

Spatial Analysis of Human Bone

By LA-ICP-MS

- Spatial analysis of bone biopsy samples at 4 μm resolution
- Substantial progress towards identifying specific fate of administered metallodrugs

Introduction

The elemental bulk composition of bone is an important indicator of health and of the impact of the diet of an individual. Elemental data is easily interpreted, but does not include information about specific regions of the bone and gives no analysis of the important mineralization processes, which lay down new bone tissue. Bones are active throughout the life of any organism and consist of a number of different tissue types. Even in mature individuals, osteoblast cells are continuously laying down new material in mineralization processes, occurring at the micron and sub-micron scale, whilst in other areas, older material is re-absorbed.

Laser sampling for ICP-MS analysis (LA-ICP-MS) has already been used in this application area, directed towards analysis of tissue in microtomed samples. The preparation of microtomed samples is already an established technique for autoradiography studies. Tissue is deep frozen, then embedded into a block of carboxymethyl cellulose. Thin (50 micron or less) slices of tissue are cut from the frozen bulk tissue. The 50-micron slices are then freeze-dried under vacuum, following which the sliced sample is robust enough to be handled and analysed at room temperature. The sample is also preserved for a long period by this process.

In recent LA-ICP-MS studies of microtomed tissue samples, quantitative bulk analyses of around 20 tissue types have been achieved. Standard blood samples, spiked with the elements of interest, which had been prepared in the same way as the unknown samples, were used successfully for instrument calibration.

In this study the New Wave Research UP213 (213 nm) Laser Ablation System was used.

The purpose of this present investigation was to further develop LA-ICP-MS protocols to yield spatially resolved analysis of bone samples in an attempt to discover the long term fate of the metallodrug Fosrenol[®]. Comparing this analysis with bulk analyses and with the composition of older bone tissue could indicate how certain elements behave in the mineralization processes.

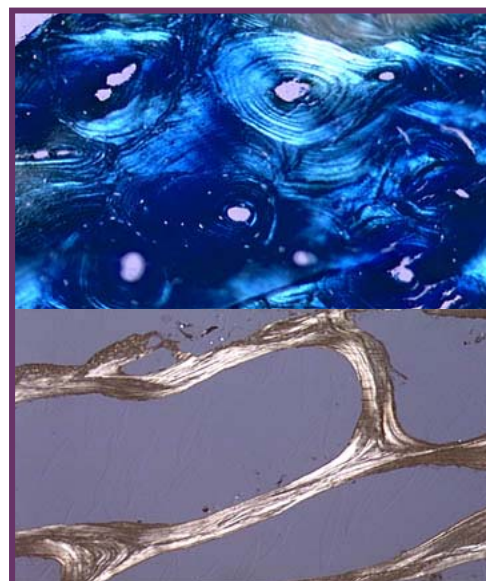


Figure 1: Cortical and trabecular bone tissues



Figure 2: Continuous laser line using a 4 μm and 10 μm crater



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Samples and Sample Preparation

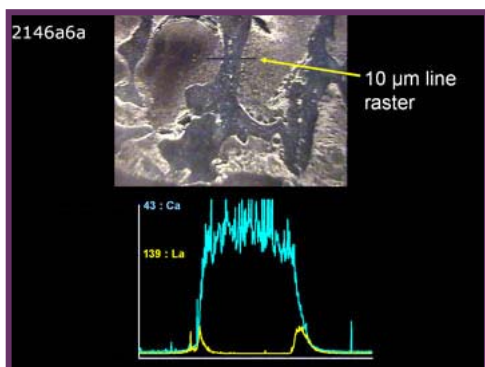


Figure 3: Bone biopsy, showing laser sampling tracks using a 10 µm crater

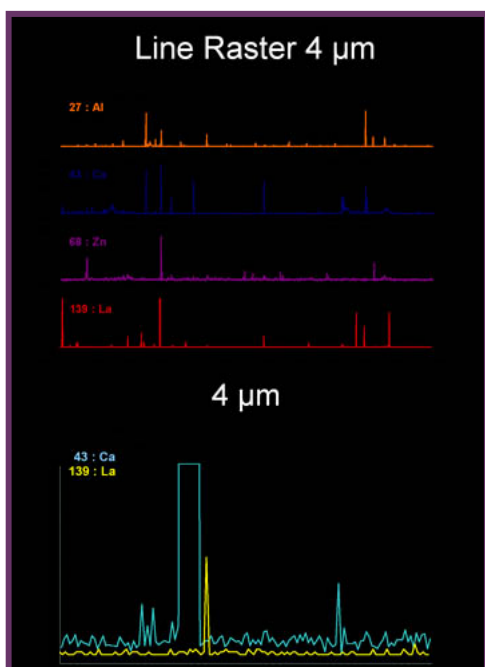


Figure 4: Laser analysis using a 4 µm crater and a more detailed view, showing the La signal separated from the Ca signal

All bone biopsies in this study were from renal failure patients receiving lanthanum carbonate (Fosrenol®) treatment. Figure 1 illustrates that bone consists of a number of very specialised tissue types. Relatively dense, hard cortical bone, found in the shafts of long bones and outer plates of smaller bones, is completely different to trabecular bone, which has a mesh-like structure, yielding a high strength and low-density tissue.

Figure 2 shows a bone biopsy, which has been sampled using two tracks of craters at two crater diameters. Multi-element time-resolved ICP-MS data is acquired continuously, during the sampling of material along a previously defined track. The resulting data for each element of interest shows how the intensity of that element varies along the sample track. When a large crater diameter (100 µm) is used, relatively little detailed information about trace and minor element distributions are produced. Using a 10 µm crater size, the spatial resolution of the analysis is greatly improved. In the example shown in Figure 3, the concentration of lanthanum is clearly much higher at the very edge of the sectioned trabecular bone than in the bulk of the specimen.

At even higher spatial resolution, (using a 4 µm crater diameter) a very narrow band of high La concentration is shown, in Figure 4, at the very leading edge of a mineralization zone.

As already discussed, many bone samples are quite complex in their detailed structure, so analytical data from even a short traverse of a single bone biopsy will often cross a number of tissue types. The bone biopsy in Figure 2 has been sampled using a 10 µm diameter pass and a 4 µm diameter pass. The multi-element data acquired during the 4 µm pass shows a series of high Ca and La zones, shown in Figure 4.

In the expanded time-base display of the data acquired after around 600 seconds, Figure 4 (zoom in) shows that there is complete separation of the simultaneously acquired Ca and La signal peaks. This shows that La signal is derived entirely from the mineralization front and not from within the bulk of the bone material.

Conclusions

Laser sampling protocols for ICP-MS analysis can be established that give detailed information about elemental distributions in individual tissue types in bone samples.

Spatial resolution below 5 µm allows the study of the occurrence of elements in the mineralization zones of bone tissue. This resolution is fine enough to show that some elements, such as La, occur predominantly in mineralization zones and are not incorporated in the bone itself.

With this physical resolution in laser sampling, LA-ICP-MS is being used to examine how metallo-drug compounds interact with different types of bone tissue to determine the long term fate in the body.

Acknowledgement

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