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Fabrication and characterization of laponite-calcium phosphate based cement for filling bone defects

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Abstract

The aim of this study was to develop a novel nanocomposite laponite-calcium phosphate bone cement (CPC) consisting of various concentrations of laponite nanoplates (LAP). Moreover, effects of LAP on the mechanical and handling properties of CPCs were studied. Results demonstrated that the rate of converting cement reactants into hydroxyapatite significantly increased, while initial and final setting time of cement gradually decreased by incorporation of LAP. Noticeably, incorporation of 2 wt. % LAP into CPC reduced the initial and final setting time. Moreover, incorporation of 2 wt. % LAP significantly improved the mechanical properties and injectability of CPCs.

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Keywords: Bone cement; Calcium phosphate; Laponite nanoplates; Mechanical properties

1. Introduction

Extensive range of synthetic materials containing metals, ceramics, polymers and cements has been introduced as synthetic alternatives for damaged bones [1]. Among these synthetic materials, due to good bioactivity, biocompatibility and adaptability with bone tissue, calcium phosphate cements (CPCs) have been attracted wide researches[2]. In addition to their excellent biological behaviour, the main profits of CPCs are injectability and in

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situ-setting ability at physiological condition, which avoid invasive surgical procedure[3]. However, weak mechanical properties of CPCs made them unsuitable for load-bearing situations[4].

Nomenclature

CPC	Calcium Phosphate cement
LAP	Laponite
SEM	Scanning Electron Microscope
FE-SEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscope
XRD	X-ray diffraction
α-TCP	alpha tri-calcium phosphate
β-TCP	beta tri-calcium phosphate
HA	Hydroxyapatite
Ca/P	Calcium to Phosphate ratio
P_2O_5	Phosphorus pent oxide
$Ca(NO_3)_2$	Calcium nitrate
L/P	Liquid to Powder ratio
CDHA	Calcium-deficient HA
SiO_2	Silicon dioxide
Na_2HPO_4	Disodium phosphate
ASTM C191-91	Standard Test Methods for Time of Setting of Hydraulic Cement by Vicat Needle

Generally, final mechanical strength of CPCs depends on the degree of cement conversion, the porosity of the cement, the type of setting product, the crystal size and/or the use of filler particles[5]. Accordingly, wide studies have focused to design and fabricate nanocomposite CPCs with high mechanical strength [1, 6-8]. The promising approach to improve the mechanical strength of CPCs is to strengthen them with nanoparticles [7].

Recently, synthetic nanoclays such as Laponite (LAP, $Na_{0.7}$ $[(Mg_{5.5}Li_{0.3})$ Si_8O_{20} $(OH)_4]^{-0.7}$) have been introduced as promising bioceramics for bone tissue regeneration. LAP is a biocompatible hydrous magnesium silicate belonging to the family of (2:1) phyllosilicates and possesses disk-shaped structure with a diameter 25 nm and thickness 1 nm[9]. In aqueous conditions, LAP nanoplates degrade into bioactive products which able to affect on formation and bone growth in different stages and cause acceleration of bone regeneration process [10, 11]. Results revealed that unique properties of LAP such as swelling ability, cation exchange capacity, gel formation ability, and adsorptive capacity are due to high specific surface area, pH-depended edge charge formed by broken bonds at the edges of clay, surface charge and appropriate interaction with organic and inorganic materials consisting of drugs, proteins and biological molecules[12]. Due to these unique properties, LAP nanoplates have been widely applied to develop nanocomposite constructs in the various polymers and ceramic matrices[13, 14]. Despite these wide researches, the incorporation of LAP nanoplates within CPCs has not been investigated, yet. The aim of this study was to develop nanocomposite LAP-CPCs consisting and study the effects of LAP concentration on the mechanical properties, setting time and injectability of bone cement.

2. Materials and Methods

Initially, CPC consisting of 98 wt. % α-tri-calcium phosphate (α-TCP) (Ca/P =1.5) and 2 wt. % hydroxyapatite (HA) (Ca/P =1.67) nanopowders was prepared. In this regard, α-TCP and HA nanopowder were synthesized using sol-gel technique. In order to synthesize HA nanopowder, 17 wt. % P_2O_5 (Merck) and 83 wt. % $Ca(NO_3)_2$ (Merck) solutions in ethanol were prepared, separately. After mixing two solutions and aging for 24 h at room temperature,

as-prepared gel was dried at 80°C and consequently calcified at 650°C to achieve HA powder. α-TCP powder was synthesized in a two-step process; in the first step, calcium-deficient HA (CDHA) with calcium to phosphorus precursor ratio of 1.5 was synthesized similar to the HA synthesis procedure as mentioned above. However, the calcification temperature was selected at 650 °C. In the second step, as prepared CDHA powder was dispersed in 30 ml distilled water and sonicated for 15 min to obtain a homogeneous solution. Consequently, in order to stabilize α-TCP at room temperature, 3 wt% colloidal silica (SiO_2) (Merck) was added to the suspension and sonicated for 15 min. The suspension was milled at 250 rpm for 12 h, dried at 110°C for 24 h, and then heated at 1250 °C with the heating rate of 10 °C/min for 2 h, followed by quenching in the furnace. In order to prepare CPC, various concentrations of LAP (Rockwood Additives Limited, UK) (0, 1, 2 and 3 wt. %) was added to the calcium phosphate precursors. Consequently, powder component and liquid phase consisting of 8% (w/v) disodium hydrogen phosphate (Na_2HPO_4, Merck) aqueous solution were mixed with a fixed powder to liquid (P/L) ratio of 0.5. After that, prepared cement paste was poured into cylindrical mold and placed in 100% relatively humidity (0.9% saline) at 37 °C for pre-determined intervals (1, 3 and 5 days). According to the LAP concentrations (0, 1, 2, and 3 wt. %), samples were named as CPC-0, CPC-1, CPC-2 and CPC-3, respectively.

3. Characterization

Setting time of CPC was measured by using Vicat needle based on ASTM C191-91 standard [15]. The injectability of CPCs was examined by using a syringe (10 ml syringe, carrying a nozzle with aperture diameter of 2 mm). 150 s after mixing, CPC paste was completely extruded from the syringe under hand pressure and the injectability of CPCs was calculated by the ratio between the paste weight extruded from the syringe and the total paste inside the syringe [16].

The chemical composition of calcium phosphate precursors as well as bone cements was evaluated by X-ray diffraction (XRD) using Philips X'pert-MPD System with Cu Kα radiation. Moreover, the crystallite size of α-TCP and HA powders were calculated by using modified Scherrer equation as below (eq. 1) [17].

$$ln\beta = ln\frac{k\lambda}{L} + ln\frac{1}{cos\theta} \qquad (1)$$

In which L and λ stand for crystallite size (nm) and wavelength of X-ray diffraction machine (λ=0.15406 nm) respectively. θ is angle of single peak, β stands for peak width at half height, and k is constant (k=0.9). The cross-section morphology and microstructural changes during setting reaction were assessed by field emission scanning electron microscope (FE-SEM) using MIRA3 TESCAN model. Moreover, transmission microscope electron (TEM, Philips EM208S 100 kV, Netherland) was applied to evaluate the morphology and particle sizes of LAP nanoplates. Mechanical test on set cement samples was measured using compression test machine (Hounsfield, H25KS). The cement pastes were poured in the form of cylindrical molds (15 mm diameter and 10 mm height), placed in the medium with 100% humidity and 37°C for pre-determined intervals (1, 3, 5 days) and dried before the mechanical test. The molds were compressed along their height at a speed of 0.5 mm/min[18].

4. Results and Discussion

Fig. 1 shows TEM image of LAP nanoparticles applied as the strengthening agent into the cement and its particle size distribution. Results confirmed that LAP nanoparticles possessed a disk-shaped structure with particle size of 42.5±11 nm.

The initial and final setting times of nanocomposite cements as well as their injectability are presented in Table 1. An increase in the amount of LAP concentration, initial and final setting times of CPC were decreased which

could be helpful for minimally invasive surgical procedure. Generally, setting of CPC takes place by the formation of a solid network and is influenced by hydrolysis rate of primary reactants. Moreover, the required Energy for the formation of this solid network is provided by surface energy produced from mixture of liquid and powder phases. Therefore, higher rate of hydrolysis reaction causes to increase super-saturation rate and consequently reduces setting time. On the other hand, getting smaller of particle size of primary reactants used to prepare CPC causes to accelerate super-saturation state as a result of raising specific surface area, thus, setting time of CPC remarkably reduces[19].

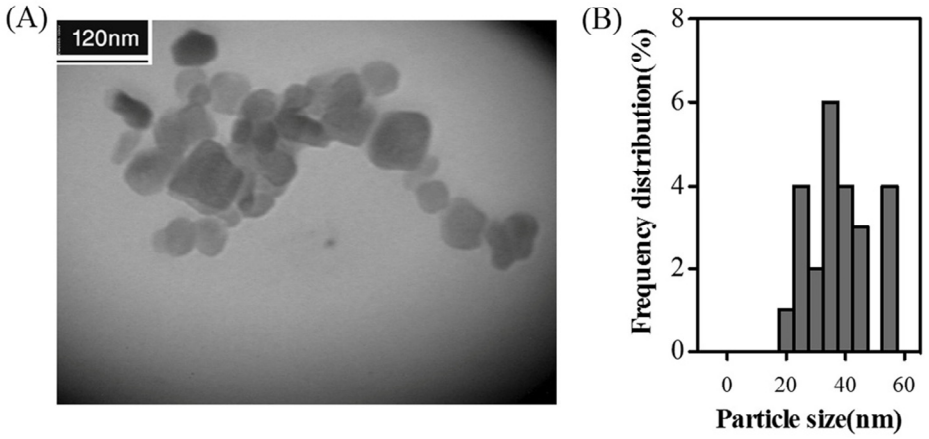

Fig. 1. A)TEM micrograph of LAP nanoplates and B) the particle size distribution of LAP.

Table1: setting time and injectability of CPC with different concentration of LAP.

Sample	Setting Time (min)		Inejectability
	Initial time	Final time	
CPC-0	6.3±2.0	14.2±4.0	87.0±1.4
CPC-1	6.1±1.3	14.0±1.1	90.50±3.5
CPC-2	5.9±1.2	13.8±1.9	91.6±3.8
CPC-3	5.0±1.5	10.0±0.8	83.2±8.7

Moreover, according to Table 1, CPC-2 showed the greatest injectability (approximately 92 %) which can be considered as well injectable. Generally, incorporation of additives such as ascorbic acid[20], reduction of reactant particle size [19] and formation of hydrogel [1] result in improved rheological properties (injectability, viscosity, and cohesion) of cement paste, homogeneous dispersion of powder and liquid phases during injection and complexly extruding the paste from syringe. Therefore, according to what mentioned above, incorporation of LAP nanoparticles into CPC, rheological properties such as viscosity and cohesion were enhanced and as a result of that, injectability of nanocomposite cement containing LAP was improved. Nevertheless, more than 2 wt.% of LAP, viscosity was extremely raised and injectability was consequently decreased. On the other hand, plasticity of nanocomposite CPC was evaluated by molding them in complicated molds such as star-shaped star. Fig. 2 confirmed that nanocomposite cement paste was able to fill edges of star-shaped molds. Thus, this cement could inject into the complicated defects and filled it completely.

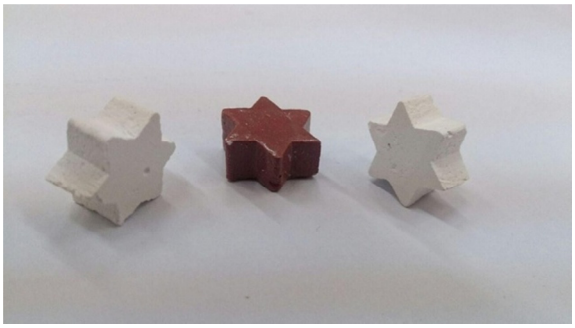

Fig. 2. Plasticity of CPC into star-shaped molds.

XRD patterns of LAP nanoplates as well as synthesized HA, α-TCP powders, CPC-0 and CPC-2 after 3 days of soaking in ringer's solution are presented in Fig. 3. Results confirmed that α-TCP and HA powders were synthesized without any secondary phase. Moreover, crystallite size of α-TCP and HA powders were calculated 28.70 and 18.70 nm by using modified Scherrer equation (1). XRD pattern of CPC-2 sample consisted of an extra peak at approximately 2θ=28°, according to the XRD pattern of LAP nanoplates, this peak was related to LAP nanoplates confirming the presence of LAP nanoparticle in the structure of cement, and the rest of peaks contributed to characteristic peaks of HA according to standard card of HA (01-086-1199 (ICSD code)). However, considering XRD pattern of CPC-0, it could be found out that except the peaks located at approximately 28 and 31° assigned to β-TCP, the rest of peaks at XRD pattern of CPC-0 were related to the characteristic peaks of HA according to standard card of HA (01-086-1199 (ICSD code)). Additionally, as obviously shown, by incorporation LAP nanoparticle into CPC after 3 days of immersing, α-TCP almost totally converted to CDHA indicating an increase in conversion rate by adding LAP. On the other hand, crystallite size of CPC-0 and CPC-2 after 3 days of immersion in renger's measured 46.08±6 nm and 36.29±4.53 nm, in sequences. As observed, by adding LAP nanoplates, formed crystals of HA got smaller, it could be due to act LAP nanoplates as Nucleation sites[19].

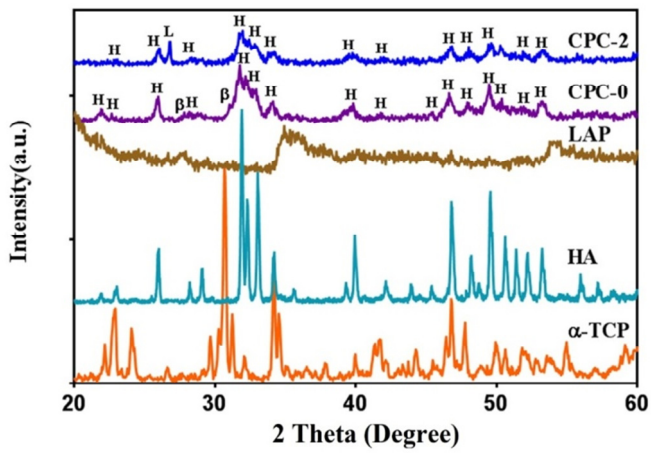

Fig. 3: X-ray diffraction of α-TCP, HA, and LAP powders as well as CPC-0 and CPC-2 after 3 days immersing in ringer's solution (H=HA, β=β-TCP, and L=LAP).

Fig. 4 presents the surface morphology of CPCs consisting of various LAP contents. The surface morphology of all samples resembled plate-liked particles with various sizes depending on the cement type. This morphology was similarly reported in previous studies and related to precipitate HA crystals[21]. These particles became smaller and narrower when concentration of LAP was gradually increased. For instance, while particle size of HA in CPC-0 was 2.84±1.05 μm, incorporation of 3 wt.% LAP nanoplates resulted in reduced HA particle size to 37.6±12.2 nm. In these CPCs, LAP nanoparticles acted as nucleating agents leading to an increase in nucleating sites. Therefore, these agents prevented growing HA particles and provided precipitation of HA crystals more homogenous.

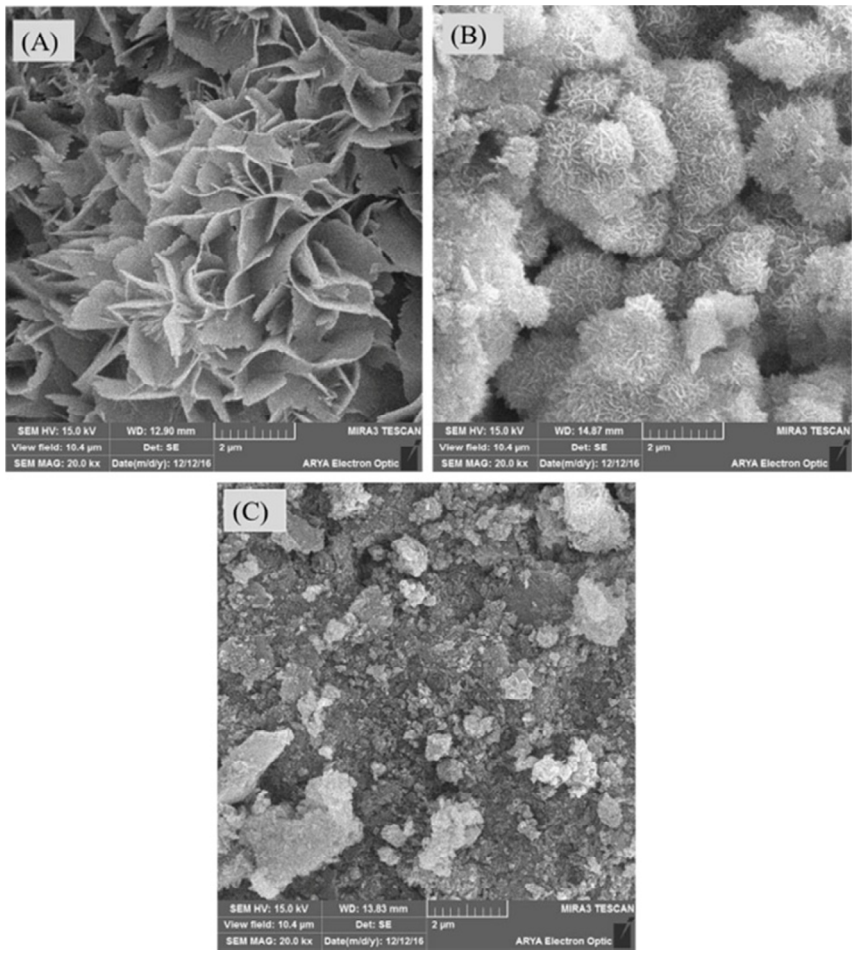

Fig. 4. FE-SEM images of (A) CPC-0, (B) CPC-2, and (C) CPC-3 after 3 days of immersion in ringer's solution.

Mechanical strength of the set cements was investigated by using compression test machine. According to Fig. 5, the incorporation of LAP nanoparticles to CPC from up 0 (CPC-0) to 2 wt.% (CPC-2) resulted in a significantly enhanced mechanical properties. In this regard, mechanical strength and young's modulus of set samples increased from 18.70±2.23 MPa to 26±2.36 MPa and from 0.50±0.15 GPa to 1.27±0.15 GPa, respectively. It could be related to micro/nanopores, which were filled by LAP nanoparticles and a significant increase in nucleating sites after addition of LAP nanoparticles. However, incorporation of higher amount of LAP nanoplates than 2 wt. % (CPC-3) resulted in reduced mechanical properties of CPC which could be due to either agglomeration of LAP nanoparticles into CPC structure.

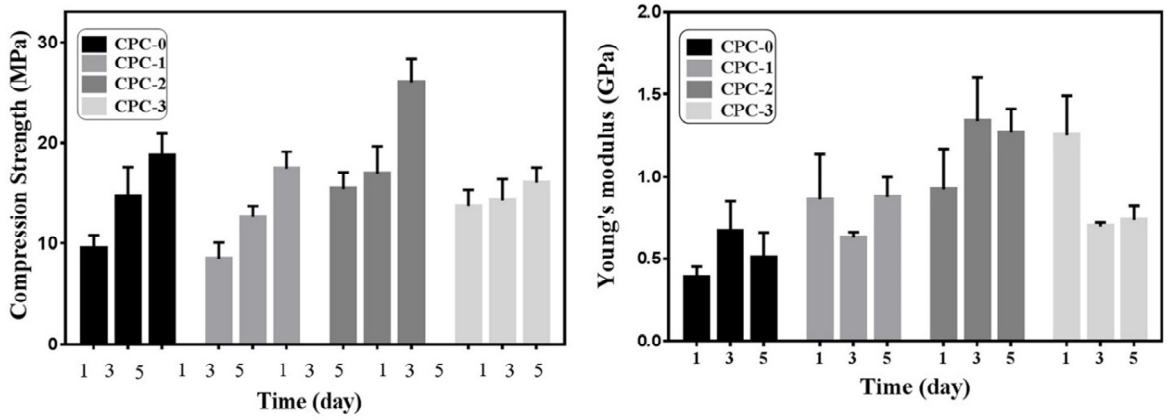

Fig. 5. (A) Compressive strength and (B) young's modulus of CPC with different concentrations of LAP (0, 1, 2, and 3 wt.%).

5. Conclusions

It could be concluded that CPCs prepared by using α-TCP and HA as the powder phase, 2 wt. % LAP nanoplates and 8 wt.% Na_2HPO_4 aqueous solution as the liquid phase revealed suitable setting time and injectability with enhanced mechanical properties. The proposed materials have shown a potential for filling bone defects in minimally invasive surgical procedures.

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