Optimisation of synthesis and study on biological activity of silver-doped calcium hydroxyapatites for tissue engineering

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INTRODUCTION

Synthetic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is extensively employed in bone tissue engineering due to its excellent osteoconductivity and biocompatibility. However, its intrinsic antibacterial activity is limited, which contributes to bacterial infections being a leading cause of implant failure. To mitigate this drawback, silver (Ag) ions have been incorporated into the HAp structure, as Ag is well established as a highly effective antimicrobial agent.

In light of the increasing interest in Ag-doped HAp as a bactericidal material for bone graft applications, and the necessity to minimize Ag-related cytotoxicity while maintaining optimal antibacterial performance, this study aimed to investigate the incorporation of Ag into the HAp structure using various synthesis methods, and to comparatively evaluate the effectiveness of these approaches.

EXPERIMENTAL

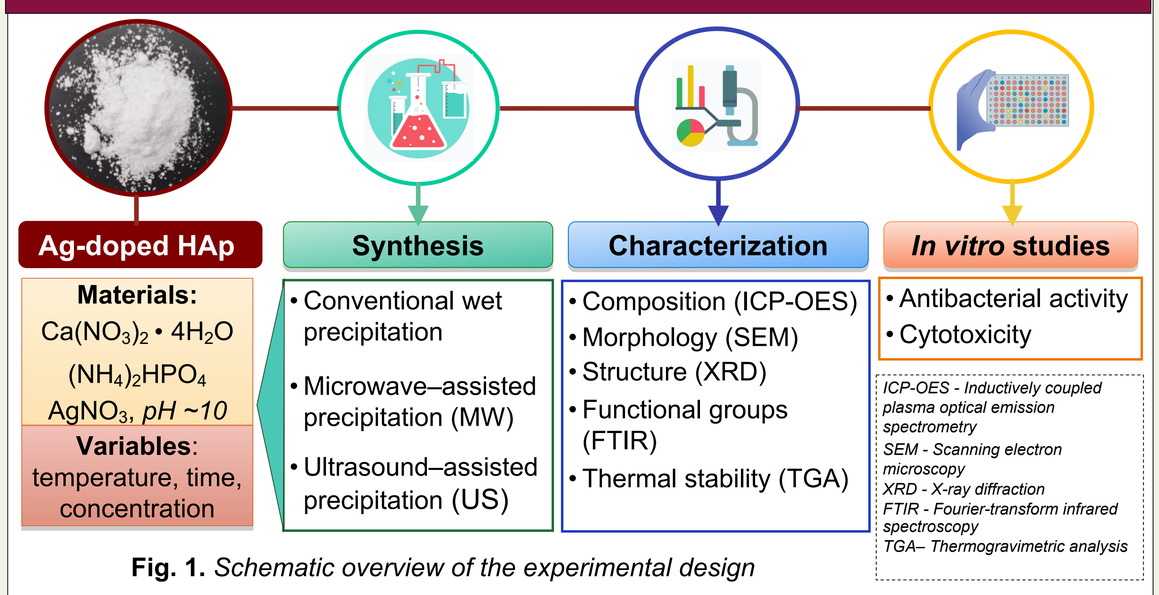


Fig. 1. *Schematic overview of the experimental design*

RESULTS

Composition

- Ag-doped HAp exhibits a low level of Ag substitution, where the incorporation of Ag is strongly dependent on synthesis parameters such as temperature and the application of US and MW irradiation.
- (Ca+Ag)/P ratio remains in the range of ~1.51-1.59, slightly lower than stoichiometric HAp (~1.67), indicating a calcium-deficient apatite phase that is commonly reported for non-stoichiometric, biologically relevant HAp systems used in bone regeneration.

Sample name	Synthesis conditions						Results	
	T, °C	Type of influence	Power	t, h	Ag, mol%	(Ca+Ag)/P ratio	Ag, mol%	(Ca+Ag)/P ratio
T60	60	-	-	4	4	1.67	0.09	1.59
T95	95	-	-	4	4	1.67	0.11	1.58
US	60	Ultrasound	200	0.5	4	1.67	0.03	1.51
MW	60	Microwave	800	0.5	4	1.67	0.01	1.57
T60+MW	60	Microwave	800	3.5 + 0.5	4	1.67	0.10	1.59
Control	80	-	-	4	0	1.67	0	1.55

Structural Characterization

- All as-synthesized samples exhibited XRD patterns characteristic of single-phase HAp, with crystallite sizes ranging from 11 nm (MW-assisted Ag–HAp) to 27 nm (pure HAp) **(Fig. 2)**.
- All characteristic bands of HAp were confirmed by FTIR spectroscopy, which also revealed the presence of CO_3^{2-} groups, indicating that the as-synthesized powders are carbonated HAp. However, a decrease in the intensity of these bands was observed following heat treatment **(Fig. 3)**.

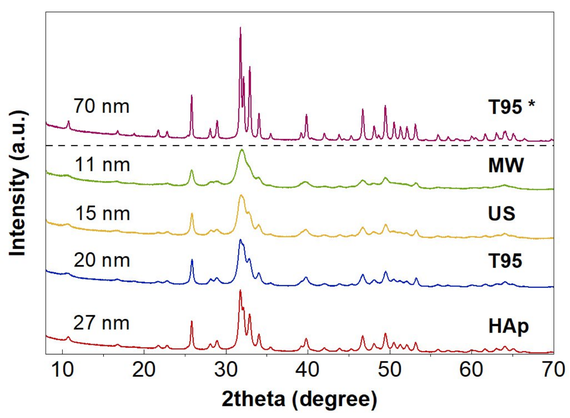


Fig. 2. *XRD patterns of the HAp and Ag-HAp. * - after heat treatment at 900ºC for 1 hour.*

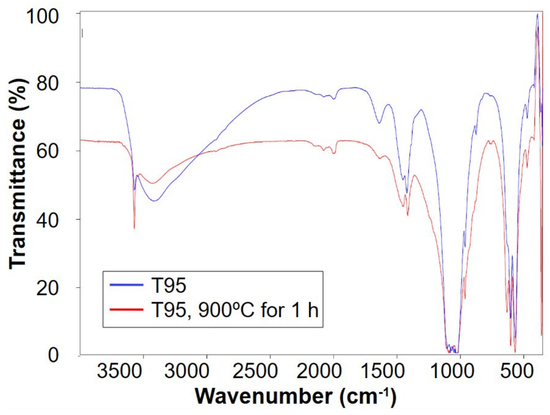
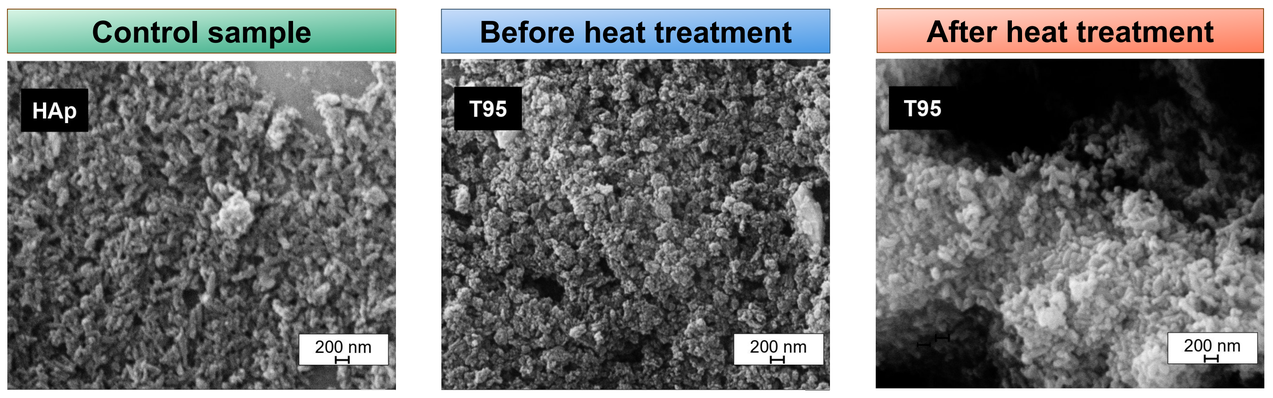


Fig. 3. *FTIR spectra of the as-synthesized and heat-treated Ag-HAp.*

Morphology

- The surface revealed agglomerated nanoparticles, with the degree of agglomeration increasing as the crystallite size decreased. The nanocrystals displayed an elongated rod-like morphology that became more pronounced following heat treatment at 900°C. Despite the incorporation of Ag, the morphology remained almost unchanged compared to the pure HAp.



Thermal Stability

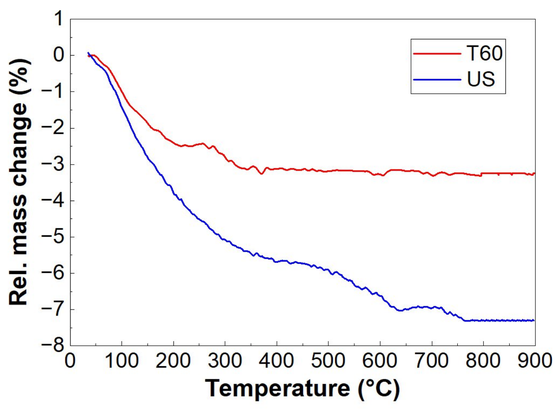


Fig. 4. *TGA graphs of Ag-doped HAp synthesized by different methods*

- The thermal stability of Ag-doped HAp decreases with diminishing crystallite size leading to the formation of secondary phases at lower annealing temperature for samples prepared under MW or US conditions.
- Heat treatment at 900°C did not induce the formation of secondary crystalline phases, with only pure HAp observed in samples prepared by the conventional precipitation method **(Fig. 2)**.
- The crystallite size was observed to increase up to ~70 nm as the temperature increased to 900°C.

Antimicrobial Activity

- A strong antibacterial effect against *E. coli* is observed only after 24 h **(Fig. 5)**. For *S. salivarius*, a delayed response is evident, with the effect occurring over a longer period **(Fig. 6)**.
- Inhibition zone analysis revealed that Ag-HAp samples prepared using a combined synthesis method exhibited the most pronounced antibacterial activity against *E. coli* (8 mm).

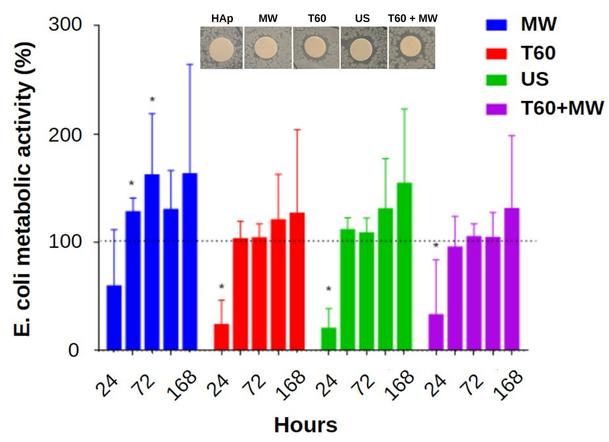


Fig. 5. *Comparative antimicrobial activity of Ag–HAp samples against Escherichia coli.*

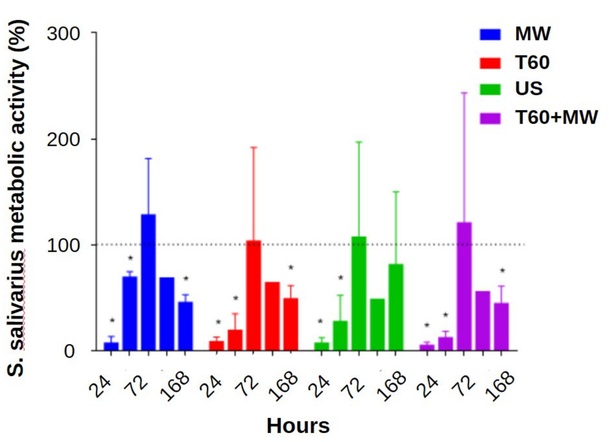


Fig. 6. *Comparative antimicrobial activity of Ag–HAp samples against Streptococcus salivarius.*

Cytotoxicity

- Investigated by exposing MG-63 human osteosarcoma cells to Ag-HAp samples and measuring metabolic activity using a resazurin reduction assay.
- A clear time-dependent increase in cell viability is observed for all investigated samples **(Fig. 7)**.
- This trend is likely governed by an initially high Ag ion release causing transient cytotoxicity, followed by reduced release and subsequent cellular adaptation and recovery.

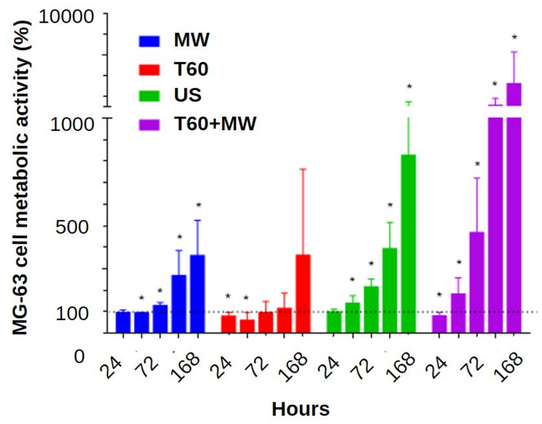


Fig. 7. *Cytotoxic activity of Ag-doped HAp. *p<0.05.*

CONCLUSIONS

➔ Ag-doped HAp powders form agglomerates of nanosized particles with low crystallinity, while preserving the characteristic hexagonal HAp structure, regardless of the synthesis method.

➔ The strongest effect in terms of biological activity was observed for Ag-HAp samples prepared via conventional precipitation synthesis combined with microwave irradiation.

➔ Further investigations on Ag ion release are currently in progress to provide a mechanistic understanding of the observed cytotoxicity and antimicrobial responses.

Acknowledgements

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