# Synthesis and physicochemical characterization of silver modified tricalcium phosphate Ag-βTCP and Ag-βTCP/poly(3-hydroxybutyrate) scaffolds for bone tissue regeneration

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## Introduction

Surgical interventions in orthopedics are at high risk for bacterial infections resulting in postsurgical complications. For this reason, biomaterials containing antibacterial agents, such as antibiotics or bactericidal ions have been extensively studied. Nowadays silver modified biomaterials have attracted much attention, due to their antibacterial activity and low toxicity. In the case of scaffolds for bone tissue engineering, calcium phosphates stand out from other compounds due to their chemical composition resembling the inorganic component of bone tissue, high biocompatibility and bioactivity. Silver-containing calcium phosphates with various Ca/P ratios such as hydroxyapatite (HAp), tricalcium phosphates (α,β TCP) or calcium pyrophosphates (CCP) have been investigated [1]. Furthermore, ceramic-polymer composites are gaining much interest thanks to their superior mechanical properties in comparison to the brittle ceramic and the possibility of functionalization with biologically active substances like drugs or nanoparticles [2]. Therefore, the aim of this study was to obtain, characterize and compare bioceramic and composite scaffolds based on Ag-βTCP and a biodegradable bacterial polymer – poly(3-hydroxybutyrate) (P(3HB)).

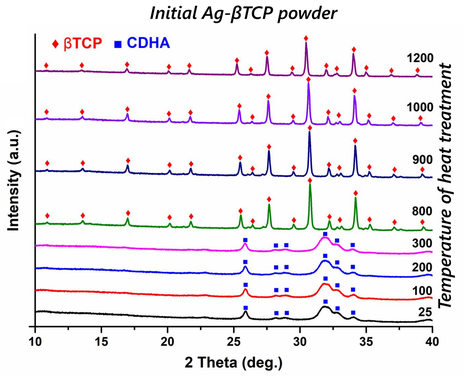
## Methods

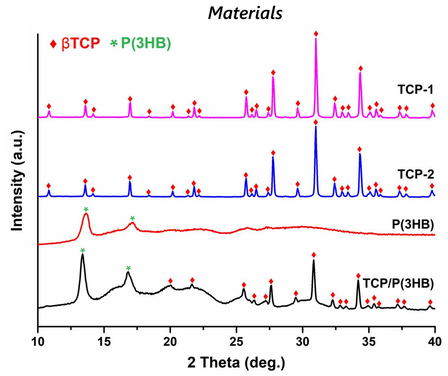
Firstly, silver modified βTCP powder was synthesized via the wet precipitation method. $H_3PO_4$ solution was dropped into $Ca(OH)_2$ to obtain the Ca/P ratio equal to 1.5. $AgNO_3$ was used as the source of silver. The amount of silver was equal 1.0 wt.%. Afterwards, the precipitate was left to mature, centrifuged, dried, grounded in a ball mill, calcined at 900 °C, grounded in an attritor mill and sieved (<63 μm). Scaffolds were prepared via the polyurethane foam replication method. Matrices were impregnated in ceramic slurry (consisting of Ag-βTCP powder, distilled water, Dispex A4040 and methylcellulose), dried and sintered at 1200 and 1150 °C. The obtained ceramic specimens were infiltrated with 5% (w/v) P(3HB) chloroform solution, dried at room temperature for 7 days and subjected to further studies. The developed scaffolds were investigated by X-ray diffraction (XRD), X-ray fluorescence method (XRF), scanning electron microscopy (SEM), hydrostatic weighing and compression tests (universal testing machine Instron). Furthermore, to assess the P(3HB) hydrolytic degradation, composites were incubated in distilled water at 37 °C up to 120 days. Obtained extracts were analyzed via UHPLC-MS.

| Symbol | Material | Temperature of heat treatment |
|---|---|---|
| Ag-βTCP | Ag-βTCP powder | 900°C |
| TCP-1 | Ag-βTCP based scaffold | 1200°C |
| TCP-2 | | 1150°C |
| TCP/P(3HB) | TCP-2 scaffold coated with P(3HB) | -- |

## Results

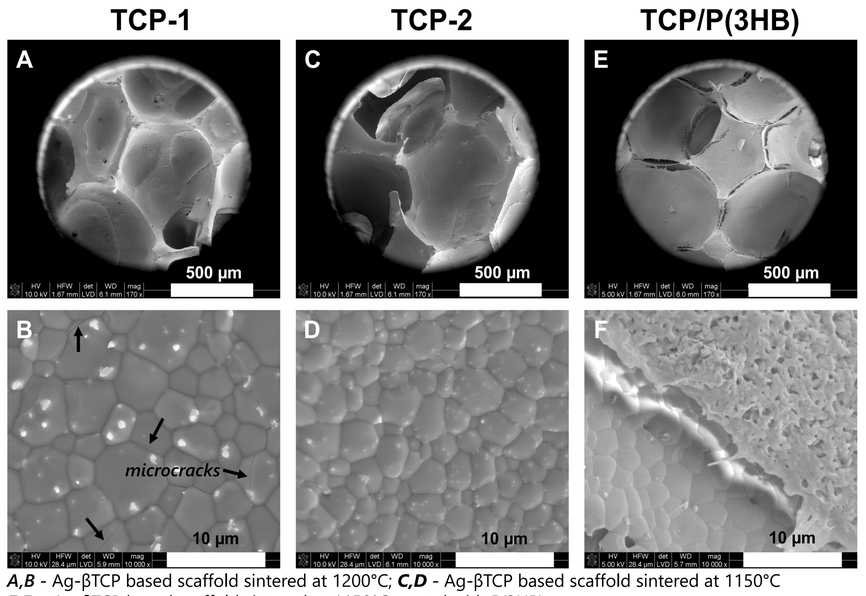
### Phase and chemical composition

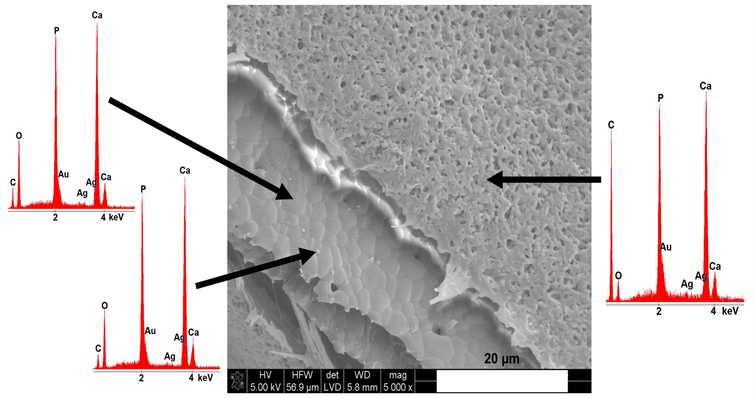



| Chemical element | Content [wt.%] | | |
|---|---|---|---|
| | Ag-βTCP | TCP-1 | TCP-2 |
| Ca | 39.351 | 39.276 | 41.062 |
| P | 18.608 | 17.33 | 17.436 |
| O | 40.262 | 41.57 | 39.535 |
| Ag | 1.097 | 1.259 | 1.127 |
| Si | 0.197 | 0.063 | 0.068 |
| Al | 0.160 | 0.088 | 0.46 |
| Mg | 0.141 | 0.099 | 0.098 |
| Na | 0.047 | 0.142 | 0.119 |
| Fe | 0.044 | 0.046 | 0.053 |
| S | 0.017 | 0.011 | 0.006 |
| Cl | 0.016 | 0.025 | -- |
| Sr | 0.016 | 0.020 | -- |

### Microstructure



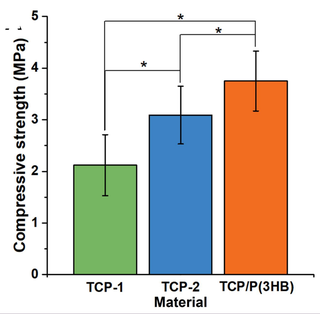



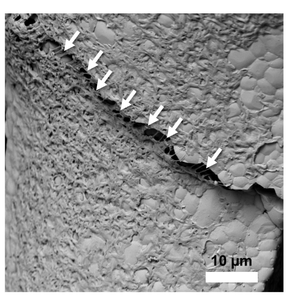

*A,B* - Ag-βTCP based scaffold sintered at 1200°C; *C,D* - Ag-βTCP based scaffold sintered at 1150°C
*E,F* - Ag-βTCP based scaffold sintered at 1150°C coated with P(3HB)

### Porosity

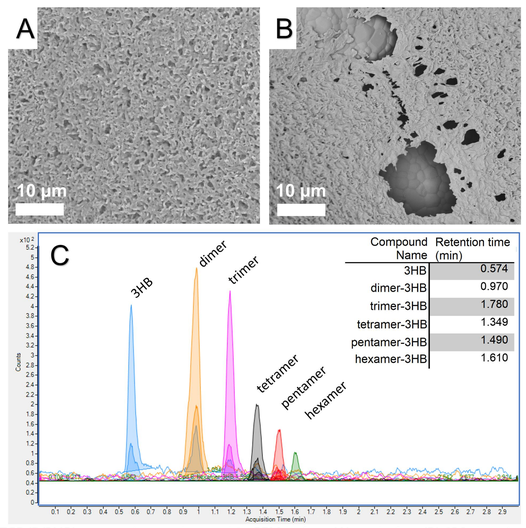
| Material | $P_{total}$ [vol%] | $P_{open}$ [vol%] | $P_{closed}$ [vol%] |
|---|---|---|---|
| TCP-1 | 70.8 ± 1.9 | 66.7 ± 2.7 | 4.0 ± 1.6 |
| TCP-2 | 71.1 ± 2.9 | 68.1 ± 4.6 | 2.9 ± 1.1 |
| TCP/P(3HB) | 71.8 ± 1.8 | 61.8 ± 3.0 | 10.1 ± 3.0 |

### Compressive strength and microstructure after compressive test




### Degradation studies – HPLC measurements




*Surface of the TCP/P(3HB) composite as observed in SEM before (A) and after (B) 120 days incubation in distilled water. Population of oligomers as evidenced by UHPLC-MS analysis (C)*

## Conclusions

Ag-βTCP and Ag-βTCP/P(3HB) scaffolds were successfully obtained. Composite materials possessed higher comprehensive strength and surgical maneuverability. Furthermore, degradation products originated from P(3HB) may nourish surrounding tissues. Thus, obtained materials were found to be a prosperous bone substitutes for tissue regeneration. Further in vitro studies are necessary.

## References

[1] Gokcekaya, O., et al., Mater. Sci. Eng. C 53, 111-119 (2015)
[2] Skibiński, S., et al., Ceram. Int. 47(3), 3876-3883 (2021)

## Acknowledgements

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