# Selective Inhibition of MG-63 Osteosarcoma Cell Proliferation Induced By Curcumin-Loaded Self-assembled Arginine-Rich-RGD Nanospheres

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**Statement of Purpose:** Osteosarcoma is the most frequent primary malignant form of bone cancer. Traditionally, chemotherapeutic agents have been used to treat osteosarcoma, yet, there has been no significant improvement in the human survival rate of osteosarcoma over the last several decades [1]. Thus, the development of safer, novel, and more targeted therapies is still necessary. Curcumin is a hydrophobic natural compound that has been found to have anti-cancer effects. The objective of this in *vitro* study was to develop a treatment with higher selectivity towards osteosarcoma cells and lower cytotoxicity towards normal healthy osteoblast cells using a self-assembled peptide nanosphere/curcumin complex.

**Methods:** Here, an amphiphilic peptide C18GR7RGDS was developed and tested as a novel curcumin carrier in aqueous solution. This peptide contains a hydrophobic aliphatic tail group leading to their self-assembly by hydrophobic interactions. In the hydrophilic head group, the RGD tripeptide can target overexpressed integrins on cancer cells, while the cationic arginine-rich sequence can facilitate the macropinocytosis pathway for cellular uptake [2]. Through transmission electron microscopy (TEM) characterization, the self-assembled structures of spherical amphiphilic nanoparticles (APNPs) in water, phosphate buffer saline (PBS) and acetic acid at different pH values were observed. Using a method of co-dissolution with acetic acid and dialysis tubing, a solution of APNP/curcumin was prepared. The successful encapsulation of curcumin in APNPs was then confirmed by Fourier Transform Infrared (FT-IR) and X-Ray Diffraction (XRD) analysis. The cytotoxicity the APNP/curcumin complexes on both osteosarcoma (OS, CRL-1427, ATCC, USA) and normal human osteoblast cell lines (HOB C-12760, PromoCell, Germany) were evaluated by methyl-thiazolyl-tetrazolium (MTT) assays (initial seeding density was $2\times10^3$ cells/well on a 96-well plate, and cells were treated by drugs for 24h). Cellular uptake studies were conducted by a confocal fluorescence microscope, in which the nucleus of the cells were stained by DAPI (Ex=358 nm, Em=461 nm), f-actin were stained by Atto Rho6G phalloidin (Ex=525 nm, Em=561 nm), and curcumin uptake was observed using a FITC filter (Ex=495 nm, Em=519 nm). All experiments were repeated at least three times with three replicates each. Differences between means were determined using ANOVA and unpaired student t-tests.

**Results:** In water and PBS, TEM images showed that the peptide could self-assemble into nanospheres with diameters of 10-20 nm (average diameter=15.6 nm). In an acetic acid solution at pH=6, the self-assembled nanospherical aggregates could still be observed. However, at a pH=2 and 4, only random cloud-like layers were observed, and the amphiphilic peptides could not self-assemble into nanospheres. By co-dissolution with an acetic acid solution followed by dialysis, a homogeneous solution of curcumin was successfully prepared in the presence of APNPs, and the resulting solution showed a significantly increased solubility of curcumin. Moreover, in the resulting solution, nanospheres with similar morphology as the pure APNPs but with comparably larger diameters around 18-30 nm were observed. After the drug loading process, the APNP/curcumin complex showed a similar FTIR spectra and XRD pattern as the pure APNPs, indicating the successful encapsulation of curcumin by APNPs. Cytotoxicity assays showed that the curcumin-loaded APNPs had a significantly selective inhibition against OS viability *in vitro*. At a curcumin concentration of 30 μM, the cell viability of OS was as low as 15% after being treated with curcumin-loaded APNPs, while over 50% of HOB were viable. Also, at a concentration between 20-30 μM, the curcumin-loaded APNPs had a higher selectivity against OS compared with the cytotoxicity of curcumin dissolved in DMSO. In the confocal microscopy images, the curcumin-loaded APNPs induced a significantly higher cellular uptake of curcumin in OS cells than HOB (Figure 1).

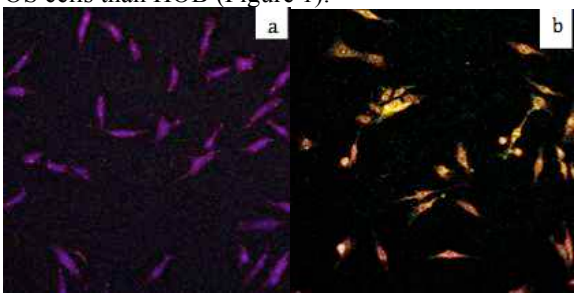


**Figure 1**. Confocal microscopy images for the cellular uptake for (a) HOB and (b) OS cells after being treated with 20 μM curcumin encapsulated by APNPs for 2h. Images were taken at a magnification of 10X. The yellow fluorescence indicates the internalized curcumin.

**Conclusions:** This study demonstrated for the first time that the arginine-rich-RGD amphiphilic peptides can serve as a drug delivery vehicle for curcumin to selectively inhibit the proliferation of osteosarcoma cells over healthy osteoblast cells. After loading with curcumin, the micelles of APNPs could induce selective inhibition against MG-63 osteosarcoma cells than normal human osteoblasts. Further studies for curcumin and gene combination delivery by APNPs, qPCR assays, and *in vivo* studies are necessary to continue this promising research.

**References:**

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