

## Final Report of Calcinosis Project funded by Raynaud's and Scleroderma Association

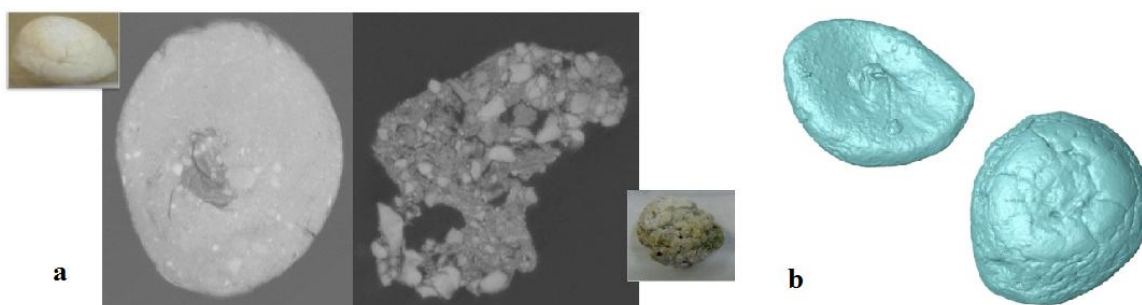
**Background and Aim:** Approximately 25- 40% of patients with systemic sclerosis will develop calcinosis, with the knees, elbows and fingertips being commonly affected. There is limited information in the literature on the composition of calcinotic lumps, although what is available (mostly X-ray diffraction data, microscopy and thermal analyses) suggests that they consist of B carbonated apatite. The aim of this research was to unite all of these methods and more to provide a detailed understanding of the structure and composition of calcinotic deposits associated with systemic sclerosis, with a view to identifying compounds which are able to break the deposits down. Examples of the samples of calcinosis donated by patients to the University of Manchester, for the purposes of this project, are shown below in figure 1.



**Figure 1. Photographs of samples used for characterisation studies.**

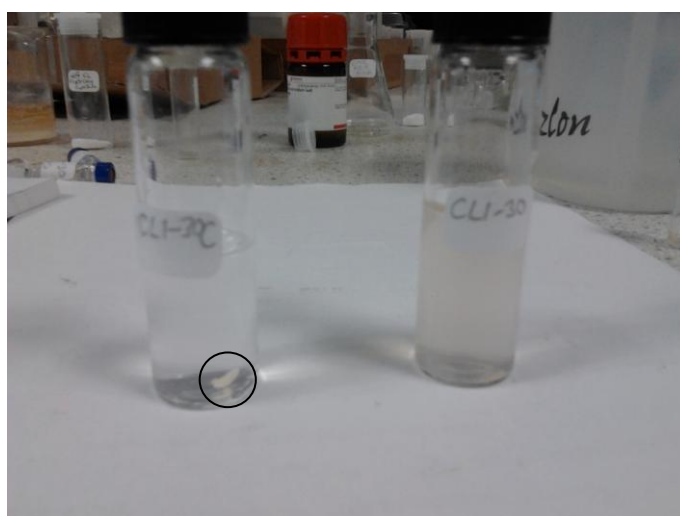
**Methods:** Micro-computed tomography (XCT), thermal (TGA), powder x-ray diffraction (PXRD), elemental, electron microscopy (SEM) and infra-red (IR) analyses were carried out to determine the elemental composition and internal structure of the deposits. The calcinotic deposits had either extruded spontaneously or were surgically removed. For dissolution studies, samples were covered with a solution of the desired reagent and sonicated. The amount of calcium taken up by the solution was measured by elemental analysis.

**Results:** These indicate that hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is the main component of the sample. The PXRD comparison between hydroxyapatite and the samples was a very good fit. The presence of carbonate was confirmed by IR and TGA studies (a loss of  $\text{CO}_2$  at 800 °C in TGA indicates carbonate embedded in the structure). The internal structures of these deposits was probed by SEM and XCT, which show that the samples have very porosities, despite having similar elemental compositions (Figure 2a). The images taken were reconstructed into images showing the surface features of the deposit using XCT (Figure 2b).



**Figure 2. a) CT slices from two of the samples with relevant photographs b) 3D reconstruction of the sample surface**

The dissolution screening indicated that picolinic and citric acid and selected aminocarboxylate calcium chelators were most effective at breaking down or dissolving the deposits, when used in conjunction with an ultrasonic bath. These compounds were DOTA (used as an MRI contrast agent when chelated with gadolinium), BAPTA (a very good calcium chelator) and to a certain extent, pentetic acid. The citric and picolinic acid provided the best uptake of calcium into solution, whereas the aminocarboxylates were better at breaking the deposit down into a fine powder, with less uptake of calcium. Figure 3, below, shows an experiment set up with a control and one of these compounds, where the control sample is intact, and the active compound has broken down the calcinotic deposit.



**Figure 3. An experiment showing a calcinotic growth (circled, left) in a solution containing no active compound and a second sample with one of the lead compounds (right), both after sonication. The disappearance of the growth is clear.**

**Conclusion:** Calcinotic deposits were found to consist of hydroxyapatite with a carbonated component. A greater understanding of the composition of these structures could lead to a better understanding of their formation, potential prevention and improved treatment. Citric and picolinic acids and aminocarboxylate compounds were identified as potential compounds for treating calcinosis.

**Future Work:** The next steps in devising a treatment are:

1. Consider combining the mild acids and aminocarboxylates as they seem to take

different roles in breaking down the deposits.

2. A mechanistic study of the role of these lead compounds could be useful. Techniques such as secondary ion mass spectrometry (SIMS) and use of quartz micro-balances could give more precise information on the chemical action of the lead compounds.

3. Examine methods to formulate these compounds for delivery into patients with troublesome calcinosis. This will be the most challenging problem.

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