Application Note: 40764

Quantitative & Spatial Elemental Analysis of Bone Samples using Laser Ablation Coupled with X Series ICP-MS

Key Words

- Biological Samples
- ICP-MS
- Laser Ablation
- Spatial Analysis
- Synthetic Standards

Introduction

Many biological studies require quantitative spatial distribution data for selected elements in tissue samples and the spatial analysis capability of LA-ICP-MS can serve as a useful tool in the context of these studies. Examples of such studies include drug efficiency testing, cancer research and investigative studies into Alzheimer's disease and Osteoporosis. In the past, the lack of matrix-matched standards has been one of the shortcomings of the analytical technique, limiting the acceptance of LA-ICP-MS for biological research. The use of matrix-matched standards is deemed to be critical to the quality of quantitative LA-ICP-MS data in order to ensure similar laser coupling efficiencies between standards and samples and hence similar analytical sensitivities.

This application note describes a novel methodology for production of homogeneous, matrix-matched standards to enable quantitative, spatial elemental analysis of bone samples. Furthermore, element migration patterns are investigated in bone samples in accordance with the development of a new metallic bone implant material - pioneered for bone fracture repairs. The study focuses on a pilot study undertaken using rabbit bones and a cross-section of bone containing the implanted material is shown in Figure 1.

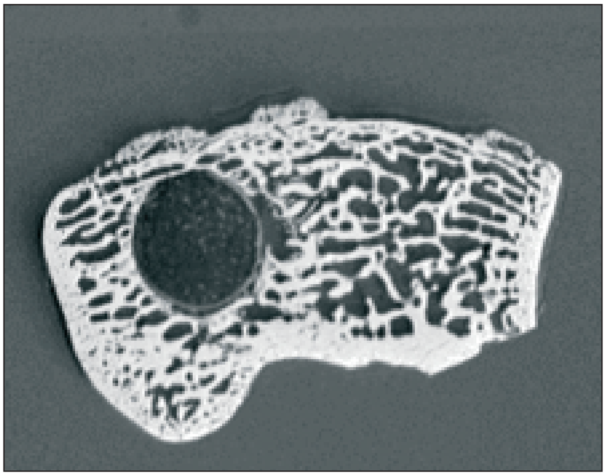

Figure 1: Cross-section of rabbit bone containing the new metallic bone implant material.

Traditionally following a complex limb fracture, metallic pins are inserted into bone material to maintain correct alignment during the healing process. However, a second operation is usually required to remove these pins when the bones have healed. A new research study is investigating the use of pins made from a unique metal alloy, which dissolves in the body over a period of a few months, eliminating the need for a second operation. However, during this dissolution process, elemental constituents from the implant will be distributed within the surrounding bone and soft tissue and some of the elements may be transported in the bloodstream to various organs within the body. It is therefore of vital importance to study the distribution of the elemental constituents derived from the implant material in order to understand their fate in the body during and after the dissolution process.

Analytical Requirements

A key requirement is to analyze the spatial distribution of major elements such as Mg, Al, Zn and Li in the bone material adjacent to the implant material as well as monitoring minor rare earth element constituents such as La, Ce, Nd, and Dy. Element concentrations are required to be measured in the area surrounding the bone implant, to assess the progress of the dissolution process and the subsequent distribution of elements in surrounding tissues. LA-ICP-MS is considered to be the technique of choice in the context of this application, providing flexible spatial analysis capability, high sensitivity and wide dynamic range.

X Series ICP-MS coupled with Laser Ablation

Thermo ELECTRON CORPORATION

Production of Matrix-Matched Synthetic Standards for Biological Sample Analysis

The availability of matrix-matched standards is a key requirement for fully quantitative LA-ICP-MS analysis and it is important to match the densities of both the standards and the samples to be analyzed. In this study, standards were prepared from hydroxyapatite which was precipitated from $CaCl_2$ * $_2H_2O$ and Na_3PO_4. Solid multi-element standards were prepared by mixing the hydroxyapatite powder with aqueous multi-element solutions and a binding reagent. Two binders (PVA and Cellulose) were evaluated in this study to fix the fine hydroxyapatite powder and to match the organic content and the density of the standards with the bone samples. Each mixture was dried and powdered to a resulting grain size of < 80 µm and the powders were pressed into pellets using a pressure of 4 t for 30 seconds. A series of standards were prepared containing elements in the concentration range of 0-100 ppm using this approach and Figure 2 shows the density of the blanks and standards depending on the % of PVA binder used for reference

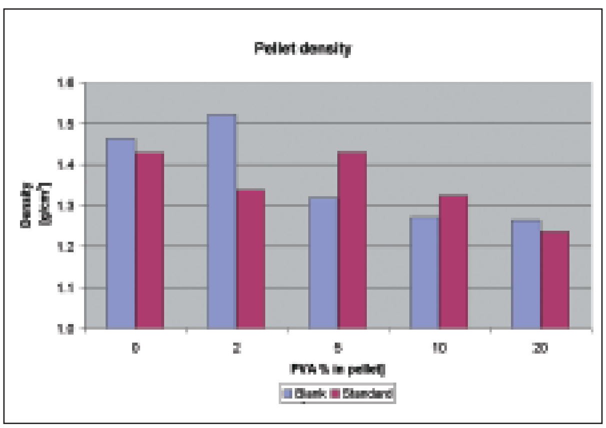

Figure 2: Variation of pellet density (g/cm³) with the percentage of binder used.

The density of natural bone can vary depending on the age of the bone and can also vary between humans and animal species. However, a natural healthy bone has a typical density of 1.8-2.0 g/cm³. The density of the tablet can be varied to up to 2.1 g/cm³ by varying the pressure at which the pellets are pressed. This approach was used to achieve a density of 2.1 g/cm³ for the calibration standard pellets used in this study.

Analytical Results

Once the density of the standard was matched to the bone, the next step was to study the homogeneity and ICP-MS sensitivity when using the two different binder materials. The standards were pressed with and without a binder and the pellets were then analyzed using the UP213 laser coupled to an X Series ICP-MS using the parameters shown in Table 1. The analysis was performed using nine spot ablations with separations of 250 µm. Figures 3a & 3b show the homogeneity and sensitivity, respectively, for several elements.

LASER PARAMETER	PRE-ABLATION	ABLATION
Crater Size	100 µm	80 µm
Laser Pattern	spot	spot
Frequency	10 Hz	10 Hz
Ablation time	5 sec	20 sec
Energy Density	4.1 J/cm²	4.6 J/cm²

Table 1: Laser Parameters

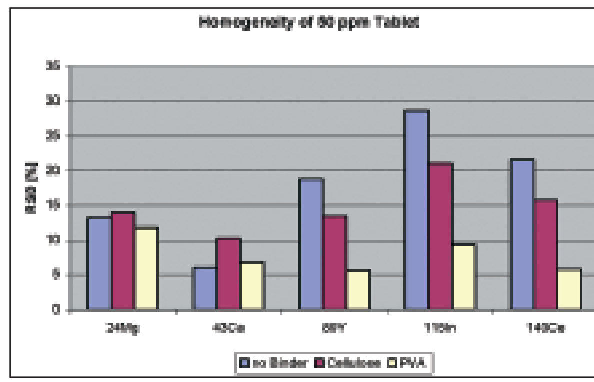

Figure 3a: Variation of pellet homogeneity with 10 % binder content.

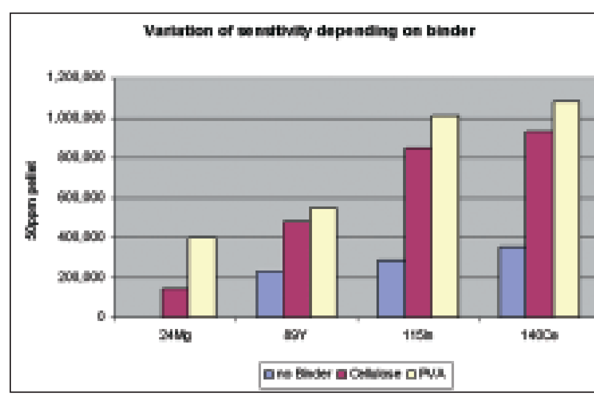

Figure 3b: Variation of ICP-MS sensitivity depending upon type of binder used (at 10 % composition).

The results in Figure 3a and 3b show clearly that PVA is the binder of choice, providing both better homogeneity and better sensitivity. Figure 4a shows how sensitivity of the elements changed with the percentage of PVA binder used. The percentages used were 0, 2 %, 5 %, 10 % and 20 %.

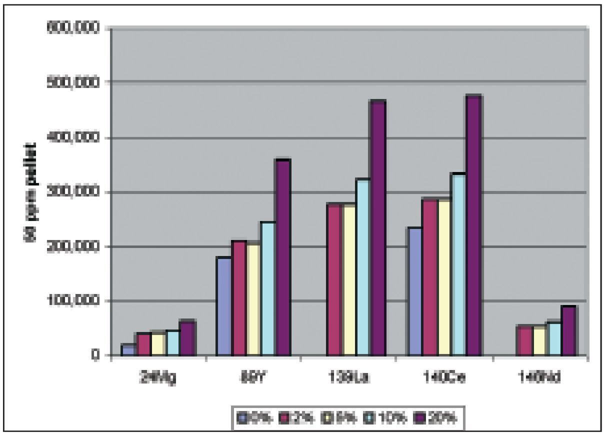

Figure 4a: Variation of sensitivity related to the percentage of PVA binder used

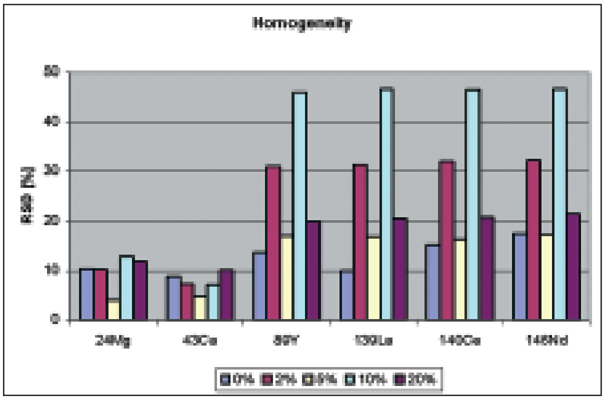

Figure 4b: Variation of pellet homogeneity related to the percentage of PVA binder used.

The best homogeneity was achieved using 5 % PVA binder (Figure 4b) and compromise conditions were achieved with respect to the analytical sensitivity of the ICP-MS. The 5 % PVA binder composition was therefore used to produce the synthetic, matrix-matched standards in this study.

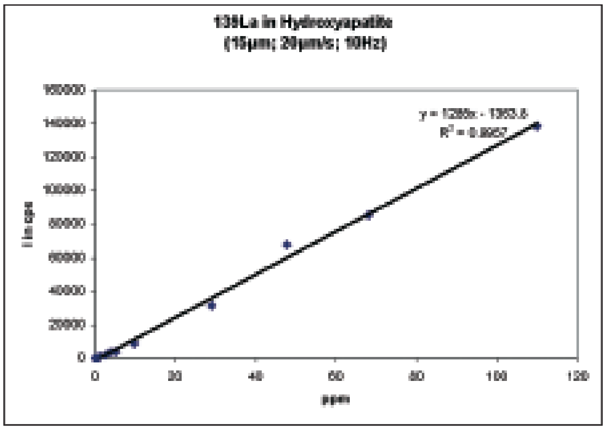

Figure 5: Example of a calibration graph using the matrix matched standards.

Figure 5 shows an example calibration curve established by analyzing the series of standards with varying concentration. The linearity of this curve (R2 0.9957) validates the described analytical approach.

Table 2 shows the typical composition of the implant material, as well as that of the natural healthy bone. The implant and bone samples were initially dissolved and measured as solutions using ICP-OES and ICP-MS instrumentation. However, all spatial sample information was lost during this sample preparation technique. Quantitative LA-ICP-MS data was then generated using the matrix-matched standards for comparison and the measured LA-ICP-MS data shows good agreement with the ICP-OES/ICP-MS solution data.

ELEMENT	TYPICAL IMPLANT CONCENTRATION (%)	ICP-OES/ICP-MS ANALYSIS OF HEALTHY BONE (%)	LA-ICP-MS ANALYSIS OF BONE AT 0.1 MM FROM IMPLANT MATERIAL	
			0 weeks after implant operation (%)	4 weeks after implant operation (%)
Mg	80-96	0.2	0.18	>1
Al	2-7	0.00002	ND	ND
Y	1-3	0.00003	ND	ND
Li	0.2-4	0.00001	ND	ND
La	0.6-6	0.00002	0.0001	0.003
Ce	0.5-15	0.00002	<0.00003	0.007
Nd	0.4-2.7	<0.00001	<0.000025	0.001
Dy	0.1-0.3	<0.000001	<0.00005	<0.00005

ND analyte not detected

Table 2: Quantitative Analytical Data

Quantitative LA-ICP-MS data shows that La, Ce and Nd concentrations increase notably in the bone material (at 0.1 mm from the implant) between week 0 and week 4 of the study. These observations provide supporting evidence for the partial dissolution of the implant material and migration of elemental constituents into the adjacent tissues.

Figure 6 shows a laser traverse across a section of implant material embedded in rabbit bone. Laser-parameters are comprised of a 15 µm crater, laser frequency of 10 Hz; scan speed of 20 µm/sec and 6.8 J/cm^2 energy at the sample surface.

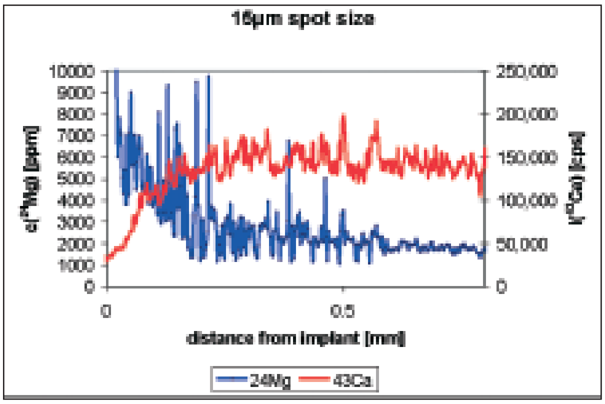

Figure 6: LA-ICP-MS data for Mg concentration (ppm) and Ca Intensity (cps) during traverse from implant (left) into bone

Elements of interest are at very low, but constant concentration levels at a distance of 0.5 mm from the implant. There is, however, a high Mg concentration of 80-95 % in the implant itself and high Mg concentrations (0.5-1 %) are observed in the bone adjacent to the implant edge (i.e. at distance <0.2 mm from the implant). The Mg concentrations are observed to decline with increasing distance from the implant edge - suggesting that Mg is migrating from the implant into the surrounding bone structure.

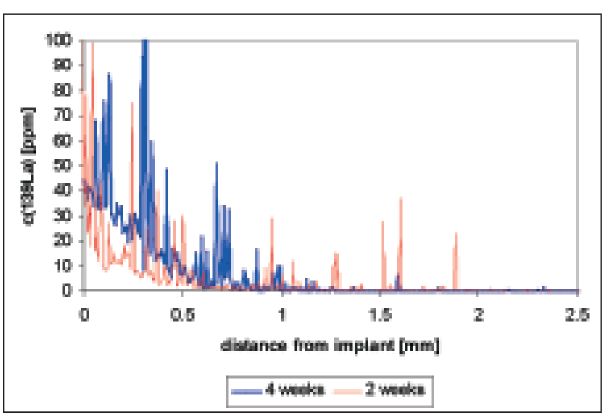

Figure 7: La data two and four weeks after the implant operation

Figure 7 shows La concentrations resultant from two laser traverses from the implant into the surrounding bone at different time intervals. The first traverse (red trace) was performed two weeks after the implant and the second laser traverse (blue trace) was performed four weeks after the implant. During this time period, the dissolution process of the implant is observed to have started and is illustrated by the higher La concentrations. The La concentration within the bone is approximately 0.0001 %. However, this concentration increases with increasing proximity to the implant material. Two weeks after the implant operation the La concentration is observed at around 0.0025-0.0003 % at a distance of 0.1-0.5 mm from the implant. However, after 4 weeks the La concentration is observed at around 0.0035-0.001% over a distance of 0.1-0.5 mm from the implant material.

Summary

The results of the first biological pilot study on the use of a new implant material are very encouraging. Partial dissolution of the implant material is observed through the application of a novel quantitative LA-ICP-MS methodology and the spatial analysis capabilities of the LA-ICP-MS system enable effective monitoring of element migration patterns during the implant dissolution process. Use of synthetic matrix-matched standards is considered a key requirement for quantitative LA-ICP-MS analyses of these biological materials and this study describes a successful standard preparation methodology to enable this approach. The X Series ICP-MS coupled with Laser Ablation serves as an invaluable tool for both qualitative and quantitative analyses of biological materials.

Acknowledgments

S. Gruhl, C. Vogt, M. Lange

University Hannover,

Institute for inorganic Chemistry, Germany