Experimental report

20/05/2020

Proposal: 8-05-432 **Council:** 4/2018

Title: Understanding bone collagen structure with Small Angle Neutron Scattering

Research area: Materials

This proposal is a new proposal

Main proposer: Hanna ISAKSSON

Experimental team: Ulf OLSSON
Elin TORNQUIST
Sophie LE CANN
Luigi GENTILE

Local contacts: Sylvain PREVOST

Samples: bone

Instrument	Requested days	Allocated days	From	To
D11	3	3	14/10/2018	15/10/2018
			25/06/2019	28/06/2019

Abstract:

An interest in understanding the structure-composition-mechanical function of musculoskeletal tissues has led to become frequent users of SAXS. SAXS data from bone tissue is heavily dominated by the mineral scattering, while we are highly interested in the behaviour of the collagen network, specifically its alterations in certain metabolic bone diseases. Therefore, we are looking for alternatives to SAXS. We believe that SANS will result in a different contrast between the phases in bone, and therefore better be able to answer our research questions related to bone collagen. A first pilot SANS experiment was carried out at SINQ, PSI. Although promising, the signal-to-noise ratio was not sufficient to resolve our question and determine the potential of the technique, and to address more relevant model systems of smaller animals. Thus, we now propose an experiment on rabbit bone ranging from newborn to adult bone as a representation of tissue with different mineral-collagen structure and orientation. We aim to obtain quantitative information of the collagen structure in bone. Data will be compared to SAXS data recently obtained from the same samples.

Understanding bone collagen structure with Small Angle Neutron Scattering

Experimental report – D11 SANS – ILL, Grenoble (06/2019)

Experiment No.: 8-05-432

A) Overview

Background: Bone is a hierarchically structured two-component material, made up of hydroxyapatite crystals interwoven in a collagen matrix [1]. Small-angle x-ray scattering (SAXS) is the state-of-the-art modality for investigating bone nanostructure. Bone fragility may be highly linked to changes in the collagen matrix, which is not well captured with x-rays. Due to the different interaction of neutrons with matter, especially hydrogen, we hypothesise that SANS can be used to look at the collagen matrix.

Aims: Investigate whether small-angle neutron scattering can be used to focus on the collagen matrix at the nano-scale of bone. Compare small-angle neutron and x-ray scattering to clarify their complementarity.

***Method*:** Compare small-angle neutron and x-ray scattering, from the same specimens and measurement positions, in terms of scattering curve characteristics.

B) Experiment

Specimens: Specimens consisted of orthogonal (longitudinal and radial, Figure 1a) sections, 1 mm in thickness, of cortical bone taken from femora and tibia of three different species (bovine, porcine, and ovine).

SANS: Each specimen was placed in an empty 2 mm Hellma quartz cuvette and measured at three vertical positions, identified using transmission measurements, along the midline. All three specimen-detector distances (SDD) were used in combination with a wavelength of 5.6 nm to cover a q-range of 0.0005-0.5 $Å^{-1}$. All measurement parameters are found in Data reduction and subsequent angular integration was done in GRASP (GRASansP Barebones v. 8.14, Charles Dewhurst, Institut Laue Langevin, France). The beamstop was masked away and the data was integrated both over 360 deg. (full integration) and along the main mineral scattering and collagen scattering directions (partial integration for anisotropy analysis). The partial integration was realised as strips centred at the beam centre and extending well beyond the region where counts were detected. The integrated data was merged using Matlab (R2019ab, The MathWorks, Inc., MA) in order to have a continuous q-range (Figure 1c).

Table1.

Background measurement was done on an empty cuvette (EC), and references on the blocked beam (Cd), and open beam (EB) were done for the same duration as detailed in Data reduction and subsequent angular integration was done in GRASP (GRASansP Barebones v. 8.14, Charles Dewhurst, Institut Laue Langevin, France). The beamstop was masked away and the data was integrated both over 360 deg. (full integration) and along the main mineral scattering and collagen scattering directions (partial integration for anisotropy analysis). The partial integration was realised as strips centred at the beam centre and extending well beyond the region where counts were detected. The integrated data was merged using Matlab (R2019ab, The MathWorks, Inc., MA) in order to have a continuous q-range (Figure 1c).

Table1, once at each SDD accept for Cd at 39 m due to it being negligible.

Data reduction and subsequent angular integration was done in GRASP (GRASansP Barebones v. 8.14, Charles Dewhurst, Institut Laue Langevin, France). The beamstop was masked away and the data was integrated both over 360 deg. (full integration) and along the main mineral scattering and collagen scattering directions (partial integration for anisotropy analysis). The partial integration was realised as strips centred at the beam centre and extending well beyond the region where counts were detected. The integrated data was merged using Matlab (R2019ab, The MathWorks, Inc., MA) in order to have a continuous q-range (Figure 1c).

Table1. Measurement parameters.

Sample	Wavelength [nm]	Beam spot size [mm]	SDD [m]	Exposure time [min]
Bovine	5.6	2	1.4	20
Ovine	5.6	2	8	40
Porcine	5.6	2	39	60

C) Ongoing analysis

Complementary small-angle x-ray scattering (SAXS) datasets were collected from the same specimens and measurement positions. The data has been compared in the overlapping q-region (0.003-0.145 $Å^{-1}$) with regard to scattering curve shape and position of curve characteristics after full (360 deg.) azimuthal integration, and q-dependent anisotropy after full (360 deg.) azimuthal integration in different q-intervals.

D) Conclusions and feedback

SANS can be used to investigate bone nano-structure. However, the SANS and SAXS data have been compared in the overlapping q-region and the results show striking similarities between the modalities. A manuscript will be submitted shortly (May 2020).

The allotted beamtime was enough to carry out all the planned measurements. Our beamtime had first started during the forced reactor shutdown in October 2018, and was later re-scheduled. During the first beamtime we had the time to try different setups and determine measurement parameters, which meant we did not have to spend time doing this during the second beamtime.

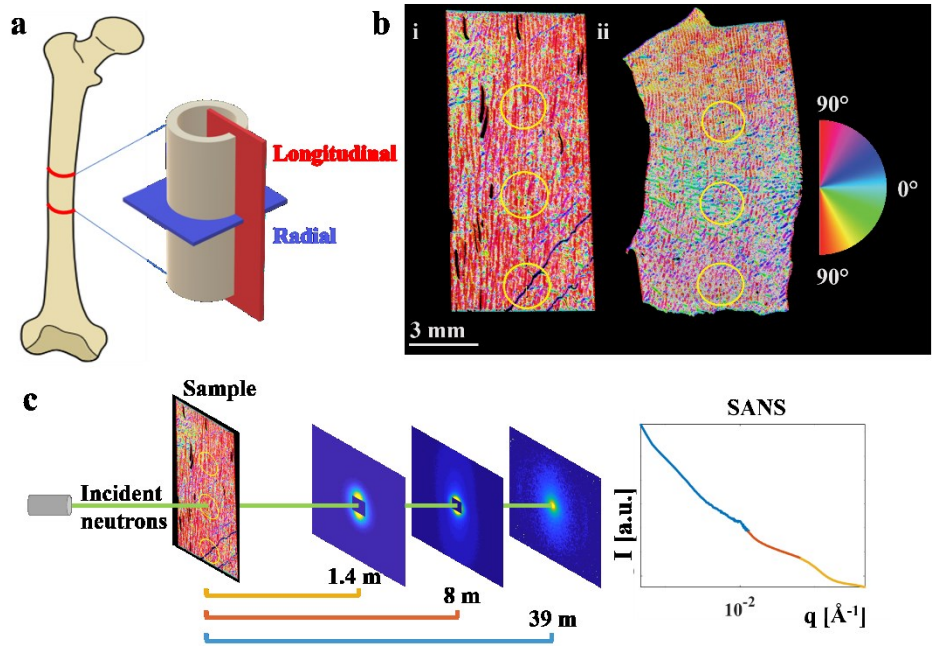

Figure 1. a) Specimens were taken as orthogonal sections (longitudinal, radial). b) The microstructural orientation was extracted from micro-CT images of the samples. c) SANS measurements were carried out with three different specimen-detector distances (SDD) in order to cover a q-range of 0.0005-0.5 $Å^{-1}$.

References

[1] Bala, Y., D. Farlay, and G. Boivin, "Bone mineralization: From tissue to crystal in normal and pathological contexts," *Osteoporos. Int.*, vol. 24, no. 8, pp. 2153–2166, 2013.