# Final Report for Short Term Mobility Programme

Programme title: Hydroxyapatite-based materials of marine origin: luminescence/imaging and drug delivery applications

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Proponent: Dr Anna Tampieri

The research activity was performed by Clara Piccirillo at CNR-ISTEC from the 27th of June to the 8th of July 2016.

The work was organised following two research lines; more specifically:

- The optimisation of the properties of hydroxyapatite-based material doped with europium.
- The preparation and the characterisation of macroporous 3-D structures made of hydroxyapatite-based materials.

For both researches, the hydroxyapatite based materials used were obtained from cod fish bones, a by-product of the food industry. Previous work done by Clara Piccirillo already showed that hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) extracted from cod fish bones has the potential to be used in biomedicine [1,2]; the studies performed during the STM aimed to imparting additional properties to these materials and/or widen their applications. More details about the two activities are described below.

## Doping with europium

This activity was previously started during a STM of Clara Piccirillo at CNR-ISTEC in November 2014. The preliminary tests done at the time showed the potential of these systems; in fact treating the cod fish bones in solutions containing europium and successively calcining them led to hydroxyapatite-based materials doped with Eu, and showing luminescence properties.

During this period, further analysis and tests were performed on these materials, to better understand their properties and potential for bioimaging.

Here is a list of the studied samples, with the corresponding Eu concentrations.

| Samples calcined at 700 $^{o}$C | Eu concentration (wt %) |
|---|---|
| A | 0.00 ± 0.00 |
| Eu1 | 1.12 ± 0.01 |
| Eu3 | 2.31 ± 0.01 |
| Eu5 | 3.64 ± 0.06 |
| Eu7 | 5.86 ± 0.06 |
| Samples calcined at 1100 $^{o}$C | Eu concentration (wt %) |
| B | 0.00 ± 0.00 |
| Eu2 | 0.80 ± 0.02 |
| Eu4 | 1.53 ± 0.01 |
| Eu6 | 2.89 ± 0.09 |
| Eu8 | 4.26 ± 0.17 |

Scanning Electron Microscopy images of some selected samples were acquired, and their morphology was related to the Eu concentration and to the preparation conditions. As shown in Figure 1, it could be seen that for samples calcined at 700 $^{o}$C higher Eu concentration led to a powder with a more compact morphology, with grains with smaller size. For calcination at 1100 $^{o}$C, on the other hand, a more sintered powder was obtained, with sintering being favoured by higher Eu concentration.

Moreover, luminescence spectra were also taken; they are reported in Figure 2. It can be seen that all samples showed clear definite peaks, both in emission and excitation, with higher intensity for higher Eu concentration.

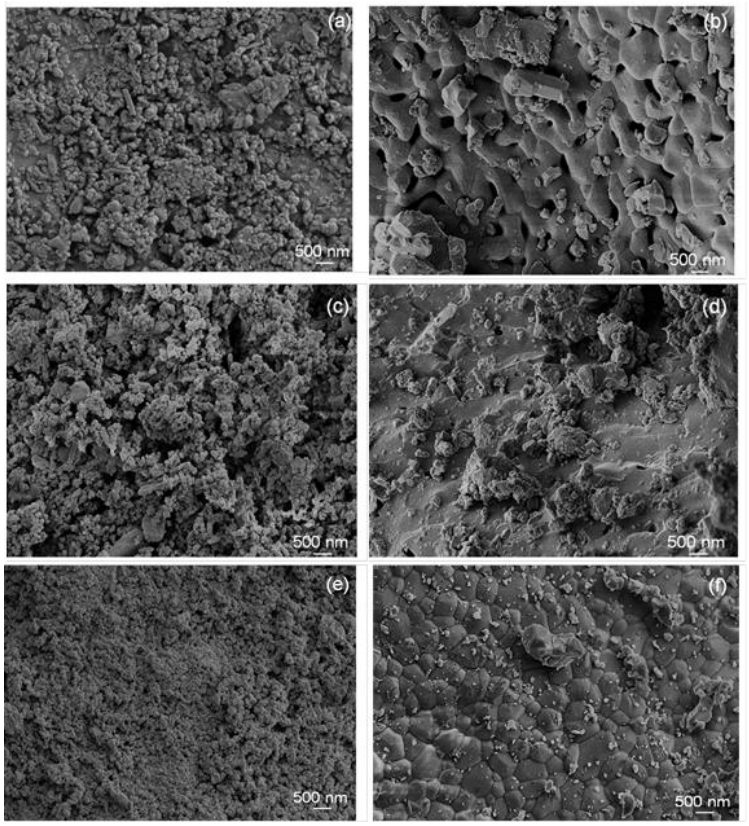


**Figure 1**. SEM micrographs of samples (a) A, (b) B, (c) Eu1, (d) Eu2, (e) Eu7, (f) Eu8.

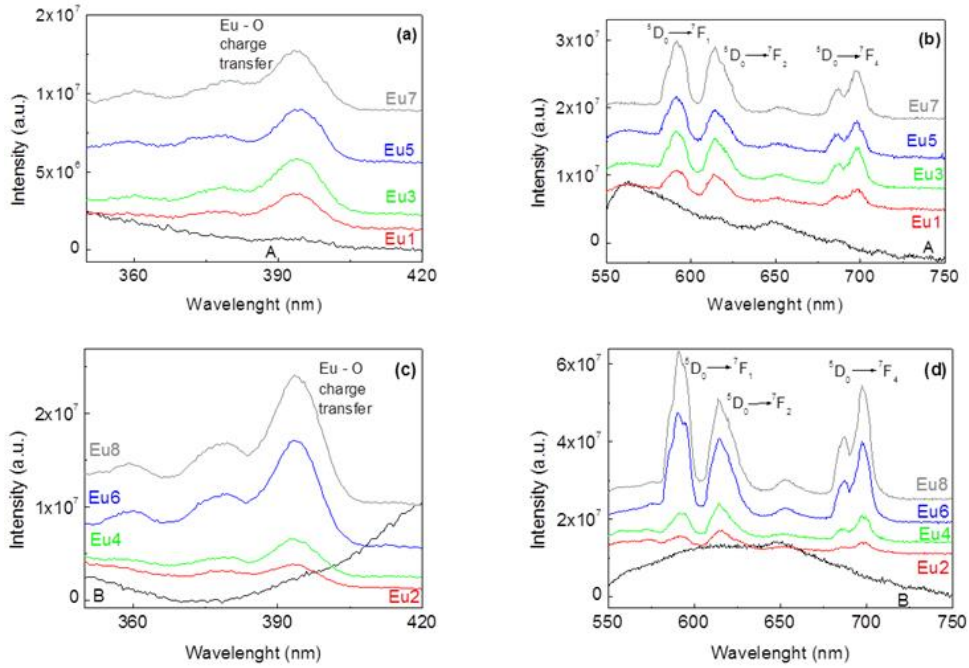


**Figure 2**. Emission and excitation spectra of the Eu-doped samples.

These data showed that these materials could potentially be used for bioimaging.

## Macroporous 3-D structures

The macroporous 3-D structures were fabricated using a protocol developed at CNR-ISTEC [3], based on the formation of a foam containing the hydroxyapatite-based materials. The powder used were made by calcining the bones at 900 $^{o}$C for 1 hour; the composition was a biphasic calcium phosphate (BCP) material made of HAp (about 70 % wt) and β-$Ca_3(PO_4)_2$ (b-TCP).

Several formulations were considered for the preparation of the foam, to see which one could lead to a macroporous structures with the best properties. In particular, different quantities of Dolapix – a deflocculating agent – were tested; the exact formulations are described in the table below, all quantities are expressed in grams.

| | **1** | **2** | **3** |
|---|---|---|---|
| Water | 28.45 | 28.45 | 28.45 |
| Dolapix | 4.95 | 2.91 | 1.98 |
| BCP powder | 90 | 90 | 90 |
| Olympicon | 1.95 | 1.97 | 1.97 |
| W53 | 0.7 | 0.71 | 0.72 |

As comparison, a macroporous structure was made using commercial HAp powder using the same foam formulation of sample 1; moreover, another macroporous was made using a formulation with the same proportion of sample 1, but with more materials (203 g of powder instead of 90 g). Previous work showed that this preparation led to structures with 85 and 65 % porosity, respectively.

The foams prepared following the protocols above were percolated into a cast with dimensions of 10x8x3 cm and left to dry. After 24 hours, the dried solid structure was calcined to 1300 $^{o}$C, as reported in literature [3].

Some preliminary analyses were performed on sample 1; Figure 3 shows SEM images of the sample 1 compared with the two samples made using commercial HAp powder. In the Figure, the first two columns from left report the images of the 65 %, 85 % commercial macroporous while the last column corresponds to sample 1. The Figure shows images with magnification of 150, 3000 and 14000 times, from top to bottom.

The micrographs at lower magnification show a clear difference in the pore dimensions and distribution between the 65 % sample and the other two; this is reasonable, since the 65 %

sample was prepared using different formulation. Comparing the samples in the images with higher magnifications, it can be seen that both sample 1 and the 85 % commercial sample show a very well sintered structure; in sample 1, however, more irregular grains with average larger size are present. This difference can be due to the characteristics of the two powders, the one of marine origin having larger particle size and lower surface area. Some preliminary treatment of the powder (i.e. high energy ball milling) may be necessary to uniform and reduce their size.

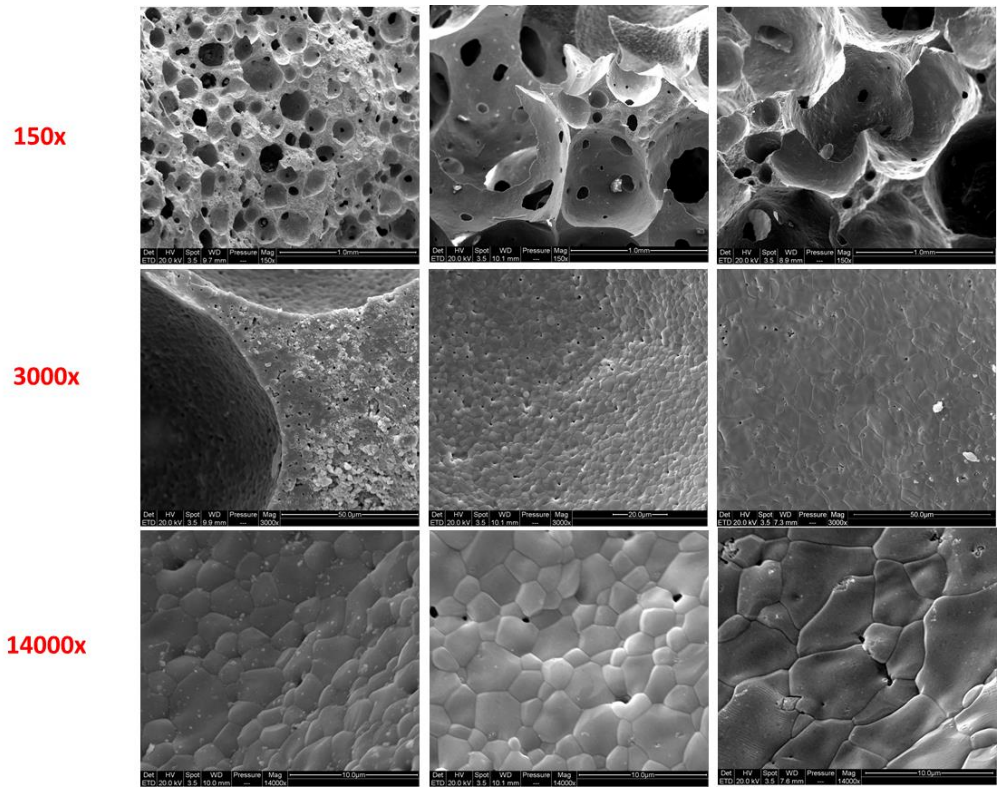


**Figure 3**. SEM micrographs of the macroporous structures.

Further tests will be performed to assess the mechanical properties of these porous materials.

## Conclusions

The results of the work performed by Clara Piccirillo during her staying in CNR-ISTEC can be summarised as follows:

- Luminescence can be effectively induced in HAp-based materials of marine origin through a simple treatment in Eu-containing solution of the bones. This treatment also affects the morphology of the powders.

- The Eu-doped materials can be used for biomedical imaging.
- 3-D porous structures can be successfully made from biphasic materials extracted from cod fish bones through a foaming process.
- The parameters of the foaming may need further optimisation.

## References


1. Piccirillo C., Silva M.F., Pullar R.C., Braga da Cruz I., Jorge R., Pintado M.E., Castro P.M.L.: "Extraction and characterization of apatites-and calcium phosphate-based materials from cod fish bones." ***Mater. Sci. Eng. C,* 33**, 103 (2013).
2. Piccirillo, C., Pullar, R.C., Costa, E., Santos-Silva A., Pintado, M.M., Castro P.M.L.: "Hydroxyapatite based materials of marine origin: a bioactivity and sintering study." ***Mat. Sci. Eng C***, 51, 309 (2015).
3. Dapporto, M., Sprio, S., Fabbi, C., Figallo, E., Tampieri, A., A novel route for the synthesis of macroporous bioceramics for bone regeneration. ***J. Europ. Ceram. Soc.***, 36, 2383 (2016).