Influence of Synthesis Technique on the Bioactivity of Chitosan-Apatite Nanocomposites

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Introduction

The work is devoted to the creation and research of the biomaterials for bone tissue growth based on hydroxyapatite and chitosan. The biocompatibility, biodegradation, and antibacterial properties of chitosan makes it an ideal material for the manufacture prostheses of bones or teeth. Our previous *in vivo* studies [1,2] have proved the optimality of the beads-shaped composite materials. The investigation suggest the formation of beads as a result of the mechanical mixing of HA and polymers (alginate, chitosan), which was reflected in the properties of composite materials.

This work simulates the process of synchronous calcium phosphate crystallites and chitosan polymer matrix formation during the synthesis of a beads-shaped composite material containing a drug.

Methods

The technology of sample synthesis is shown in Fig.1. More specifically, a 6% chitosan solution was added to the 0.5 M calcium acetate solution (pH 6.86) at various mixing ratios (Table 1). Mixtures were shaking for 3 hours at 37 °C, followed by dripping into 50ml of 0.3M sodium dihydrogen phosphate (pH = 7.4) through a medical drip. After cooling, sodium dihydrogen phosphate solution was replaced with 50 ml of 1% TPP (tripolyphosphate) solution (pH ~ 9.0), in which the formed beads were left to further stabilize their form by ionic gelation through the interaction of amino groups of chitosan and P_2O_5 groups of TPP. After 12 hours, the beads were washed with distilled water and dried at 37 °C.

Samples were analyzed by SEM and EDA (FEI Inspect S50). X-ray diffraction (XRD) structural studies performed using the Shimadzu XRD-6000 diffractometer with Cu-Kα radiation. The Ca/P ratio was determined using the X-ray fluorescence spectrometer ElvaX Light SDD (XRF analysis). The bioactivity of the samples was studied by keeping them for 30 days in Simulated Body Fluid (SBF), which is similar in composition to physiological liquid.

Results

XRD analysis of synthesized samples indicates the formation of a low-crystalline fraction of amorphous calcium phosphate (ACP). After calcination in a muffle furnace at 950 °C for 1 hour, the XRD spectrum shows the expected presence of a high-temperature fraction of TCP, monetite, and calcium pyrophosphate formed due to presence of TPP (Fig. 2).

The elemental composition of the samples before and after being in SBF, according to the EDA, is shown in table 2 and Fig. 3.1.

SEM shows that with increasing calcium content in the mother solution, the surface becomes denser due to the increase of calcium phosphate amount. Most obvious surface changes are observed in sample №3. After keeping in the SBF solution for 48 days, its surface is the most uniform (Fig.3.2), and calcium and phosphorus ions are evenly distributed on the surface (Fig.3.3). The surface content of N and C is almost the same in all samples, and the Na content associated with TPP decreases sharply by 2.5, 4.6, 15.4 times in samples 1, 2, 3, respectively. The increase in Ca content was observed. The atomic ratio of Ca/P in all samples is about 1.0.

Conclusion

In this work, the beads-shaped material was obtained by the method of synchronous synthesis of calcium phosphate and polymer matrix. The results of EDA and XRF studies confirm that a new layer of calcium phosphate is formed in the physiological solution on the surface of the composite material, which is a precursor of brushite and hydroxyapatite. This fact claims the high bioactivity of the surface of chitosan-calcium phosphate beads formed by this technology.

References

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2. Sukhodub L.B., Sukhodub L.F., Litsis O., Prylutskyy Y.I. // Mat Chem and Phys.-2018.-217, -P. 228-234.

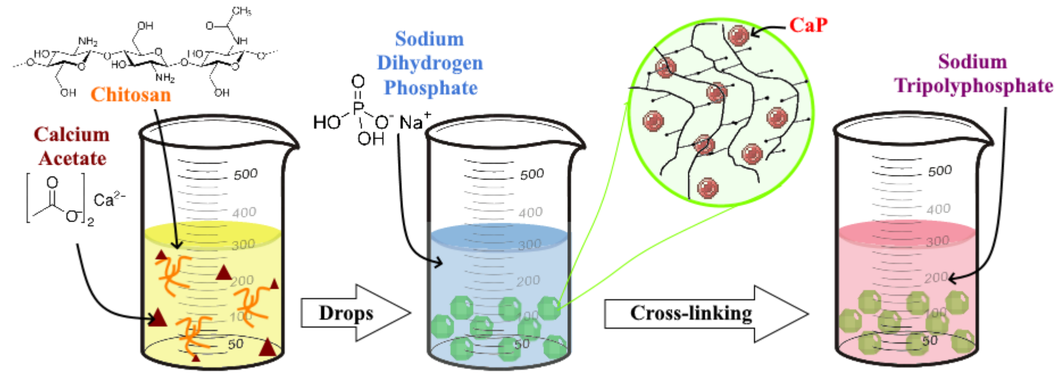

Fig. 1 The schematic illustration of the synthesis process.

Table 1 Characteristics of samples and synthesis conditions

Sample	Weight ratio Ca/CS (g/g)	Calcium acetate $C_4H_6CaO_4$ 0.5M, pH=6.86 ml	CS 6%, ml	Sodium dihydrogen orthophosphate NaH_2PO_4 0.3M, pH=7.06	MW, 300 W	Sodium tripoly phosphate Na5P3O10, 1%
1	0.15	0.25	2.5	abundance	5 x 15 sec	12 h
2	0.45	0.75	2.5		5 x 15 sec	12 h
3	0.75	1.25	2.5		5 x 15 sec	12 h

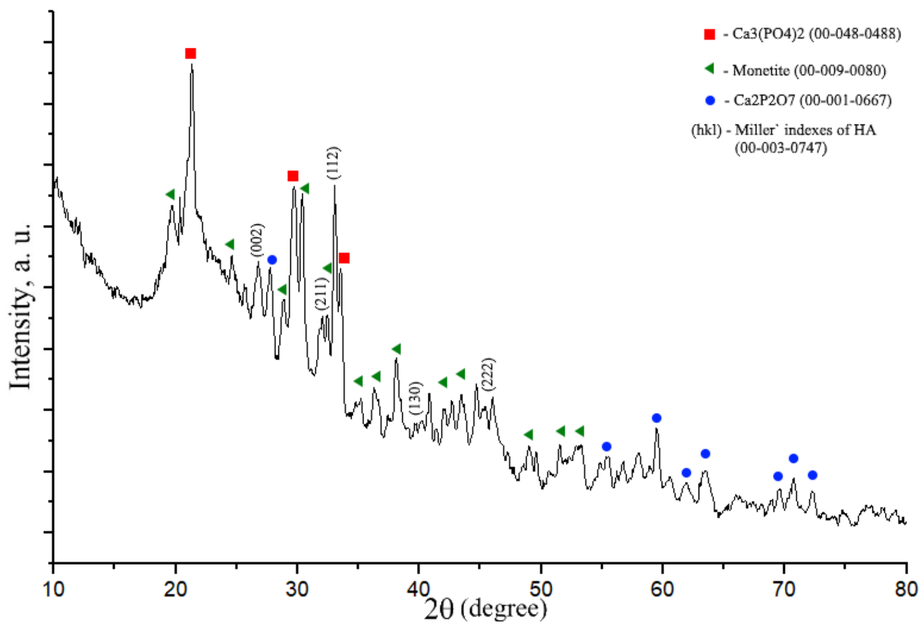

Fig. 2 XRD spectrum from sample 3, annealed at 950 °C for 1 hour.

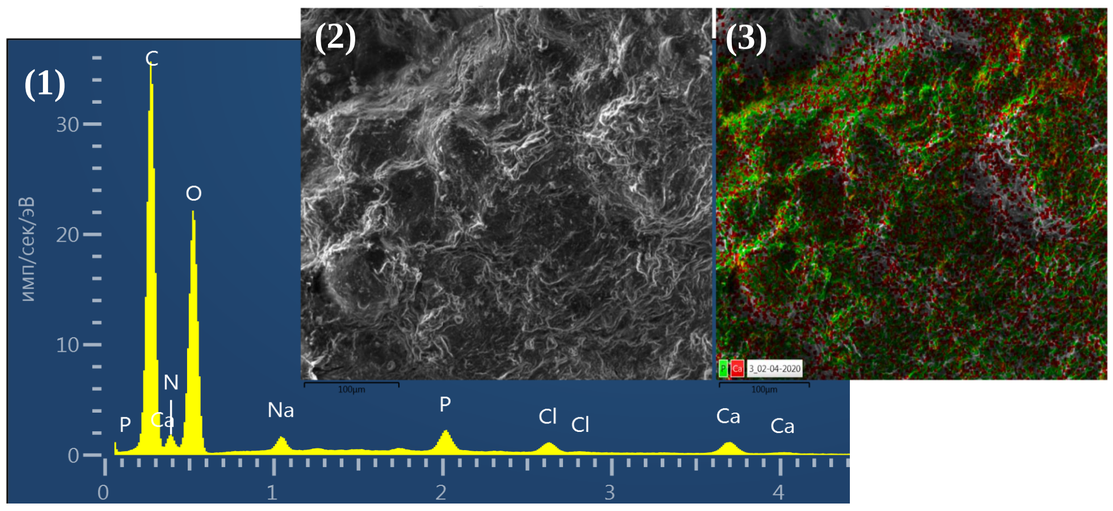

Fig. 3 Elemental analysis (1) of the Sample 3 and SEM micrograph (2) after 48 days in SBF; (3) map of the location of calcium and phosphorus ions on the surface of the granule**.**

Table 2 The elemental composition of the samples before and after being in SBF solution

Element	EDA, вес.%					
	Sample 1		Sample 2		Sample 3	
	Before SBF	After SBF	Before SBF	After SBF	Before SBF	After SBF
Ca	0.61	1.11	0.66	1.5	1.51	2.79
P	4.5	0.87	5.21	1.32	7.92	2.41
Na	2.44	0.97	4.33	0.94	7.84	0.51
C	44,59	47,77	37,94	46,53	31,24	39,22
Ca/P w%	0.135	1.275	0.126	1.136	0.19	1.157
Ca/P at%	0.106	1.0	0.097	0.88	0.147	0.89

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