THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF
MATERIALS SCIENCE AND ENGINEERING

Development of Injectable Bone Grafts for Spinal Repair

Year 1: Establishing suitable substitution degrees of HAP with magnesium and strontium using a continuous system.

1st Year Confirmation Report

From 3rd September 2018 – 30th October 2019

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1. Introduction and Aims

Spinal fusion is a major surgery used after the failure of conservative treatment of neuropathic pain [1, 2]. The procedure of spinal fusion involves the insertion of an implant (spacer, graft or cage) within the intervertebral space after discectomy of the dysfunctional spinal motion segment. Those implants are stabilized with fusion devices such as pedicular screws, interpedicular fixation plates, and intervertebral spacers [3, 4] (Figure 1).

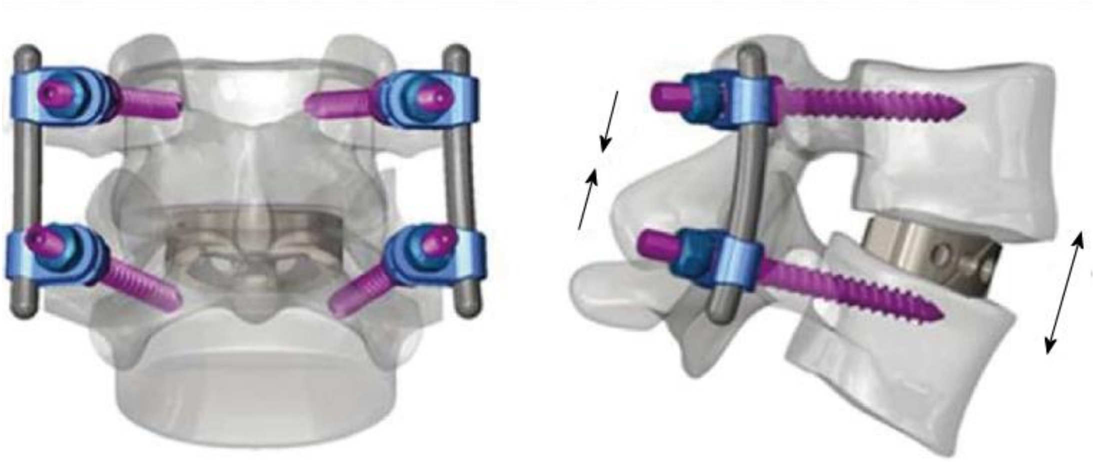

Figure 1 Example of lumbar interbody fusion cage stabilized with pedicle-screw stabilization [5].

Spinal fusion cages are hollow in their geometry and the surgical gold standard is to fill the space with bone autograft [3]. Hydroxyapatite (HAP) with its excellent osteoinductive properties is also routinely used as a spinal fusion cage filler [6-10]. A slow degradation rate and limited bioactive properties are major drawbacks for the *in vivo* use of HAP [11, 12]. These limitations could be overcome through the modification of HAP with Mg and Sr, which were shown to improve the solubility and bioactivity of HAP [7, 13-15]. This project aims to develop a multi-substituted hydroxyapatite with magnesium (Mg) and strontium (Sr) with a continuous method for the usage as fillers for spinal fusion cages.

The main objectives of this project are

1. Development of a suitable synthesis method for the continuous synthesis of multi-substituted HAP
2. Identification of optimal substitution degrees for HAP
3. Identification of a suitable material to create mouldable scaffolds and the fabrication method necessary for the application of the material

2. 1st year activities

The first 5 months I spend at the host institution, Sheffield University doing literature research on the topic and gaining a general understanding in the field. Furthermore, I undertook training in laboratory techniques and attended courses in ethics, transferable skills. I attended departmental seminars which gave me the opportunity to learn about different fields in material science.

Methods

During my secondment I tested several continuous synthesis methods for hydroxyapatite and further optimized the synthesis conditions. Within the tested methods; precipitation using a column to generate laminar flow seemed to be the most promising system for the synthesis of HAP (Figure 2).

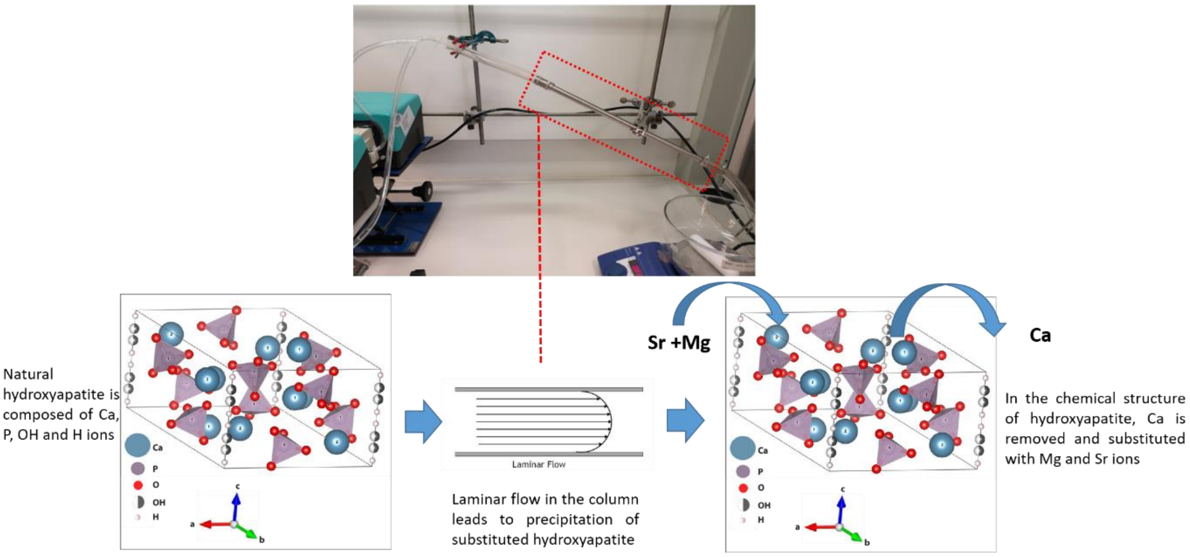

Figure 2 A column is used to create laminar flow and precipitate substituted hydroxyapatite.

In the process of HAP synthesis an acid and a base are used as calcium, phosphorus and substituent (strontium and magnesium) sources for the synthesis of substituted HAP. Acid and base are mixed in a column and further filtered, washed, dried and ground (Figure 3).

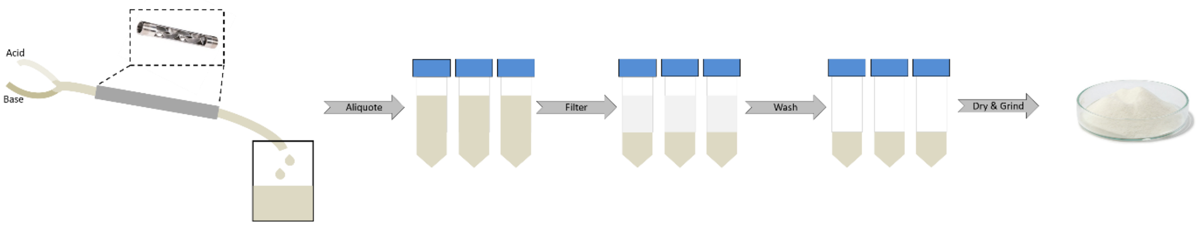

Figure 3 . Process of HAP synthesis: Acid and base are mixed and precipitated through a column (grey). The mixture (green) is filtered and the supernatant (light grey) is discarded. Samples are washed and dried and grinded to a fine powder.

Furthermore a design of experiments approach was used to establish an optimal range of HAP substitution degrees. Although different groups have worked on substitution for HAP, an optimal substitution degree for the combined substitution with Mg and Sr suitable as bone graft has yet to be defined [14-18]. Table 1 shows the minimum and maximum values used for the design of experiments.

Table 1 Different substitution degrees of Mg and Sr

	Mg	Sr
Conc 1	20	20
Conc 2	5	20
Conc 3	20	5
Conc 4	5	5

2.1. Results

Experimental evaluation of the product was carried out via FTIR, ICP, XRD and statistical analysis. Different concentrations lead to the formation of different phases as shown on XRD analysis (Table 2).

Table 2 XRD results with different phases synthesised using different concentrations

Concentration	1	2	3	4
Brushite	72.4%	-	19.4%	23.2%
Amorphous phase	27.6%	-	71.2%	-
HAP	-	100.0%	9.4%	76.8%

ICP analysis showed that the substitution degree affects the incorporation amount of Mg and Sr (Figure 4).

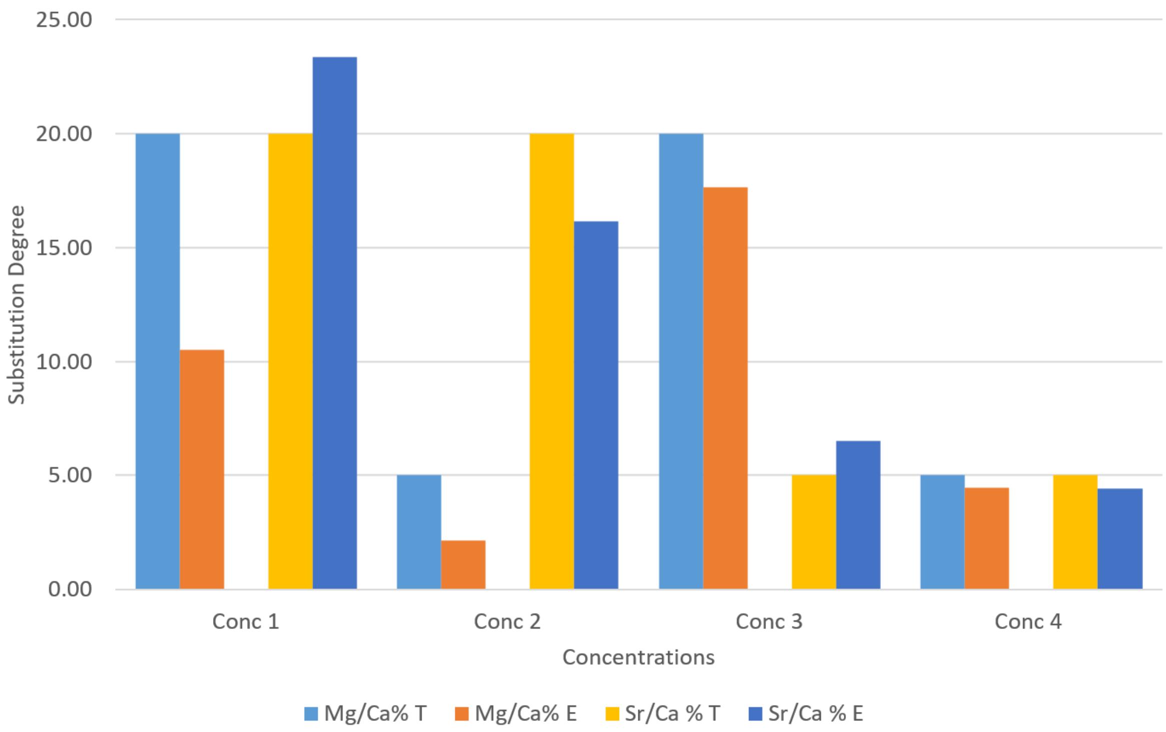

Figure 4 The effect of the substitution degree on Mg and Sr incorporation. %T = theoretical substitution degree, %E = experimental substitution degree. Analysed via ICP

3. Future work

After finishing the first secondment at the industrial partner, the project will continue with obtaining cell culture standard operating procedures SOP which will build on from ESR2 of the SPINNER project. In the next step the *in vitro* effect of the obtained substituted HAP powders will be evaluated.

As a next step preliminary experiments on polymers for the incorporation of HAP substitutes have to defined. Methacrylated gelatin with its shear-thinning behaviour is a promising candidate for the incorporation of substituted HAP within the scaffold matrix [19]. Previous groups have already shown the incorporation of HAP into methacrylated gelatin [20-22].

As last step and part of the project, spinal cages will be filled with our composite polymer-SrMgHAP material and a mechanical testing will be performed.

4. Education and trainings

As part of the program I attended ethics, transferable skills and unconscious bias as compulsory seminars. As a PhD student I am entitled to benefit from seminars offered by The University of Sheffield. Thus, I attended:

- Laboratory Demonstration
- Careers Focus: How to Network
- DDP Managing Yourself and your PhD
- Tools for literature searching

In order to work according to the university regulations I attended several online trainings and in person trainings in CoSHH management and working with liquid nitrogen.

5. Conferences and workshops attended

The following conferences/workshops have been attended:

- 1st SPINNER meeting and workshops in spine anatomy, physiology, biomechanics and surgical-therapeutic approaches. Tuttlingen, Germany. 4th December 2018 – 10 December 2018.
- White Rose Biomaterials and Tissue Engineering Group (BiTEG) conference. Sheffield, UK. 18th December 2018
- 2nd SPINNER meeting and workshops in scientific writing, project management and product development. Bolgona, Italy.18th February 2018 – 22th February 2019
- Research, Innovation and Entrepreneurship workshop: Bringing researchers together. Marie Curie Alumni Association, Italian Chapter. Bologna, 24th May 2019
- ESB-ITA, Bologna 30th September – 1st October 2019

6. References

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