ESRF	**Experiment title:** Trace element mapping in human and animal bone and in mineralized rat oteoblast cell cultures, with particular interest to strontium	**Experiment number**: LS2137
Beamline: ID18F	**Date of experiment**: from: 17/04/2002 to: 25/04/2002	**Date of report**: 28/08/2002
Shifts: 20	**Local contact(s)**: Dr. Andrea SOMOGYI, Dr. Michael DRAKOPOULOS	*Received at ESRF:* *28/08/2002*

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Report:

Background:

Recently, in the frame of a retrospective epidemiological study we noted increased bone strontium (Sr) levels in dialysis patients with osteomalacia, a bone disease characterized by a defective mineralization.(1) In a Sr-loaded chronic renal failure rat we were able to localize the element at the osteoid/calcification front, a critical site in bone mineralization, by both histochemistry and EPXMA.(2) However, as in dialysis patients, the bone Sr concentration are 10 to 30-fold lower these techniques appear to be not sensitive enough to allow a spatial localization of the element in bone sections.
The mechanism by which Sr impairs mineralization is not yet clear. This might either be due to a pure physicochemical process or an indirect interference by affecting osteoblast function. To further elucidate these issues it might be of interest to check whether this metal (i) is incorporated in the hydroxyapatite crystal lattice (ii) disturb the crystal lattice.

Materials and Methods:

Human bone: 5µm slices of human bone biopsies embedded in glycol methyl methacrylate, were used for localization of Sr. These biopsies have been taken for diagnosis of aluminum-related bone disease and foregoing epidemiological studies on the spectrum of renal osteodystrophy in the current dialysis population.(3)
Rat Bone: Bone samples of strontium loaded chronic renal failure rats and animals that have not received this compound (controls) were, prior sectioning to 5µm thin bone slices embedded in methyl methacrylate after fixation in Burkhardt solution and ethanol.
Cell Culture. The osteoblastic osteosarcoma cell line UMR-106 was grown to confluence after which Sr was added to the culture medium at various concentrations ranging from 0 to 100 mg/l. Under appropriate cell culture conditions cells start to mineralize. This process seems to be mediated by the presence of Sr in a dose-dependent way. For the purpose of microanalysis, mineralized cell cultures were fixated in Burkhardt fixative before embedding in glycol methyl methacrylate to allow the preparation of µm-thin slices. Alternatively, mineralized cells were washed with demineralized water, scraped off from the culture dish, centrifuged and air dried to form mineralized pellets.

Results:

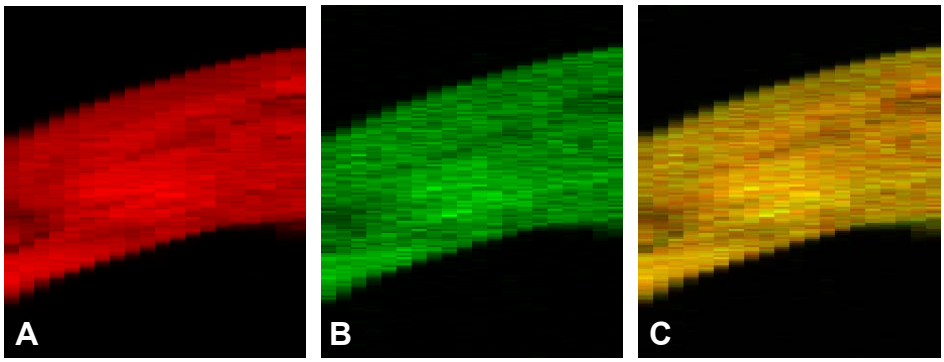

Figure 1: XRF multi-element mapping of human trabecular bone in a bone biopsy from a dialysis patient with osteomalacia, (A) CaKα image, (B) SrKα image and (C) combination image. These mappings indicate the colocalisation of both elements in the mineralized parts of the trabeculae.

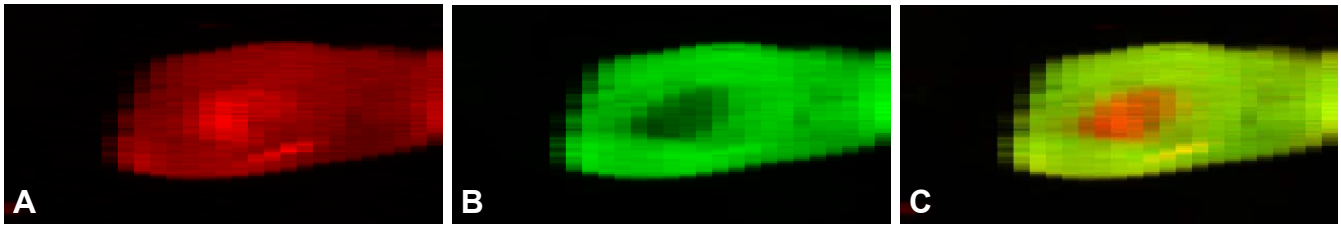

Figure 2: XRF multi-element mapping of trabecular bone of strontium loaded chronic renal failure rats with osteomalacia. CaKα image (A), SrKα image (B) and the combination image (C) indicate regions of high and low Sr-content within the calcium rich (mineralized) parts of the trabeculae.

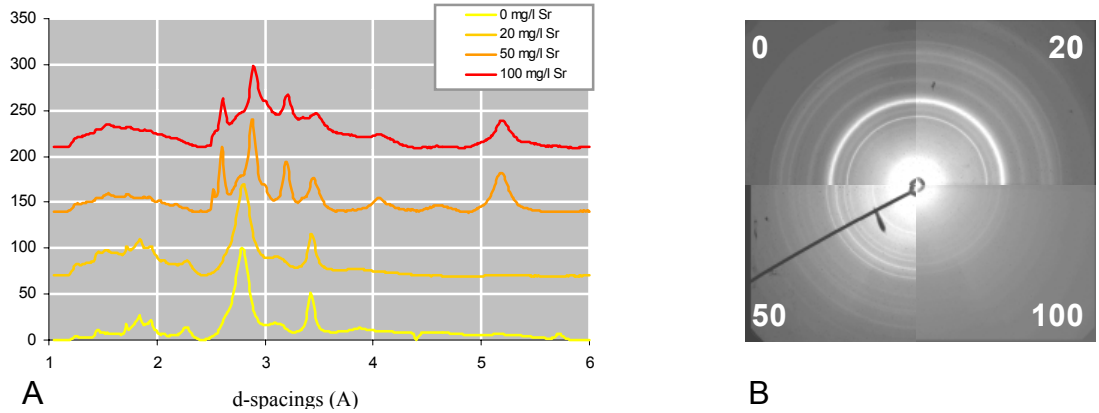

Figure 3: (A) XRD spectra expressed as d-spacing of the mineral phase from cell cultures loaded with 0 mg/l Sr^{2+} (control) 20, 50 and 100 mg/l Sr^{2+}. The mineral phase of the control culture could be identified using a XRD-database as calcium hydroxyapatite, the natural occuring mineral of bone. As shown by figure 3-A other mineral phases appear with increasing strontium concentration. Moreover a more diffuse mineralization at the highest strontium concentrations could be observed from the non-integrated diffractograms (B).

References

1. D'Haese PC, Schrooten I, Goodman WG, Cabrera WE, Lamberts LV, Elseviers MM, Couttenye MM, De Broe ME 2000 Increased bone strontium levels in hemodialysis patients with osteomalacia. Kidney Int **57**:1107-1114.

2. Schrooten I, Cabrera W, Goodman WG, Dauwe S, Lamberts LV, Marynissen R, Dorrine W, De Broe ME, D'Haese PC 1998 Strontium causes osteomalacia in chronic renal failure rats. Kidney Int **54**:448-456.

3. Couttenye MM, D'Haese PC, Van Hoof VO, Lemoniatou E, Goodman W, Verpooten GA, De Broe ME 1996 Low serum levels of alkaline phosphatase of bone origin: a good marker of adynamic bone disease in haemodialysis patients [see comments]. Nephrol Dial Transplant **11**:1065-1072.