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Evaluation of Antibacterial Activity of Calcium Phosphates Based Bone Cements for Biomedical Applications

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Abstract

In orthopedics and traumatology surgeries, the repair of bone deformities is a concern. Pure and Cobalt (Co) doped Calcium phosphate (CaP) cements were successfully prepared in this study. The particles that had been manufactured were characterized. Three different methods were used to study the structure of the materials: X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Scanning electron microscope (SEM). The lattice characteristics, degree of crystallinity, and particle size of pure CaP bone cement were reduced dramatically with the incorporation of Co^{2+} ions. The functional groups of CaP bone cements were detected via the FTIR technique. Antibacterial properties of pure and Co doped CaP bone cement were evaluated qualitatively against Escherichia coli (E. Coli, gram -), Methicillin-sensitive Staphylococcus aureus (MSSA, gram +). Methicillin-resistant Staphylococcus aureus (MRSA, gram +), Methicillin-resistant coagulase-negative staphylococci (MR-CoNS, gram +), and Pseudomonas aeruginosa (P. aeruginosa, gram -) bacteria for 24 h at 37 ºC. After analyzing the data, it was found that the inclusion of Co^{2+} ions inhibited the growth of E. coli and MSSA bacteria. Antibacterial bone cements are potential material for preventing infection-related bone healing failures.

Keywords: Bone cements; Bone Cements; Calcium Phosphate; Cobalt; Antibacterial properties.

1. Introduction

Around the globe, the frequency of bone fractures and osteoporosis is skyrocketing. Many methods have been used to cure bone deformities. Some of these therapies are expensive and take a long time. Bone cement is a well-known biomaterial used to fix bone deficiencies or serve as a binding agent between bone tissue and metallic implants. It is a disease that affects the bones and marrow and is known as osteomyelitis. Infectious agents, such as fungi and bacteria, may cause this condition. Bacteria are often to blame [1-2]. Patients with osteomyelitis are treated with antibiotics to prevent bone loss. This disease is brutal to cure because of the bone's complexity. Conventional treatments need surgery and long-term antibiotic use [3]. Some surgeries might leave patients with more extensive bone abnormalities that need subsequent surgery. To heal the diseased region, long-term usage of high-dose and long-term antibiotics may result in resistance and organ issues [4]. The quantity and concentration of antibiotics used in long-term antibiotic treatments are inadequate to clear the infection and produce significant adverse effects. Additional expenditures are associated with prolonged hospital stays related to intravenous antibiotic treatment [3,5].

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Calcium phosphate (CaP) bone cements are often employed as bone replacement materials. Additionally, CaP speeds up wound healing in bodily fluids [6]. CaP bone cements have a significant drawback in terms of antibacterial characteristics.

Cobalt (Co) is a rare metal needed to produce the B12 vitamin in animals. The Co has been shown to be a safe daily source of no more than 0.012 μg [7]. According to Wu et al., the inclusion of Co^{2+} ions increased the antibacterial properties of bioactive glass scaffolds against E. coli bacteria. [8]. When Co^{2+} ions were tested for hazardous behaviour, Kulanthaivel et al. found no toxic behaviour [9]. The Co^{2+} ions were shown to serve a crucial function in bone repair [10]. Co^{2+} ions doping helps mend osteoporosis-related bone deformities and regenerate bone [11].

2. Experimental

The β-tricalcium phosphate (β-TCP) materials have been prepared using the following protocol. The $(NH_4)_2HPO_4$ (Merck) solution was prepared by dissolving 15.85g into 200 mL of water, labeled as solution A. The $Ca(NO_3)_2.4H_2O$ (Merck) solution was designed by dissolving 42.51g into 200 mL of water, marked as solution B. Solution A was included in solution B. At the same time, the mixture was agitated for 30 minutes, and the pH was adjusted to 7 using NH_4OH (Merck). Five minutes of irradiation in the microwave (ARÇELİK MD 500) were applied to the white residue [12]. An oven was set to 80 ºC for 24 hours for drying the white precipitation. After drying, the powder was calcined at 1000 ºC for 2 hours in a furnace. Co^{2+} ions doped β-TCP was prepared using the same protocol, adding about 2.62 g of Co $(NO_3)_2{\cdot}6H_2O$ (Merck). To prepare CaP (or Co-CaP) bone cements, About 1 g of β-TCP (or Co-β-TCP) materials mixed thoroughly with 0.5 g of Monocalcium phosphate monohydrate (Merck) till gain homogenized powder. About 0.8 ml of distilled water was added to the mixed powder and crushed using mortar and pestle to get a homogenized paste [12]. Then, the paste was cast using Teflon molds (10 mm) and carefully removed from the molds with the help of a metal bar. The discs are allowed to dry for 1-2 days.

Characterization of materials

X-Ray Diffraction (XRD, MODLE Rigaku Ultima IV) was used to gauge the powders' purity, crystallinity, and other lattice properties. A step of 0.03 was used to take the diffraction patterns from 10 to 80 in the 2 range. The crystallinity and crystallite size were calculated using integrated software. FTIR spectroscopy (Bruker IFS 66/S) identified the functional group vibrational bands in the manufactured materials using this technique. Recordings were made in the wavelength region of 4000 - 400 cm^{-1}, corresponding to room temperature data. The shape of particles was examined using Scanning electron microscopy (SEM, ZEISS ULTRA PLUS).

Antibacterial properties of pure and Co doped CaP bone cements cement were evaluated qualitatively against Escherichia coli (E. Coli, gram -), Methicillin-sensitive Staphylococcus aureus (MSSA, gram +). Methicillin-resistant Staphylococcus aureus (MRSA, gram +), Methicillin-resistant coagulase-negative staphylococci (MR-CoNS, gram +), and Pseudomonas aeruginosa (P. aeruginosa, gram -) bacteria. Qualitative assessment was conducted out using the disc diffusion approach. The discs were put on the plates and incubated for 24 hours at 37 ºC.

3. Results and discussion

The phase identification of pure CaP and Co doped CaP cements set at the lab temperature was achieved, and the findings are shown in Figure 1. CaP bone cement diffraction peaks in all samples were in excellent agreement with the reference phase (JCDPS NO: 09-0080). No peaks were observed at 11.5º and 23.31º, revealing the absence of additional CaP phases in the produced cements, such as brushite phase. The XRD patterns of Co doped CaP cements show crystalline phase with expansion along with a, b axis, and reduction along with c axis. Furthermore, crystallinity and crystallite size significantly decreased with increasing Co^{2+} ions into the CaP structure. This behavior could be attributed to substituting bigger-sized Ca^{2+} ions (0.099 nm) with smaller-sized Co^{2+} ions (0.074 nm) in the CaP lattice.

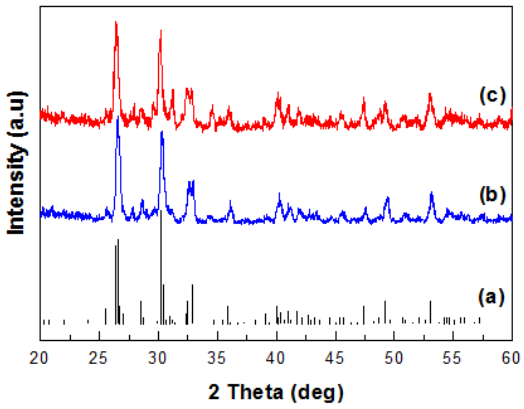

Fig. 1. XRD pattern of standard phase (Monetite) (a), pure CaP (b) and Co- CaP (c).

The functional group present in the pure CaP and Co^{2+} doped CaP bone cements were evaluated using FTIR spectra, and in which the outcomes are shown in Figure 2. The stretching mode of P-O-P and P=O bonds appeared, the stretching mode of C-O bond existed in two locations were resulted from atmospheric CO_2, and the bending mode of O-H bond was also detected in the pure CaP sample. With the addition of Co^{2+} ions into the CaP structure, there were no noteworthy changes in the position of bands. However, with the incorporation of Co^{2+} ions, the intensity of PO_4^{3-} bands were decreased.

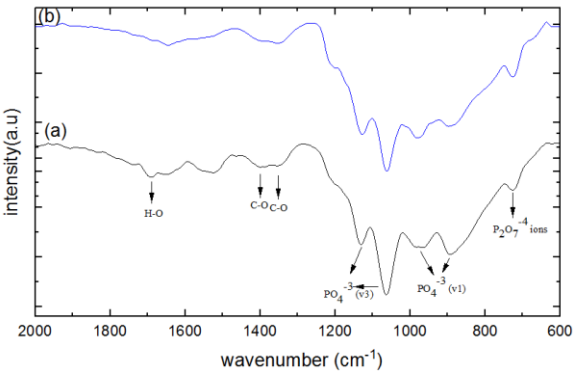

Fig. 2. FTIR graph of pure CaP (a) and Co doped CaP (b) cements.

SEM micrograph images of pure CaP and Co doped CaP bone cements are presented in Figure 3. CaP was found to generate tiny irregularly shaped structured particles using SEM (Figure 3(a)), which then changed to a loosely packed plate-like morphology with an uneven size distribution when doped with Co^{2+} (Figure 3(b)). The plates did not reveal a particular orientation, indicating isotropic behavior more or less [13].

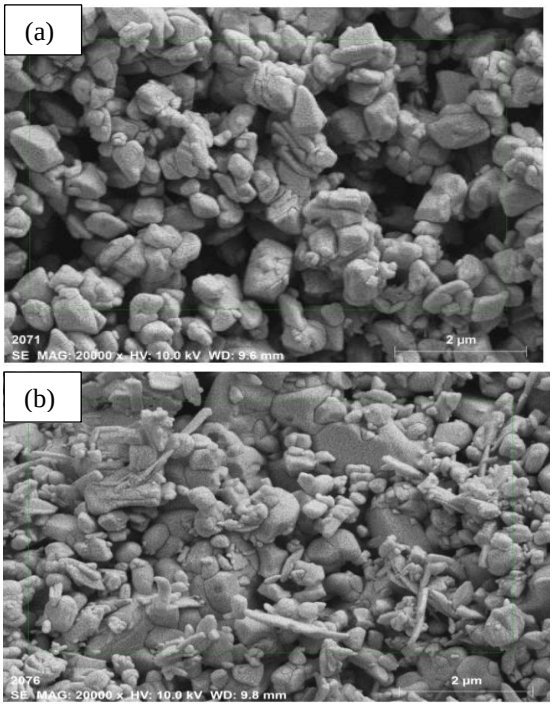

Fig. 3. SEM images of pure CaP (a) and Co- CaP (b).

Antibacterial properties of pure and Co doped CaP cements were evaluated qualitatively against E. coli, MRSA, MR-CoNS, MSSA, and P. aeruginosa bacteria (Figure 4). The pure phase of CaP and 1Co-CaP (0.78 g of Co^{2+}) ions doped CaP (1Co-CaP) showed no antimicrobial behavior against the used bacteria. The growth of E. coli and MSSA bacteria was suppressed when the quantity of Co^{2+} ions (2Co-CaP) was increased. The mean diameter of the inhibition zone for E. coli and MSSA bacteria was 19.5 mm, and 23.2 mm, respectively. Furthermore, the inhibition zone increased with increasing Co^{2+} ions into the CaP structure. The inhibition zone rose to 25.7 mm (E Coli) and 27 mm (MSSA).

This growth inhibitions of E. coli and MSSA bacteria might be ascribed to the leaching of Co^{2+} ions from the surface of Co-CaP cements into the culturing medium, then the positive ions (Co^{2+}) are attracted to the bacterial cell's negatively charged phosphate and carboxylic group nucleic acids [14]. The increased concentration of Co^{2+} on the cell wall is caused by the buildup of Co^{2+} on the bacteria's cell wall, The CorA system (inorganic metal transport) may subsequently impede endogenous respiration and infiltrate the cytoplasm of cells, resulting in cell death by leaking of intracellular chemicals [15,16]. Furthermore, a reduction in the pH value of the environment for Co-CaP cements could be another factor responsible for the death of bacteria [17].

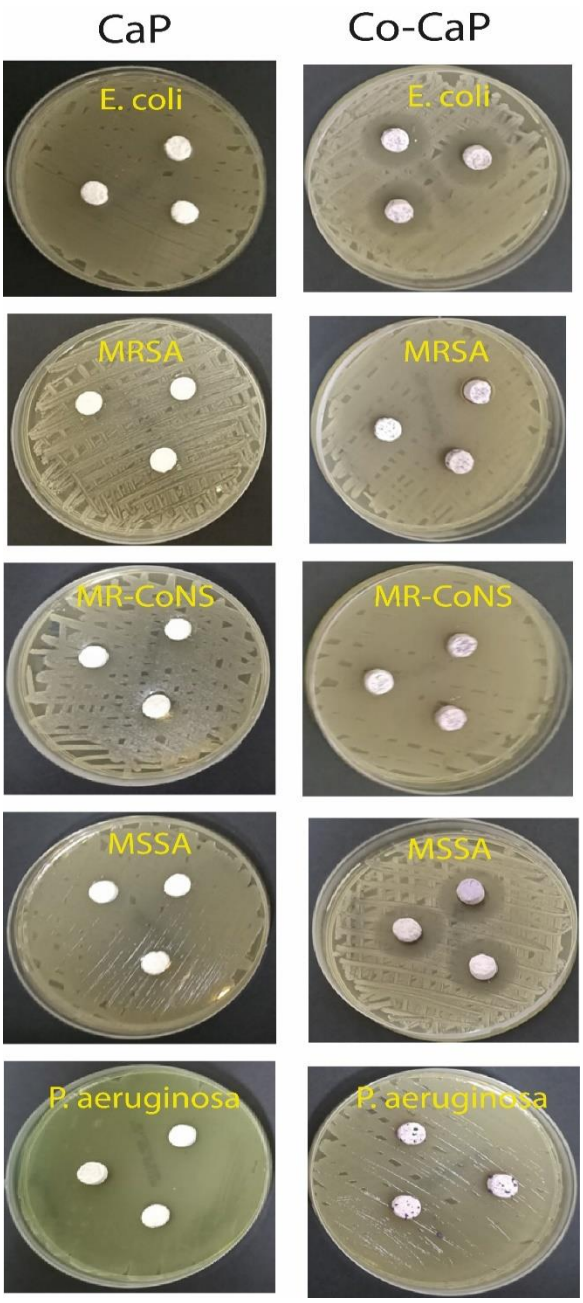

Fig. 3. Evaluation of antibacterial activity of pure CaP and Co-CaP materials at 37 ºC for 24 hours.

4. Conclusions

The CaP and Co doped CaP bone cements were successfully synthesized. The lattice parameters of CaP crystals increased with the addition of Co^{2+} ions. At the same time, the degree of crystallinity and crystallite size significantly decreased with an increasing amount of Co^{2+} ions in the CaP structure. By adding Co^{2+} ions into CaP bone cements, E. coli and MSSA bacteria were prevented from growing. The materials prepared in this study have shown good physicochemical and antibacterial properties and are therefore would be a promising material for inhibiting any infection-causing failure in bone repair. However, before using them as biomaterials in medical applications, further studies such as *in vitro* bioactivity, in vitro mechanical analysis, and *in vitro* cell culture analysis should be conducted.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

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Table 1 : Lattice parameters of pure CaP and Co doped CaP cements.

Sample ID	a(Å)	b(Å)	c(Å)	CV(Å)$^{3\,(a)}$	DC(%)$^{(b)}$	ACS (nm)$^{(c)}$
Standard phase (Monetite)	6.906	8.577	6.634	309.4	---	---
CaP	6.883	8.593	6.643	310.0	70	20.1
Co-CaP	6.913	8.572	6.633	309.1	64.83	12.5

[a] Cell Volume.

[b] Degree of Crystallinity

[c] Degree of Crystallinity

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