ESRF	**Experiment title:** Identification of the mineral phase of in vivo and in vitro models of uremia-related vascular calcification	**Experiment number**: SC-1605
Beamline:	**Date of experiment**: from: 03/11/2004 to: 06/11/2004	**Date of report**: 01/03/2005
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Background:

Chronic renal failure is associated with a strong increase in cardiovascular risk, which is responsible for approximately 50 % of the mortality in the hemodialysis population. Increased vascular calcification is a prominent feature of vascular disease in uremic patients. In recent years, the insight has emerged that ectopic calcification is a tightly regulated process, and results from an imbalance between inhibitors (such as fetuin and matrix Gla protein) and inducers of mineralisation (such as elevated phosphate levels). Several recent studies indicate that cultured vascular smooth muscle cells can undergo major phenotypical changes under the influence of elevated extracellular phosphate levels. This so-called osseous metaplasia is parallel to the bone formation process and characterised by: (i) expression of osteoblast-specific differentiation markers; (ii) deposition of bone matrix proteins ; (iii) loss of the vascular smooth muscle cell differentiation marker α-smooth muscle actin; (iv) the deposition of mineral. Although the mechanisms and mediators of vascular calcification in chronic renal failure are the topics of recent challenging research, the identity of the mineral phase formed in this context remains uncertain.

Methods:

To further elucidate the mechanism of uremia-related vascular calcification, two complementary experimental models of uremia-related vascular calcification were developed, in which vessel calcification was induced in rats with chronic renal failure by (i) phosphate suppletion and (ii) vitamin D treatment. Additionally, mineralization was induced in primary cultures of human vascular smooth muscle cells by exposing these cells to (i) elevated phosphate levels, (ii) bone morphogenetic protein type 2 (BMP-2). Given the small sample sizes of (i) rat aorta with 100 µm wall thickness which is merely partially calcified (estimated thickness of the mineralised zone: 5-20 µm), (ii) vascular smooth muscle cell cultures in which only a fraction of cells undergoes mineralisation, techniques with high sensitivity and high spatial resolution are needed to enable mineral identification. In this context, the micro-diffraction set-up of the ID18F beam line (beam spot size applied: 2x10 µm) meet the requirements to successfully identify the mineral phase present in these samples.

Results:

Vitamin D induced vascular calcification

In this model of uremia induced vascular calcification based on the X-ray diffraction analysis (XRD) three different situations were found. In some animals, a calcium-phosphorus containing precipitate was present in which no mineral phase could be identified using XRD (Figure 1), hereby indicating the presence of an amorphous phase. In other animals the precipitate was identified as poorly crystalline apatite (Figure 2) while in a third group the mineral consisted of two phases: poorly crystalline apatite and whtilockite (Figure 3).

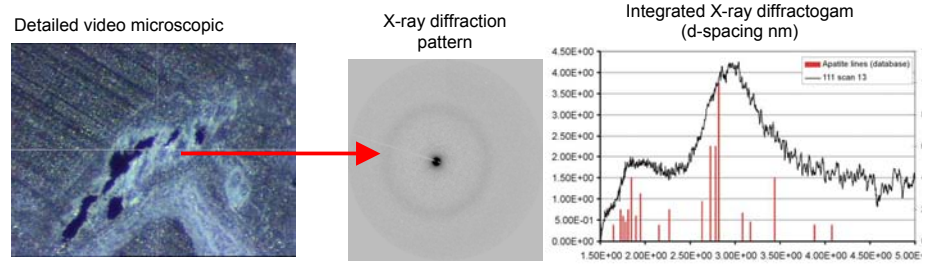

Figure 1: Presence of an amorphous precipitate in a calcium-phosphaorus rich region of in the intima media in vitamin D treated uremic rats

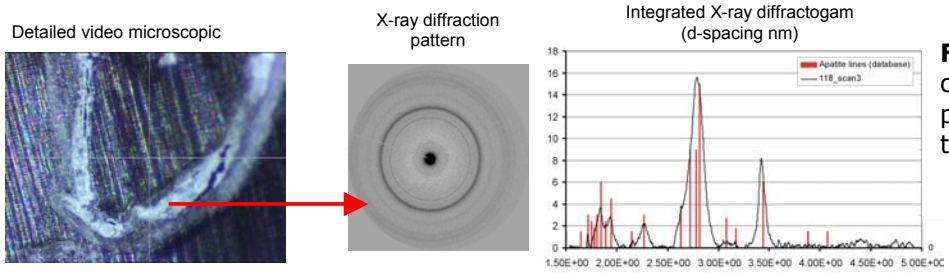

Figure 2: Identification of poorly crystalline apatite as mineral phase in the aorta of vitamin D treated uremic rats

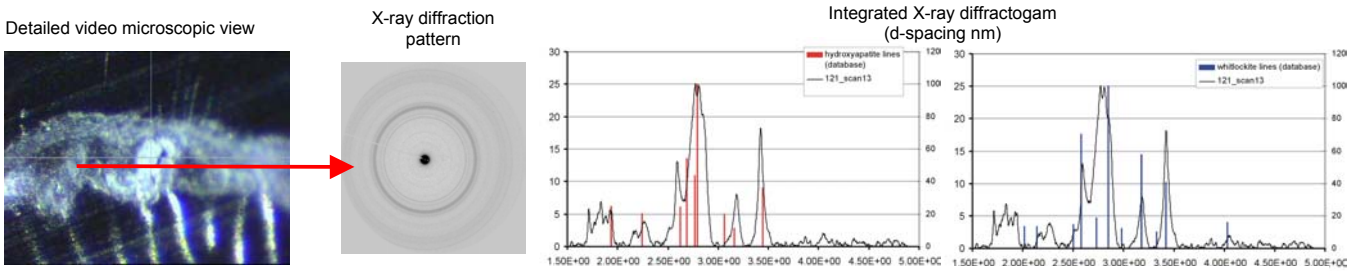

Figure 3: Presence of both apatite and whitlockite in the intima media of aorta of uremic rats treated with vitamin D

Phosphate induced vascular calcification

Micro-X-ray diffraction analysis of embedded sections of calcified aorta from chronic renal failure rats placed on a high phosphorus diet revealed the mineral to be hydroxyapatite. In this model, no phases other than poorly crystalline apatite were found.

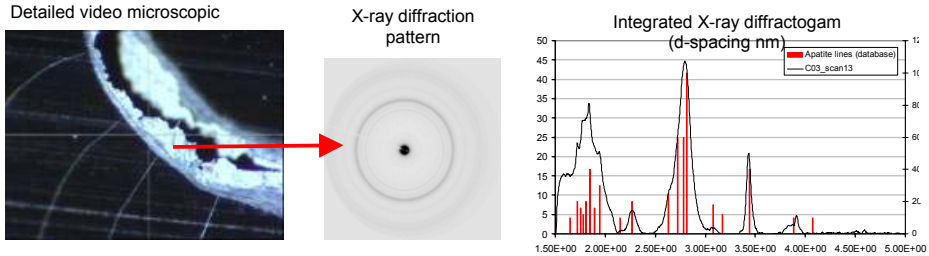

Figure 4: A high phosphate diet gives rise to the deposition of poorly crystalline apatite in the vascular wall of rats with chronic renal failure

Mineralized human smooth muscle cell cultures

The mineral deposited in human vascular smooth muscle cells upon treatment with high phosphate concentrations was shown to be poorly crystalline apatite. No other mineral phases were detected.

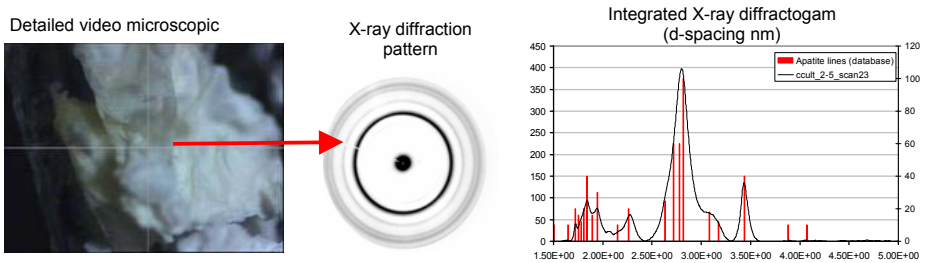

Figure 5: Mineralization in human vascular smooth muscle cells is accompanied with the deposition of apatite