

Microwave Assisted Synthesis of Cobalt Phosphate Nanoparticles and Their Antiproliferation against Human Lung Cancer Cells and Primary Osteoblasts in Vitro

Guoqiang Zhou (Corresponding author)

College of Chemistry and Environmental Science

Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education

Chemical Biology Key Laboratory of Hebei Province, Hebei University

Baoding 071002, China

Tel: 86-312-507-9359 E-mail: zhougq1982@sohu.com

Wenying Wang, Guangqi Gu & Yang Li

College of Chemistry and Environmental Science

Chemical Biology Key Laboratory of Hebei Province, Hebei University

Baoding 071002, China

Tel: 86-312-507-9795

Ying Liu

CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety

National Center for Nanoscience and Technology

Beijing 100190, China

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Abstract

In this research, different nanostructures of cobalt phosphate nanoparticles were successfully prepared. Platelike and spherical cobalt phosphate were made by microwave synthesis method without any use of capping agent as structure directors. The reaction was completed under two different microwave irradiation power (500 W and 800 W) and times (5 min and 10 min) for the production of two types of cobalt phosphate nanoparticles. The synthesized nanoparticles were characterized by field emission scanning electron microscopy (FE-SEM), powder X-ray diffraction (XRD), BET, fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS). The SEM images showed that the flowerlike nanostructure was an arrangement of cobalt phosphate plates with thickness of 80 nm and the average size of spherical cobalt phosphate nanoparticles was about 40 nm. Antiproliferation activity of cobalt phosphate nanoparticles as a function of particle concentration against human lung cancer cells and primary osteoblasts were carried out in vitro. The spherical nanoparticles showed better antiproliferation activity than the platelike nanoparticles and primary osteoblasts were more sensitive than human lung cancer cells to cobalt phosphate nanoparticles.

Keywords: Cobalt phosphate nanoparticles, Microwave synthesis, Antiproliferation, Osteoblasts

1. Introduction

Nanomaterials are defined by the U.S. National Nanotechnology Initiative as materials that have at least one dimension in the 1- to 100- nm range (Stix G, 2001). Due to their unique physicochemical properties such as a

large specific surface area and greater reactivity, nanotechnology has become one of the leading technologies. The enhanced production and applications of manufactured nanoparticles have raised questions concerning their potential toxicological effects (Oberdorster G, 2005). Cobalt phosphate ($\text{Co}_3(\text{PO}_4)_2$) was introduced as violet pigment by Salvetat in 1895. Its hue fluctuates between pink and violet because of state of hydration (Dalia J, 2009). $\text{Co}_3(\text{PO}_4)_2$ can be used as catalyst, for example, amorphous $\text{Co}_3(\text{PO}_4)_2$ can be used as catalyst in the oxidation of water in the presence of sun light and for the selective reduction of NO with C_3H_6 or CH_4 (Matthew W K, 2009). $\text{Co}_3(\text{PO}_4)_2$ and its derivatives especially LiCoPO_4 are taken into account as candidates which can be used as cathodes in lithium-ion batteries (Jang I C, 2010).

Usage of nanostructures of $\text{Co}_3(\text{PO}_4)_2$ is a key to improve its applications. As materials dimensions approach the nanoscale, certain properties become scale dependent. These include optical color, conductivity, electron affinity and catalytic activity (Clinton F J, 2009). So $\text{Co}_3(\text{PO}_4)_2$ which has proper nanostructure can perform significantly better in battery cathodes usage and catalytic applications (Lee M T, 2009). Recently, it has been reported that microwave irradiation can be used to prepare high purity nanoparticles with narrow particle size distributions. Microwave heating has been known since the early 1940s, and has been used in preparative chemistry and material synthesis since 1986 (Giguere R J, 1986). Microwave irradiation is an efficient and distinct heating method, and has attracted researcher's interest owing to its unique features such as short reaction time, rapid volumetric heating, energy saving and high reaction rate (Ela S E, 2009). The greatest advantage of microwave irradiation is that it can heat a substance uniformly through a glass or plastic reaction container, leading to a more homogeneous nucleation and a shorter crystallization time compared with those for conventional heating (Norihito K, 2010). This is beneficial to the formation of uniform colloidal materials.

In this study, $\text{Co}_3(\text{PO}_4)_2$ nanoparticles with different nanostructures were synthesized by microwave irradiation process. And the synthesized $\text{Co}_3(\text{PO}_4)_2$ nanoparticles were characterized by field emission scanning electron microscopy (FE-SEM), powder X-ray diffraction (XRD), BET, Fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS). The antiproliferation effects of two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles on human pulmonary adenocarcinoma cells A549 and primary osteoblasts (OBs) were investigated. The results indicated that the $\text{Co}_3(\text{PO}_4)_2$ nanoparticles inhibited the proliferation of two type cells following dose and time dependent manner. The spherical nanoparticles showed better antiproliferation activity than the platelike nanoparticles and OBs were more sensitive than A549 cells to $\text{Co}_3(\text{PO}_4)_2$ nanoparticles.

2. Experimental

2.1 Materials and reagents

All the chemicals used in this investigation were analytical grade materials and used without further purification. Cobalt sulfate heptahydrate ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, $\geq 99\%$), sodium dodecylbenzene sulfonate (SDBS, $\text{C}_{18}\text{H}_{29}\text{NaSO}_3$, 99%), sodium phosphate monobasic dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, $\geq 99.0\%$) and urea (CON_2H_4 , $\geq 98\%$) were purchased from Sigma Aldrich. Human pulmonary adenocarcinoma cells A549 were purchased from the ATCC (Beijing, Zhongyuan LTD, China). Kunming (KM) mice were obtained from Experimental Animal Center of Hebei Medical University. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 and trypsin were purchased from GIBCO, USA. New born calf serum was from Hangzhou Sijiqing Organism Engineering Institute. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), penicillin, streptomycin, collagenase type II were from Sigma Aldrich. Deionized water was used to prepare aqueous solutions.

2.2 Synthesis of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

The $\text{Co}_3(\text{PO}_4)_2$ nanoparticles were synthesized using microwave method. 100 ml of 3 mM $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 3 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.3 mg of $\text{C}_{18}\text{H}_{29}\text{NaSO}_3$ and 6 mg CON_2H_4 were mixed in a beaker to get an aqueous solution. This solution was divided in two parts and processed according to different steps respectively. One part of the solution was stirred vigorously for 3 min and then heated in a microwave oven (Galanz 800 W) at 500 W power for 3 min. After boiling the mixture, the device power was adjusted to 200 W and continued heating 2 min. The other part was stirred vigorously for 3 min and then heated in a microwave oven at 800 W power for 10 min. Then the reactor device was taken out and cooled with water. The precipitate was separated by centrifugation (3000 rpm, 5 min) and washed with ethanol 5 times. The wet precipitate was maintained at 100°C in a laboratory oven for 1 h.

2.3 Characterization

The morphology and size of synthesized $\text{Co}_3(\text{PO}_4)_2$ nanoparticles were measured by field emission scanning electron microscope (JSM-7500F, JEOL, Japan). A minute drop of nanoparticles solution was cast on to a carbon-coated copper grid and subsequently drying in air before transferring it to the microscope. X-ray powder

diffraction was performed on a Bruker D8 Advance X-ray diffractometer employing Cu-K α radiation with 40 kV and 50 mA (D8 ADVANCE, Bruker, Germany). For surface area measurements, the Branauer, Emmett and Teller (BET) method was used with TriStar II 3020, a volumetric adsorption apparatus (TriStar II 3020, Micromeritics, USA). The typical bonds were detected by fourier transform infrared spectroscopy (Nicolet380, Thermo, USA). The FTIR spectra obtained using the improved KBr pellet method by grinding down the resin beads prior to recording. The size distribution of the nanoparticles in medium was evaluated by dynamic light scattering (Delsa Nano C, Beckman, USA). Data were analyzed based on six replicated tests.

2.4 Cell culture

Human lung alveolar carcinoma epithelial cells (A549) were cultured in RPMI 1640 under a humidified atmosphere (5% CO₂ plus 95% air) at 37 °C. All media were supplemented with 10% heat inactivated new born calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. The isolation and culture of OBs were following the method previously described (Zhang J C, 2010).

2.5 Cell proliferation assay

A549 Cells and OBs (2×10^3 cells/100 µl) were seeded onto 96-well plates and incubated overnight at 37 °C under a 5% CO₂ atmosphere. The medium in the wells was then replaced with fresh medium containing nanoparticles (5-80 µg/ml) and incubation continued for 24 and 48 h. The effects of the nanoparticles on cell viability were determined using the MTT assay. Briefly, 10 µl of MTT solution was added to each well and the plates incubated for 4 h. The supernatant was removed and DMSO (100 µl) was added to solubilize the MTT. The absorbance at 570 nm of each well was measured with a microplate spectrophotometer (BioRad Model 3550, USA). Cells incubated without nanoparticles were used as a control. The cell viability was calculated according to the formula: $A_{\text{sample}} / A_{\text{control}} \times 100\%$.

3. Results and discussion

3.1 Morphology characterization

Two types of Co₃(PO₄)₂ nanoparticles were successfully synthesized using microwave irradiation method. The morphology and size of synthesized Co₃(PO₄)₂ nanoparticles were examined by field emission scanning electron microscopy (FE-SEM). The results showed that morphology and size of nanoparticles varied with different radiation power and time. Fig. 1B is the local magnified panel of Fig. 1A and shows the typical images of the platelike samples. The Co₃(PO₄)₂ plates with thickness of 80 nm were connected with each other forming 3D nanoflowers (Fig. 1C). When the microwave power reached 800 W and reaction time extended to 10 min, the stable and nearly spherical nanoparticles with average diameter of 40 nm were synthesized (Fig. 2). It seems that when the microwave radiation power and reaction time increase, the size of synthesized nanoparticles becomes smaller.

3.2 XRD analysis

The phase composition and structure of obtained samples were examined by X-ray powder diffraction (XRD). Fig. 3A and B showed the XRD patterns of platelike and spherical Co₃(PO₄)₂ nanoparticles, respectively. All of diffraction peaks could be indexed to pure monoclinic phase of Co₃(PO₄)₂ with lattice constants $a = 7.557 \text{ \AA}$, $b = 8.365 \text{ \AA}$ and $c = 5.067 \text{ \AA}$ (JCPDS Card No. 13-0503). However, the diffraction peaks obtained from platelike nanoparticles were slightly sharper than the diffraction peaks obtained from spherical nanoparticles. According to Scherrer equation, it is clearly indicated that the product crystallinity improved and particle size increased with the decreasing of microwave power and reaction time. These results are consistent with SEM observations. No other impurities can be detected.

3.3 BET and FTIR analysis

The BET surface areas for platelike and spherical Co₃(PO₄)₂ nanoparticles were 4.15 and 5.25 m²/g, respectively, which suggest that the smaller sized particles have larger surface areas. The FTIR spectra of the synthesized Co₃(PO₄)₂ featured phosphate absorption bands (Fig.4). The triply degenerated asymmetric stretching and bending vibrations of PO₄³⁻ were at 1030 and 570 cm⁻¹. The peaks at 3000 ~ 3500 and 1627 cm⁻¹ corresponded to the remaining water. The Co-O peaks were at 854 and 703 cm⁻¹.

3.4 Size distribution in medium

The SEM images provided information on the size and shape of nanoparticles, however, it could not provide information on whether the nanoparticles existed in single or aggregated forms in the test medium, as the nanoparticles form aggregates when dried on the microscopic observation slide. The size distribution in the test medium, therefore, was investigated using a DLS method, which showed that the average size of platelike and

spherical $\text{Co}_3(\text{PO}_4)_2$ in the test medium were 336.3 ± 52.2 nm and 132.1 ± 37.4 nm, respectively (Fig. 5). This suggested that the nanoparticles exposed to the cells did not exist as single particles, but tended to aggregate in the test medium.

3.5 Effects of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles on proliferation of cell lines

Human lung alveolar carcinoma epithelial cells A549 and OBs were continuously treated with two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles for 24 or 48 h at concentration range from 5 to 80 $\mu\text{g/ml}$. $\text{Co}_3(\text{PO}_4)_2$ nanoparticles inhibited the proliferation of two cell lines following dose and time dependent manner. Similar growth inhibition pattern was observed in A549 cells and OBs. For the total range of concentrations, the most pronounced inhibition was found at 48 h ($p < 0.01$). None of $\text{Co}_3(\text{PO}_4)_2$ concentrations induced 50% growth inhibition towards A549 cells at 24 h. But at a concentration of 80 $\mu\text{g/ml}$ $\text{Co}_3(\text{PO}_4)_2$ induced an inhibition of 60% in OBs during 24 h (Fig. 7 A). It seems that OBs are more sensitive than A549 cells to $\text{Co}_3(\text{PO}_4)_2$ nanoparticles. The zone of inhibition studied in the two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles synthesized were significantly different as shown in Fig. 6 and Fig. 7. For both cells, the inhibitory effects of spherical nanoparticles were higher than the platelike nanoparticles. We know that the size of spherical $\text{Co}_3(\text{PO}_4)_2$ is smaller than the platelike $\text{Co}_3(\text{PO}_4)_2$. It is logical to state that the inhibition of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles to the cells depends on the particle size. The smaller sized particles which have larger surface areas available for interactions with cells than the larger sized particles, hence they show higher cytotoxicity to cells.

4. Conclusions

In summary, platelike and spherical $\text{Co}_3(\text{PO}_4)_2$ nanoparticles were synthesized successfully using microwave irradiation technique. $\text{Co}_3(\text{PO}_4)_2$ nanoparticles with average diameter ~ 80 nm for 500 W and ~ 40 nm for 800 W were highly stable as they retained their pink and violet color for a longer duration. SEM images showed that microwave power and reaction time play an important role in the formation of the $\text{Co}_3(\text{PO}_4)_2$ nanoparticles. In the process of $\text{Co}_3(\text{PO}_4)_2$ crystal growth, stirring, power, time and its physicochemical nature were responsible for the formation of structures. XRD pattern showed that pure nanostructures with high crystallinity had been made. Antiproliferation activity of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles revealed that the zone of inhibition was significantly different in case of two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles, while the smaller size nanoparticles showed slightly better antiproliferation effect. In our study, $\text{Co}_3(\text{PO}_4)_2$ nanoparticles can inhibit the proliferation of A549 cells and OBs following dose and time dependent manner and OBs showed more sensitivity than A549 cells to $\text{Co}_3(\text{PO}_4)_2$ nanoparticles.

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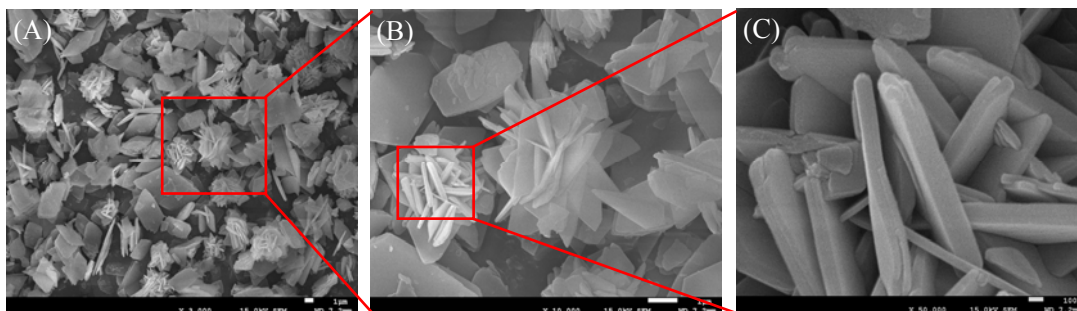


Figure 1. Field emission scanning electron microscope (FE-SEM) images with different magnifications (A-C) of the platelike $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

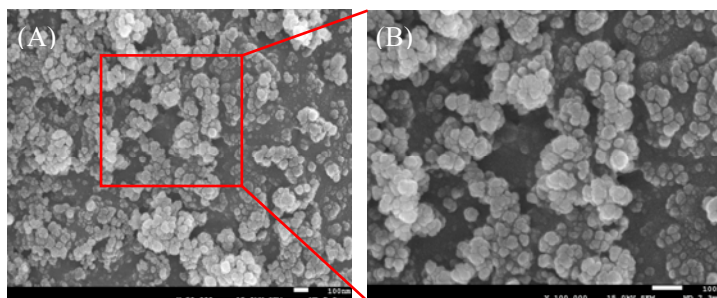


Figure 2. Field emission scanning electron microscope (FE-SEM) images with different magnifications (A-B) of the spherical $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

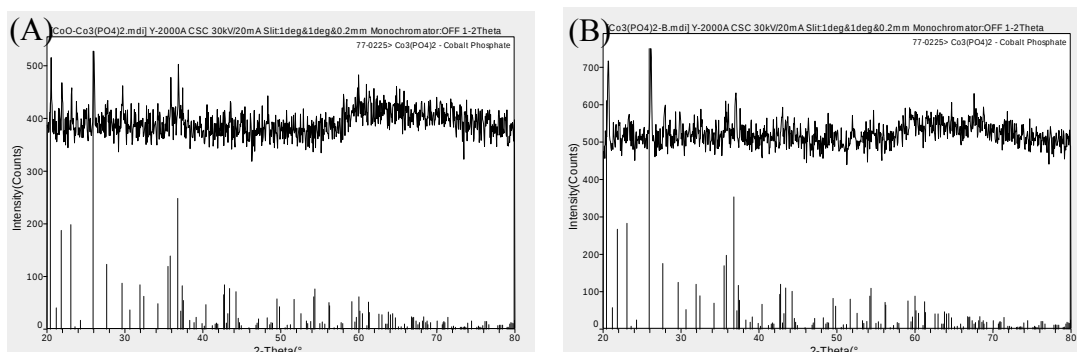


Figure 3. XRD patterns of two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles formed by microwave heating
(A) The platelike $\text{Co}_3(\text{PO}_4)_2$ nanoparticles; (B) The spherical $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

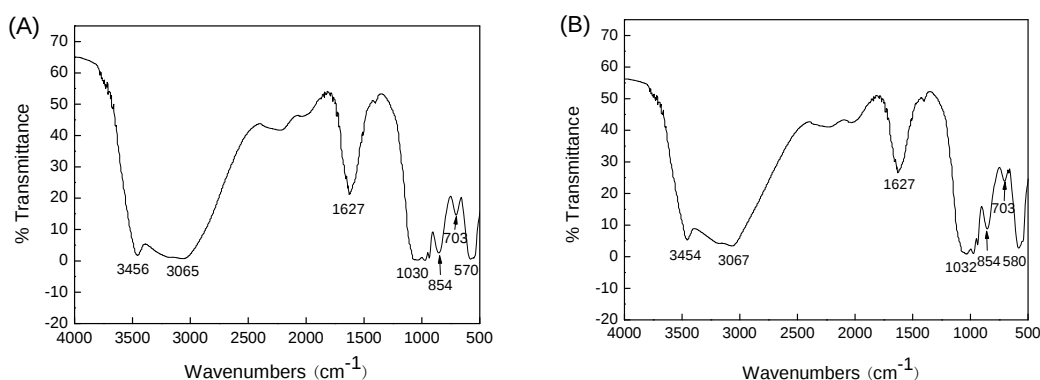


Figure 4. FTIR spectra of samples formed by microwave synthesis method
(A) The platelike $\text{Co}_3(\text{PO}_4)_2$ nanoparticles; (B) The spherical $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

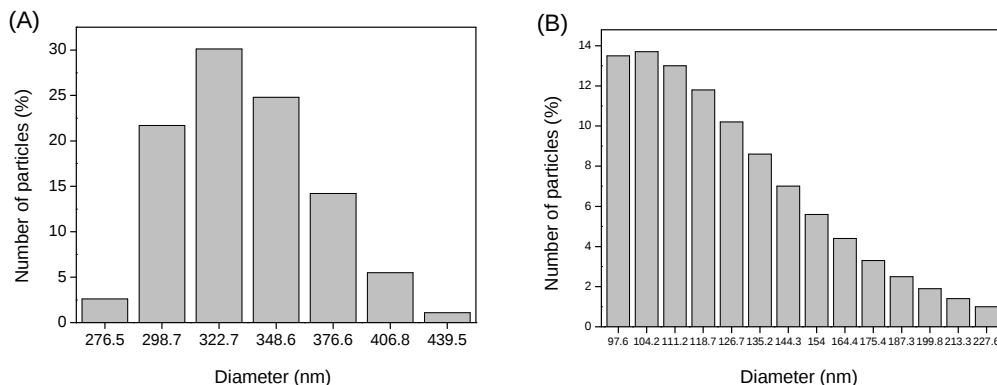


Figure 5. Size distribution of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles in medium as measured by dynamic light scattering (DLS)
(A) The platelike $\text{Co}_3(\text{PO}_4)_2$ nanoparticles; (B) The spherical $\text{Co}_3(\text{PO}_4)_2$ nanoparticles

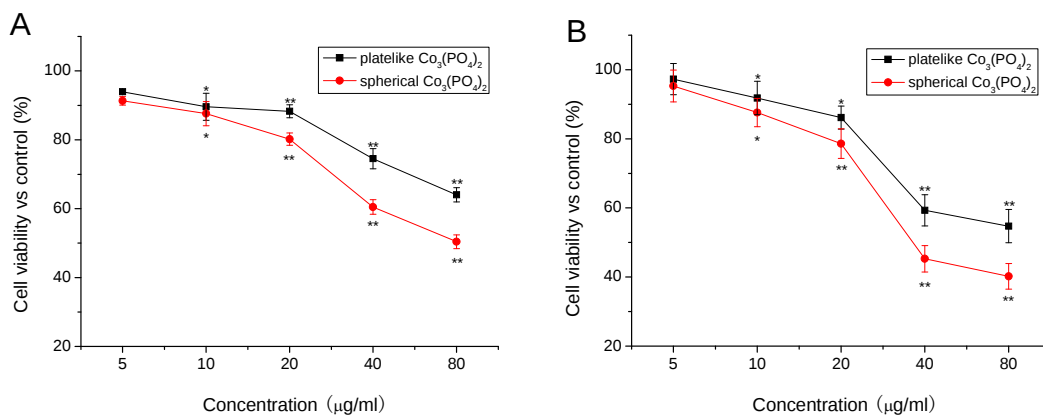


Figure 6. Effects of two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles on the proliferation of A549 cells
(A) 24 h; (B) 48 h. (* $P < 0.05$, ** $P < 0.01$ compared with the control group, $n = 6$)

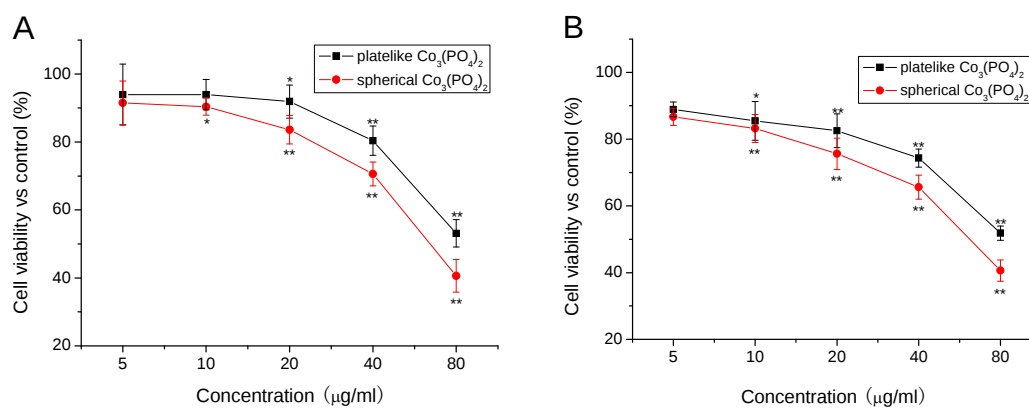


Figure 7. Effects of two types of $\text{Co}_3(\text{PO}_4)_2$ nanoparticles on the proliferation of primary mouse osteoblasts (A) 24 h; (B) 48 h. (* $P < 0.05$, ** $P < 0.01$ compared with the control group, $n = 6$)