



Apacite nanocomposites: A novel bioactive, biocompatible and osteogenic product originated from atmospheric carbon dioxide processed spontaneously in Calcoline



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HIGHLIGHTS

- Apacite nanocomposites have been synthesized from atmospheric CO₂ in Calcoline.
- Calcoline plays as CO₂ trapper-solubilizer, reaction medium and reactant.
- The nanocomposites are composed of apatite-calcite in weight ratio of 53%–47%.
- Apacite nanocomposites shows improved bioactivity and cellular behavior.
- The osteogenic differentiation of hMSCs is improved significantly on Apacite.

GRAPHICAL ABSTRACT



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ABSTRACT

Apacite (apatite-calcite) nanocomposites have been synthesized using an environmentally friendly, affordable, and one-step method relying on deep eutectic Calcoline (calcium chloride-choline chloride). The deep eutectic system plays as an all-in-one system providing all requirements for the one-step synthesis of Apacite nanocomposites including reactants, reaction medium, particle growth controller, and platform for carbon (atmospheric CO₂) capture and conversion into calcite. The characterization results confirm the synthesis of nanoparticulate Apacite composites composed of apatite crystallites with an average size of 25 nm and calcite crystallites with a mean size of 35 nm in the phase ratio 53 wt% apatite: 47 wt% calcite. The biological results obtained on *in vitro* bioactivity and behavior of hMSCs emphasize on favored non-toxicity, bioactivity, and osteogenic properties of as-synthesized Apacite nanocomposites. It seems that the nanoscale structure, high aspect ratio, low crystallinity, improved dissolution and ion release of Apacite nanocomposites provide the optimum conditions for cellular growth and differentiation without signs of toxicity.

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1. Introduction

Bone is a metabolically active tissue with hierarchical architecture constituted by soft organic and stiff inorganic materials [1,2]. The organic fraction of bone is composed of collagen fibers containing tropocollagen, which contribute to bone toughness [1]. The mineral part of bone mainly consists of hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$, HA) nanocrystals contributing to bone strength and stiffness [1,3]. HA found in bone is a non-stoichiometric or calcium deficient HA known as biological HA, which is highly bioactive and resorbable as well as can be produced by osteoblasts over and over throughout the life. This makes bone to be alive and self-restorative rather than being inert and dead [4].

Bone defects and functional disorders have become a global healthcare issue leading to the emergence of the orthopedic market valued at \$4.3 billion in 2015, which it is expected to reach at \$46.5 billion by 2024 [5]. Beside tremendous therapeutic methods, bone grafting has turned out to be a successful approach to accomplish bone reconstruction and regeneration. Bone substitute grafts include autogenous grafts, allografts, xenografts, and synthetic grafts [6]. The drawbacks in using autogenous grafts, allografts, and xenografts, including limited supply and donor site, requirement of secondary operation, and problems associated with diseases such as HIV and hepatitis, result in shifting from natural grafts to synthetic alternatives during the past decades [6]. Meanwhile, several synthetic bioactive materials have been successfully employed as bone substitute grafts such as HA, β -TCP, and bioactive glasses [7]. Synthetic HA nanostructures have been extensively studied as bone substitute biomaterials owing to their osteoconductive capabilities and biocompatibility as well as structural and functional similarity to the apatite phase in living bone [1,7]. This physicochemical similarity has led to a wide range of biomedical applications for nanoapatite-based bioceramics including various orthopedic and dental applications [8,9]. However, the low resorbability kinetics of the synthetic apatite resulted from its high crystallinity and low rate of dissolution causes the limited practical experience of HA in bone tissue engineering [10,11]. An overview of the scientific literature reveals that calcium carbonate (calcite, CaCO_3) nanoparticles show enhanced resorption caused by their high solubility, which can meet practical biomedical needs [12,13]. In addition, CaCO_3 has gained great interests in biomedical fields due to abundance in nature, biocompatibility and its functions in pharmaceutical applications including drug delivery and neurological signaling [13,14]. Therefore, the nanocomposites based on calcite and apatite can satisfy the required bioactivity (resulted from apatite) and resorbability (resulted from calcite) demanded for real clinical applications [15,16]. Moreover, the nanoscale structure of the apatite-calcite composites would further contribute to strengthening the aforementioned properties [16].

Considering their biomedical importance, in recent years, several methods have developed for the synthesis of apatite-based nanostructures; chemical precipitation [17], hydrothermal synthesis [18], sol-gel method [19], and mechanochemical processing [20] are among the most important synthesis methods of apatite nanostructures. The disadvantages encountered with the developed methods such as strictly controlled reaction conditions, using expensive reagents, consumption of large amounts of harmful solvents, and being multi-stage and time-consuming, have led researchers to seek alternative synthesis methods [21].

In recent years, the synthesis methods based on green solvents, especially deep eutectic solvents (DESs) have attracted much attention. DESs are a new generation of green solvents mainly obtained by complexation of quaternary ammonium salts (e.g., choline chloride) with hydrogen bond donors (e.g., urea) or metal chlorides. Owing to their outstanding properties, including high solubilizing ability, high ionic conductivity, good thermal stability, low vapor pressure, green entity, biocompatibility, and ease of preparation, the DESs are in the process of

establishing themselves as one of the foremost candidates for developing one-pot and green methods toward synthesis nanomaterials [22]. As an attempt toward developing a/an economical, green, and one-step synthesis method, herein, apatite-calcite (here named as Apacite) nanocomposites have been synthesized using deep eutectic calcium chloride-choline chloride (named as Calcoline). The deep eutectic system serves as an all-in-one system that provides all necessary requirements for the one-step synthesis of Apacite nanocomposites including reagents, reaction medium, a platform for atmospheric carbon (as a source for calcite) capture, and formation/growth control of the nanocomposites. The microstructure of the synthesized nanocomposites has been analyzed using XRD, FESEM, TEM, EDS, FTIR, and Raman techniques. To prove the possible biomedical applications of the synthesized nanocomposites, the *in vitro* bioactivity, cytotoxicity and osteogenic properties of Apacite nanocomposites have been assessed using mesenchymal stem cells.

2. Material and methods

2.1. Preparation of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ -choline chloride deep eutectic system (DES)

The DES is prepared by mixing calcium chloride hexahydrate ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 98%, Sigma-Aldrich) and choline chloride ($\text{C}_5\text{H}_{14}\text{ClNO}$, 99%, Sigma-Aldrich) powders in a molar ratio of 2:1 followed by heating at 95 °C for the desired time to obtain a homogeneous and viscous liquid. The resulting DES is named as Calcoline.

2.2. Synthesis of apatite-calcite nanocomposites

Apatite-calcite nanocomposites are prepared using a novel and one-step method using atmospheric CO_2 as a carbon source for calcite nanoparticles. In a typical synthesis procedure, 100 ml of Calcoline is heated up to 100 °C under stirring on a magnetic stirrer. 10 ml of a 2.5 M aqueous solution of potassium phosphate tribasic ($\text{K}_3\text{PO}_4 \cdot 7\text{H}_2\text{O}$, 98%, Sigma-Aldrich) is prepared which is slowly dropped into Calcoline. The resulted solution is held under stirring at 100 °C for 3 h. The obtained precipitates after the desired time are filtered, washed several times with deionized water and finally dried in a vacuum oven at 60 °C for 12 h. The as-synthesized fine powders are called as Apacite nanocomposites.

2.3. Characterization of Apacite nanocomposites

X-ray diffraction (XRD, Siemens D-500 diffractometer, angular range: $2\theta = 20\text{--}70^\circ$, scan speed: 2°min^{-1} , step size: 0.02°) method is used to analyze the crystal structure of Apacite nanocomposites. The average size of calcite and apatite crystallites is estimated from Debye-Scherrer formula [23]:

$$D = \frac{0.94\lambda}{\beta \times \cos \theta} \quad (1)$$

where D is the average crystallite size, λ is the wavelength of $\text{CuK}\alpha$ radiation ($\lambda = 0.154 \text{ nm}$), θ is Bragg diffraction angle, and β is full width at half maximum (FWHM) of (2 1 1) and (1 0 4) diffraction planes for apatite and calcite crystallites, respectively.

Semi-quantitative phase analysis of as-synthesized Apacite nanocomposites is performed by XPert HighScore Plus software (version 2.2b) using Rietveld refinement.

Field-emission scanning electron microscopy (FESEM, Tescan Mira 3 LMU) equipped with energy dispersive X-ray spectroscopy (EDS, Bruker, Quantax 200) is employed to study the morphology and elemental composition of as-synthesized Apacite nanocomposites.

Transmission electron microscopy (Philips CM 30) operated at 200 kV is used to measure the particle size of Apacite nanocomposites.

Fourier transform infrared spectroscopy (FTIR, PerkinElmer

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