samples BVTV ranged from 23.8 to 88.6% (mean = 55.8% and median=55.8%). The outlet pressure time characteristics ranged from 0.11 to 0.77 s (mean = 0.33 s and median = 0.25 s). A significant correlation was found between BVTV and the pressure characteristic time (ρ(Spearman) = 0.82, p-value = 0.0005). The streaming potential's characteristic time was several times higher than the pressure characteristic time. However, in the current version of the experimental set up, the reproducibility for the streaming potential measurement remains poor. Further improvements in the home-made Ag/AgCl electrodes need to be achieved.

In conclusion, a new experimental device has been developed allowing to measure fluid-related parameters during bone explant bone loading, distinguishing the vascular and lacuno-canalicular networks. In the future, such set up could be used to perform mechanobiological experimental while monitoring bone fluid behaviour.

Reference:

1. Weinbaum S *et al.* J Biomech 1994. https://doi.org/10.1016/0021-9290(94)90010-8.

2. Gauthier R *et al.* J Struct Biol 2018. https://doi.org/10.1016/j.jsb.2018.08.006.

3. Follet H *et al.* J Orthop Res 2011. https://doi.org/10.1002/jor.21275.

4. Yu B *et al.* J Struct Biol 2024. https://doi.org/10.1016/J.JSB.2024.108111.
5. Lemaire T *et al.* J Mech Behav Biomed Mater 2011. https://doi.org/10.1016/j.jmbbm.2011.03.007.