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Inhibitory effect comparison of the needle, spherical, and mesoporous hydroxyapatite nanoparticles on MCF-7 breast cancer cell line proliferation: An *in vitro* assay

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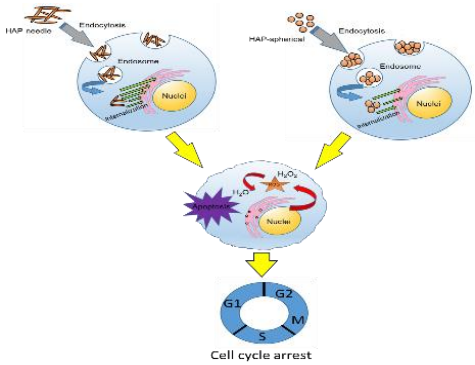
Abstract

Hydroxyapatite (HAp), the main calcium phosphate and the most inorganic compounds in hard tissues, exhibits sound cytocompatibility and osteogenic activity for clinical bone replacement and tissue engineering. HAp nanostructures depict the more special characteristics than HAp microstructures in various features such as physical, chemical and biological properties. Besides, outstanding characteristics of HAp in bone tissue engineering, recently, inhibitory effects of nanoscaled HAp in different tumor cells proliferation and especially breast cancer was fully detailed and discussed in the literature. Here for the first time, we propose the capability of three needle, spherical, and mesoporous HAp nanoparticles for inhibition of cancer cell proliferation *in vitro*. The comparison of the three morphologies of HAp nanoparticles was carried out by MTT assay. The results showed that the proliferation of the cancer cell line was reduced by more than 73% after treatment with the Hap nanoparticles for 3 days. The best inhibitory effect was obtained for the needle-shaped HAp nanoparticles that was assigned to their diffusivity into the cell membrane. These results propose that nanoscaled HAp could inhibit cancer cell growth and proliferation, so these nanomaterials can be considered as promising materials in clinical cancer therapy.



Keywords: *Cancer cell proliferation, Inhibitory effect, Nanoscaled hydroxyapatite, Mesoporous shape, MTT assay.*

Introduction

Hydroxyapatite (HAp) is a kind of the calcium phosphate complexes that acts as the inorganic mineral in bones and teeth of animals and humans.[1] Moreover, HAp is a biocompatible and bioactive ceramic that has excellent binding properties to biomolecules such as proteins and DNA.[2] Furthermore, HAp nanostructures and nanoparticles have demonstrated exceptional characteristics varying considerably from bulk or HAp micro-structures and microparticles including better osseointegrative properties,[3] enhanced fracture toughness and hardness,[4] improved adsorption capacity to biomolecules and drugs as nano-carriers, and great solubility in acidic pH environment of cellular endolysosomes.[2,5,6] Bone is the first place for breast cancer metastasis, a disorder that could increase fracture, spinal cord compression, hypercalcemia, pain, and a devastating decrease in the quality of life.[7] The inhibitory effect of nanoscaled HAp on the cancer cells proliferation has been explored and proposed in 1990.[2] Recently, it generated serious attention in biomaterial science and clinical trials.[2,7-9] In our previous reports, we remarked that tricalcium phosphate nanostructures prepared with the co-precipitation method reduced the proliferation of breast cancer cell lines.[6,10,11] To our knowledge, the influence of the nanoscaled HAp morphology on the inhibitory effect of breast cancer cells proliferation has not been investigated. Therefore, we studied the comparison of three morphologies of nanoscaled HAp (needle, spherical, and mesoporous nanoparticles) on reducing the breast cancer cells proliferation *in vitro*. Here, structures, physicochemical characteristics, and the inhibitory effect of nanoscaled HAp as a safe drug on MCF-7 breast cancer cell line was scrutinized.

Experimental

The HAp nanostructures with different morphologies were synthesized by cetyl trimethylammonium bromide (CTAB) and poly(ethylene Glycol) (PEG) surfactants published in our earlier research.[12] In brief, the HAp nanostructures were prepared with the co-precipitation approach. The structure of the HAp nanostructures was evaluated by scanning electron microscopy (SEM). Also, cell culture and viability were carried out according to the protocols reported in our previous study.[7] Briefly, after 48 h, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was prepared at 1 mg mL^{-1} concentration in phosphate buffer solution (PBS) and passed through a 0.2 µm filter. Then, 22 µL MTT plus 200 µL Dulbecco's modified Eagle's medium (DMEM) were added to each well, except for the cell-free blank wells. The

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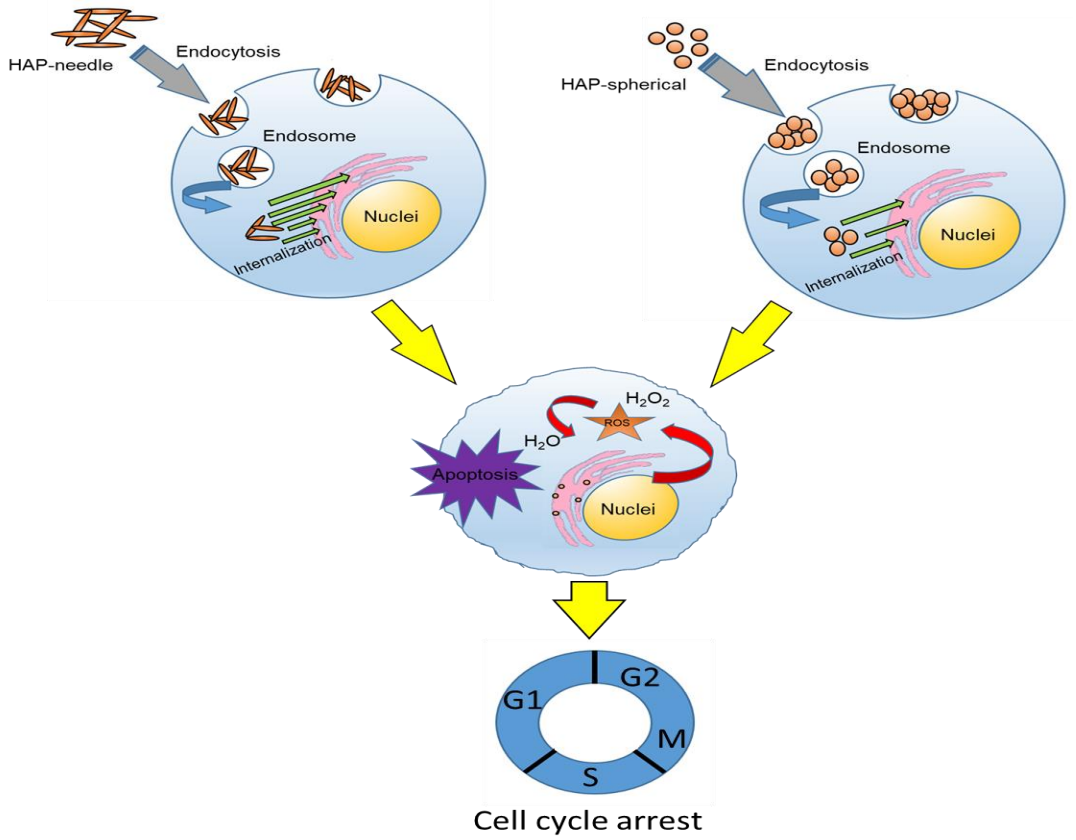

Figure 1. *The schematic presentation of the nano-HAp cell infiltration with different morphologies and subsequent toxicity mechanism.*

cells were incubated for 4 h at 37 °C with 5% CO_2, 95% air, and complete humidity. After 4 h, the MTT solution was removed and replaced with 100 µL dimethyl sulfoxide (DMSO). The plate was further incubated for 15 min at room temperature, and the optical density (OD) of the wells was determined with a plate reader (Biotek) at 570 nm wavelength, the reference wavelength was, however, 630 nm. Schematic representation of the experimental procedure is shown in Figure 1.

Results and discussion

The structure of the nanoparticles was investigated by SEM to justify the particle size and morphology. The average size of the spherical nanoparticles was estimated as 54 nm (Figure 2a) while needle nanoparticles had 246 nm in length and 43 nm in width (Figure 3a). Based on Figure 4a, the size of the mesoporous HAp particles were estimated to be 51 nm approximately.

In this study, the inhibitory effect of spherical HAp nanoparticles and needle HAp nanoparticles on breast cancer cell proliferation were compared using the MTT test. The inhibitory effect of the HAp nanoparticles with different morphologies on the proliferation of the breast cancer cell line, MCF-7, was assessed with different concentrations of the HAp nanopowders (50, 100, 150, 200, 300, 400, 500, and 600 mg L^{-1}). According to Figure 2-4b, HAp nanoparticles could decrease the MCF-7 cells proliferation. Figure 2b depicts in lower concentrations of spherical HAp nanoparticles,

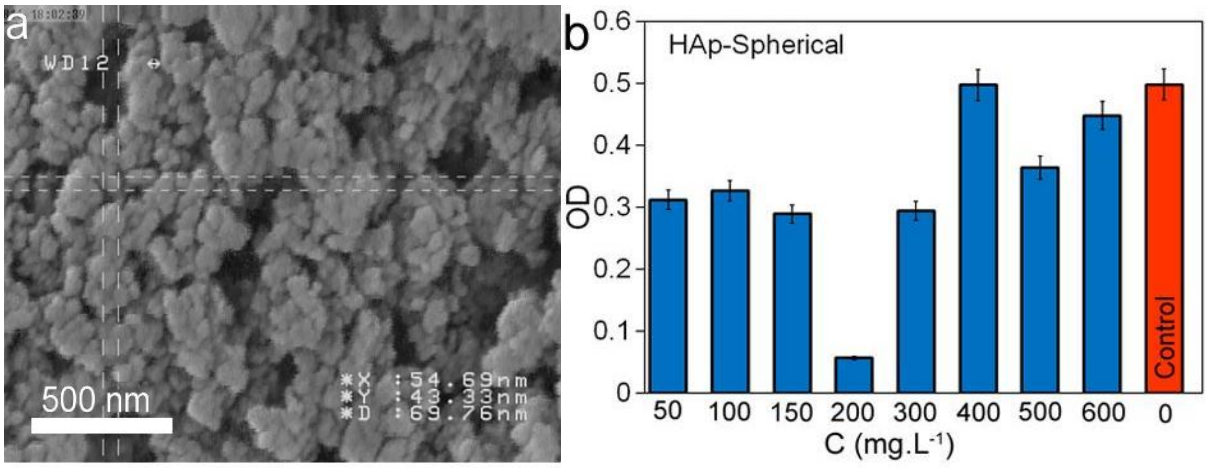

Figure 2. *(a) SEM micrograph of spherical HAp nanoparticles, (b) concentration influences of spherical HAp nanoparticles on MCF-7 breast cancer cell proliferation.*

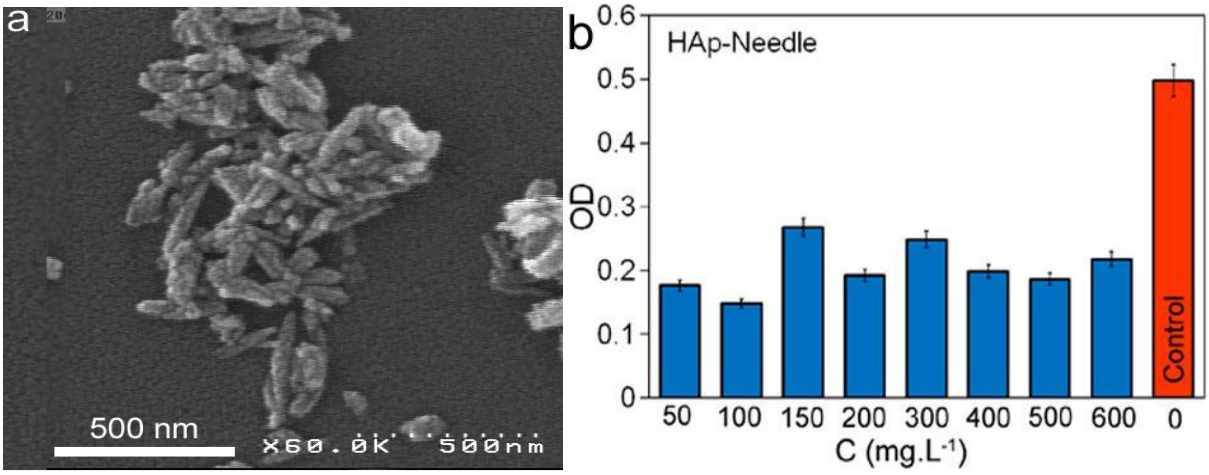

Figure 3. *(a) SEM micrograph of needle HAp nanoparticles, (b) concentration influences of needle HAp nanoparticles on MCF-7 breast cancer cell proliferation.*

the inhibitory influence is less than higher concentrations. The optimal concentration for inhibition is 200 mg L^{-1}, demonstrating that in this concentration, enough amount of nanoparticles could diffuse to the cells and inhibit the cell proliferation. In concentrations higher than 200 mg L^{-1}, the particles may be agglomerated that could reduce the diffusivity through the plasma membrane. Based on Figure 3b, the inhibitory effect of needle HAp nanoparticles is excellent in different concentrations, demonstrating that the needle shape of HAp could reduce cell growth and proliferation. The optimal concentration of needle HAp nanoparticles is 100 mg L^{-1} for inhibition of breast cancer cell proliferation (by more than 73%). This may be due to the higher diffusivity of the needle HAp nanoparticles into the cell membranes. Figure 4b shows the inhibitory effect of mesoporous HAp nanoparticles. As can be seen, the mesoporous nanoparticles have an inhibitory effect in most concentrations. The maximum inhibitory effect is observed at the concentration of 50 mg L^{-1}.

Recently, the inhibitory features of nanoscaled HAp have been well detailed in different cancer cells, including gastric,[13] breast,[7] osteosarcoma,[2] liver,[8] colon,[14] and other[15] cancer cells. All earlier surveys exhibited that there were significant differences in the inhibition level of nanoscaled HAp on various kinds of cancer cell proliferation. Han et al. reported that nanoscaled HAp had the capability for inhibition of cancer cell proliferation *in vitro* and *in vivo*. Our *In vitro* assessment showed that after treating different cancer cells by nanoscaled HAp for 3 days, cancer cells proliferation was inhibited by more than 65% while the nanoparticles could inhibit the normal cells by less than 30%. The *in vivo* results indicated that the injection of nanoscaled HAp in transplanted tumor resulted in a considerable decrease in tumor size (almost 50%). The inhibition influences of nanoscaled HAp are mostly ascribed to the excessive quantity of its endocytosis into the cancer cells which subsequently is ended up with the protein synthesis inhibition in the cells. The large amounts of nanoscaled HAp particles internalized into the cancer cells accumulates over endoplasmic reticulum. This phenomenon is claimed to be the main reason for inhibiting the protein synthesis through reducing the mRNA binding to ribosome.[2] This phenomenon demonstrates that there is a high adsorption capacity between the nanoparticles and ribosome as well as arrest cell cycle in G0/G1 phase. Also, the HAp nanoparticles exhibited neither no ROS-contained cytotoxicity nor minimal toxicity to the normal cells. These interesting results seriously proposed that the nanoscaled HAp particles could inhibit the proliferation of three cancer cell lines including MGC-803, Os-732, Bel-7402 that possessed a potential application in future cancer therapy.[2]

Two important probabilities may cause the inhibition effect of nanoscaled HAp on the gene expression/protein synthesis. The coupling of the ribosome and nanoscaled HAp reduces the coupling of ribosome and mRNA, or mRNA attached to nanoscaled HAp cannot obtain the suitable coupling site in the ribosome. Moreover, nanoscaled HAp has insignificant coupling bioactivity to mRNA.[16] Furthermore, it was reported that there was approximately no coupling between mRNA and nanoscaled HAp.[2] Also, the coupling

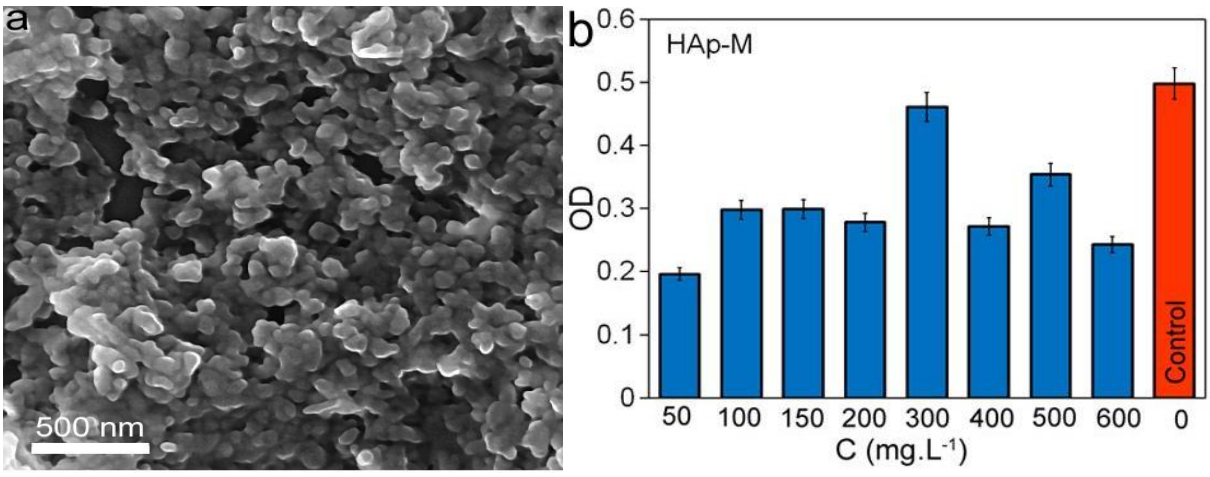

Figure 4. *(a) SEM micrograph of mesoporous Hap nanoparticles, (b) concentration influences of mesoporous HAp nanoparticles on MCF-7 breast cancer*

between ribosome and mRNA was reduced considerably after nanoscaled HAp treatment.[2] It seems that nanoscaled HAp could reduce the protein synthesis through its interaction with the ribosome that the attachment of mRNA to the suitable binding site is decreased in the ribosome, so nanoscaled HAp causes inhibiting the cell proliferation.

Conclusion

Briefly, the inhibitory effect of needle, spherical, and mesoporous HAp nanoparticles on breast cancer cells proliferation were investigated and compared. Inhibition features of nanoparticles on the growth and proliferation of MCF-7 breast cancer cell lines were explored *in vitro*. Based on the MTT assay, the inhibitory effects of all nanoparticles on the MCF-7 cell line was dependent on the conecntartion of nanoparticles. The best inhibition characteristic was reached out to be more than 73% for needle-shape HAp nanoparticles at the concentration of 50 mg L^{-1} on MCF-7 cells.

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