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Facile synthesis of lithium carbonate nanoparticles with potential properties for bone repair applications

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ABSTRACT

Lithium carbonate nanoparticles were synthesized by facile chemical route in the presence of organic capping agents. Nanosized Li_2CO_3 particles of ~86 nm in size were produced from LiOH solution and preferably using PVA as a capping agent. FTIR-ATR analysis revealed that Li_2CO_3 phase is formed during calcination of the freeze-dried synthesis product. *In vitro* tests shown that nanoparticle concentrations up to 600 μg/mL do not disturb cell viability and promote the osteogenic differentiation of stem cells by Li^+ ions (9.7 mM) leached from the nanoparticle. The biological properties exhibited by the Li_2CO_3 nanoparticles make of them attractive for bone repair applications.

1. Introduction

Lithium, a key ingredient for batteries, is also a therapeutic element with properties to stimulate the cellular mechanisms of bone tissue formation [1]. Local application of Li^+ ions enhances bone healing by increasing bone mineral density or accelerating the bone formation process [2]. Likewise, bioceramics such as calcium phosphate cement [3] and microbioactive glass [4,5] have shown a superior osteogenic and angiogenic [6] properties when doped with lithium.

Elements produced in nanoparticle form offer greater surface area for biological interactions, a more controlled release of ions, and the possibility of designing nanocomposite materials. Bioactive nanoparticles in powder are also demanded in bone reconstruction and tissue engineering. The synthesis of zero-valent lithium 5 nm – colloidal nanoparticles has been reported by chemical reduction of Li^+ ions [7], which however requires sophisticated separation methods [8]. In this respect, lithium carbonate (Li_2CO_3) nanoparticles can be produced in their powder form as demonstrated by Lu et al. [9] by precipitation reactions in a membrane microreactor.

In this work, we reported the synthesis of powdered Li_2CO_3 nanoparticles by using facile chemical routes and studied the influence of different capping agents on particle size. The cytocompatibility and ability of the nanoparticles to enhance the alkaline phosphatase (ALP) activity in stem cells is also demonstrated.

2. Materials and methods

2.1. Synthesis of nanoparticles

Nanoparticles were synthesized from a lithium hydroxide (LiOH) solution in the presence of polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), or cetyltrimethylammonium bromide (CTA) as capping agents. Briefly, 0.5 g of capping agent compound was dissolved in 50 mL distilled water at room temperature for 1.5 h. Then, 1 g of LiOH was added and the reacting solution was kept under stirring at room temperature for 1 h. Afterward, the suspension was frozen at −80 °C, lyophilized and calcined at 700 °C in air for 5 h at a heating rate of 4 °C/min to obtain a fine white powder.

2.2. Nanoparticle characterization

The particle size and morphology of the synthesized products were analyzed by scanning electron microscopy (SEM) with a Jeol JSM-IT300LV microscope equipped with energy dispersive X-ray detector Aztec EDS (Oxford Instruments) for microanalysis. Particle size distribution was estimated by using the analysis tools of the microscope software. The data were analyzed using One-way ANOVA analysis and Tukey's multiple comparison tests in Graph Pad Prism software at $p < 0.05$.

The percentage yield of the synthesis process was estimated by dividing the mass of the synthesis product by the mass of LiOH used in the reaction according to the following expression: % Yield = (mass of synthesized product/LiOH mass) × 100.

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Table 1
Mean particle size of products and synthesis yields.

	Particle size (nm)	Yield (%)
PVA/nLi	86.6 ± 10.8	86
PVP/nLi	108.5 ± 19.3	71
CTA/nLi	101.9 ± 17.5	48

A selected nanoparticle product was further analyzed by attenuated total reflectance with Fourier transform infrared spectroscopy (ATR-FTIR) on an Agilent Cary 630 ATR-FTIR spectrometer. X-ray diffraction (XRD) pattern was measured on a Siemens D 5000 diffractometer using CuKα radiation within a 2θ range of

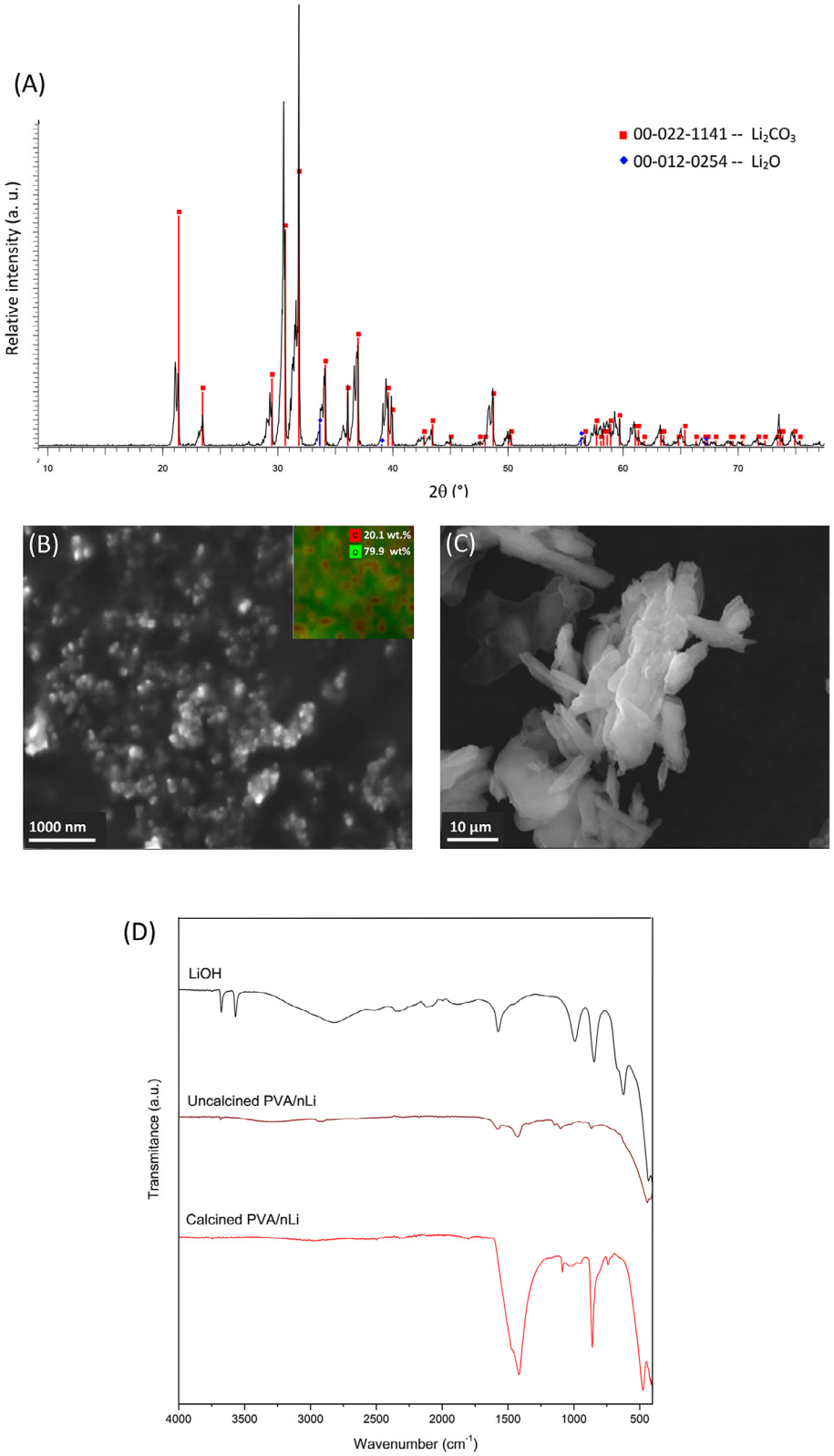

Fig. 1. XRD analysis (A) and SEM image (B) of PVA/nLi particles. SEM image of traditional Li_2CO_3 microparticles (C). FTIR-ATR analysis of PVA/nLi synthesis products (D).

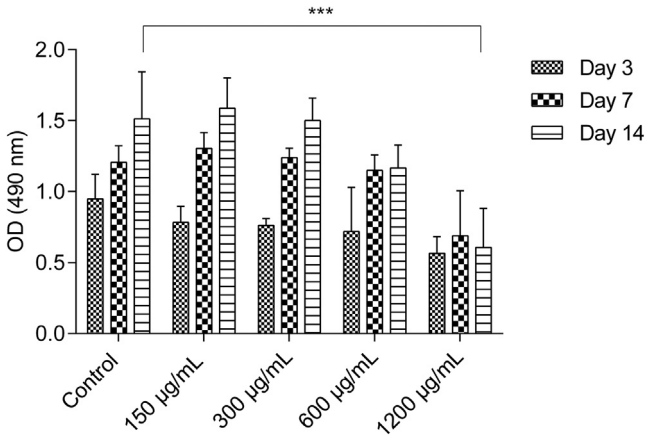

Fig. 2. Viability of DPSCs cultured in media conditioned with PVA/nLi nanoparticles.

5–80° at a scanning speed of 1.2°/min. Phase identification was carried out using the ICDD Powder Diffraction File (PDF2).

2.3. Cell culture

The viability of Dental Pulp Stem Cells (DPSCs) cultured in nanoparticle conditioned medium with 150, 300, 600, and 1200 µg/ml of PVA/nLi was assessed at 3, 7 and 14 days of incubation. Cell viability was determined with CellTilter 96® Aqueous One Solution Cell Proliferation Assay (Promega Corporation). ALP activity was determined by absorbance measurements at 405 nm in the supernatant of lysed cells caused by dephosphorylation of p-nitrophenyl phosphate substrate.

The release of Li^+ from nanoparticle in the cell culture media at 37 °C were measured by flame emission photometry in a JENWAY PFP7 flame photometer using the lithium emission line at 671 nm.

3. Results and discussion

Table 1 presents the particle size of the obtained products and the yields of each synthesis condition. The product synthesized in the presence of PVA was found to exhibit the smallest and uniform particle size. PVA was the only capping agent that produced particles with a size smaller than 100 nm. In addition, the synthesis method using PVA exhibited the highest yield of nanoparticle powder. Polar —OH groups of PVA molecules have a stronger interaction with the Li^+ cations than that produced through amide groups of PVP, which stabilize more strongly the cations, controls the nanoparticle growth and prevent their aggregation. A similar effect has been observed in the synthesis of silver nanoparticles from Ag^+ cations by using PVA and PVP [10]. By contrast, the use of cationic CTA yielded larger particle sizes, even when surfactant micelles have been found influence the shape and size of nanoparticles [11].

The nanoparticles produced with PVA were chosen for further structural analysis and bioactivity testing because it exhibited the smaller particle size and higher synthesis yield. XRD analysis (Fig. 1a) revealed that nanoparticles present a highly crystalline structure corresponding to that of Li_2CO_3 phase (ICDD: 00-022-1141) with monoclinic space group C2/c. Some peaks of Li_2O were also detected, suggesting the presence of traces of the metallic oxide. SEM image of PVA/nLi particles (Fig. 1b) confirms their nanometric nature, which clearly contrasts with that of traditional microsized Li_2CO_3 particles (>10 µm) (Fig. 1c). EDX analysis and most of the conventional solid-state analysis techniques do not detect lithium because of the too low energy of Li (Z = 3) K X-rays. However, C/O molar ratio of PVA/nLi (1:3) matches with that of CO_3^{2-} anion. The formation of PVA/nLi from LiOH was followed by ATR-FTIR (Fig. 1d). The O—H stretching vibrations of LiOH around 3600 cm^{-1} and for H_2O in LiOH at 1573 cm^{-1} disappear or decrease their intensity in the freeze-dried synthesis product (uncalcined), while PVA/nLi spectrum presents the antisymmetric C—O stretching vibrations of CO_3^{2-} at 1419 cm^{-1} and those of out-of-plane deformation modes at 853 cm^{-1} [12]. These results indicate that the freeze-dried product is transformed into Li_2CO_3 during the calcination period. The conversion of NaOH into Na_2CO_3 has been also detected by FTIR after thermal treatment of the alkali hydroxide at 120 °C in air [13]. Similarly, in the present study, calcination of LiOH produces the thermal decomposition of PVA and simultaneous formation of the Li_2CO_3 phase. Our single-phase synthesis method is proved to be simpler than the nanoparticle preparation process carried out in a microfiltration membrane dispersion reactor [9], which also involves a multiphase system with interphase mass transfer considerations.

Nanoparticles with potential for bone regeneration applications should be cytocompatible and stimulate the osteogenic differentiation of stem cells. Fig. 2 show that PVA/nLi do not induce

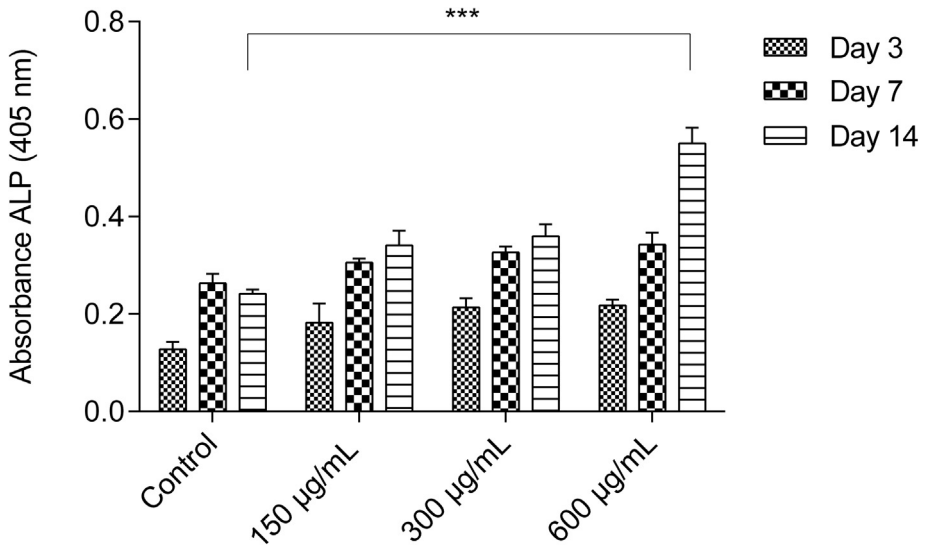

Fig. 3. ALP activity in DPSCs cultured in media conditioned with PVA/nLi nanoparticles.

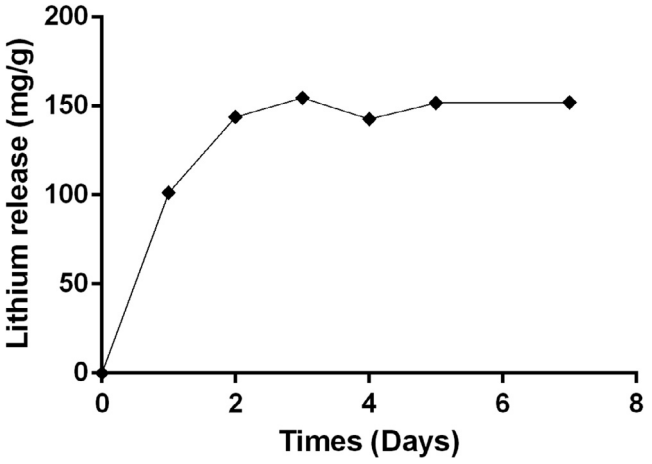

Fig. 4. Cumulative Li^+ release from PVA/nLi in cell culture medium. The amount of lithium released is expressed as Li^+ mass per nanoparticle mass.

statistically significant changes in cell viability at concentrations up to 600 μg/mL, above which only a marginal decrease is observed. The ALP enzymatic activity was measured for the nanoparticle concentrations that did not provoke a decrease in cell viability. ALP is an important marker of the osteogenic differentiation process that is produced when bone forming cells lay down the bone extracellular matrix [14]. From Fig. 3 it can be seen that the ALP activity is significantly increased relative to control in DPSCs cultured with 600 μg/mL PVA/nLi after 14 days of incubation ($p < 0.001$). Li^+ ions are known to activate the Wnt/β-catenin signaling pathway that promotes stem cell differentiation into specialized bone-forming cells. In the current study, the capacity of the PVA/nLi to stimulate the cell differentiation can be attributed to the Li^+ ions generated by dissolution of the Li_2CO_3 nanoparticles. Lithium analysis in the culture medium containing 150, 300, 600, and 1200 of PVA/nLi revealed Li^+ concentrations of 1.47 mM, 3.90 mM, 9.71 mM and 16.75 mM, respectively. These results indicate that Li^+ concentrations around 9.71 mM would be more favorable to promote the osteogenic differentiation process, which agrees well with reported lithium concentrations that upregulated differentiation markers and accelerated osteogenesis after local administration of conventional lithium salts [15]. Due to that lithium compounds dissolve readily in water, nanosized PVA/nLi particles are expected to provide a more controlled release of therapeutic lithium concentrations for long-term bone treatment. Li^+ release from nanoparticle became stabilized after 2 days around 150 mg/g (10.2 mM) (Fig. 4), showing a sustained release rate of the element. In addition, powdered nanoparticles are preferred materials for the design of advanced biomaterials, such as 3D nanocomposite scaffolds for bone tissue engineering or osteoinductive surfaces to promote titanium implant osseointegration.

4. Conclusions

Nanosized Li_2CO_3 particles can be successfully synthesized by a facile chemical route and preferably using PVA as a capping agent. The nanoparticles produce significantly high cell viability and promote the osteogenic differentiation of stem cells. The biological properties exhibited by the nanoparticles are promising to be exploited in bone regeneration applications.

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