Synthesis of magnesium and strontium substituted hydroxyapatite-polycaprolactone composites

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INTRODUCTION: Synthetic hydroxyapatite is therapeutically used as bone graft substitute, bone filler, or as coatings to support attachment of bone to metal implants. However, the slow degradation rate of hydroxyapatite compromises its osteogenic activities and use as bone filler. In this project we aim to create a multisubstituted HAP (sHAP) with Mg and Sr with a continuous flow method to increase its solubility, osteogenic integrity and bioactivity. Furthermore, we incorporate sHAP into a polymer matrix based on polycaprolactone (PCL) to create a composite bone graft substitute.

METHODS: HAP was synthesized by a wet, continuous method in a mixing column using orthophosphoric acid and calcium hydroxide as phosphorous and calcium precursors and magnesium nitrate and strontium nitrate as Mg and Sr precursors. Different substitution degrees were synthetized (Table 1). The synthesis of substituted HAP (sHAP) was characterized via Fourier-transform infrared spectroscopy (FTIR) and inductively coupled plasma optical emission spectrometry (ICP-OES). Crystallinity was analysed via X-ray diffraction (XRD). The first round of dose-dependent cytotoxicity was performed on Mg substituted HAP (SintLife®) [1] and investigated using Y201 mesenchymal stem cells (MSCs) [2] via a reasazurin metabolic assay in a transwell system using different doses of MgHAP/ml media (Min: 100 µg, Med: 505 µg Max: 1000 µg). Different concentrations of non-substituted HAP were incorporated into a 4-arm methacrylated polycaprolactone (PCL) matrix for preliminary testing of HAP-PCL (%w/w) (0 % HAP, 10 % HAP, 20 % HAP, 30 % HAP). HAP-PCL scaffolds were examined for their cytotoxicity on Y201 MSCs via a metabolic resazurin assay. In order to avoid the compound effect of serum, all experiments were performed in optimized conditions to support cell growth/differentiation using serum and xeno free medium (StemMACS™ MSC Expansion Media Kit XF, human (Miltenyi Biotec)).

Table 1 Tested substitution degrees of Mg and Sr [%]

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

RESULTS: High amounts of Mg and Sr could be incorporated into the HAP structure. However, a higher amount of substitution decreased the overall incorporation of Mg and Sr into the lattice structure. Results showed that in our used serum-free media these MgHAP had no dose-dependent cytotoxicity on Y201 cells *in vitro*. HAP-PCL films could be successfully fabricated through cast molding and showed a lower metabolic activity for HAP incorporated PCL. However, the metabolic activity among different concentrations of HAP-PCL was comparable.

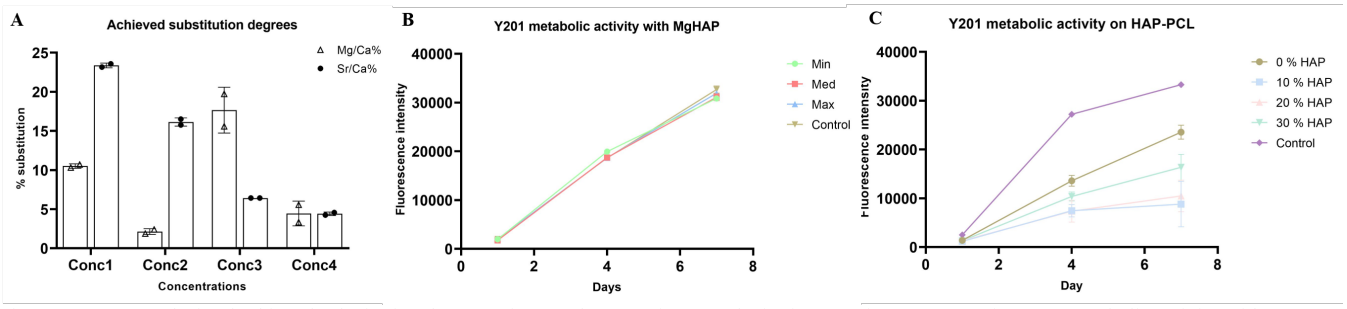

Figure 1 A: ICP analysis of achieved substitution degrees of Mg and Sr: y-axis = % substitution, x-axis = concentrations. B: Metabolic activity with resazurin assay on Y201 cells treated with MgHAP: time points: day 1, day 4, day 7; y-axis = fluorescence intensity, x-axis = day. C: Metabolic activity with resazurin assay on Y201 cells on with HAP-PCL films: time points: day 1, day 4, day 7; y-axis = fluorescence intensity, x-axis = day.

DISCUSSION: Substituted HAP has no cytotoxic effects and is currently being investigated for its osteogenic activities. sHAP incorporated into a PCL matrix showed to also not be cytotoxic and is further investigated for its osteogenic potential.

SIGNIFICANCE/CLINICAL RELEVANCE: The current state of the art methods to fill spinal fusion cages (for spinal fusion surgery) are autologous bone grafts, which are associated with donor site morbidity, slow healing process, and a potential risks for infections. Substituted hydroxyapatite-PCL composites could be used as bone filler (but not limited to) for spinal fusion cages.

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