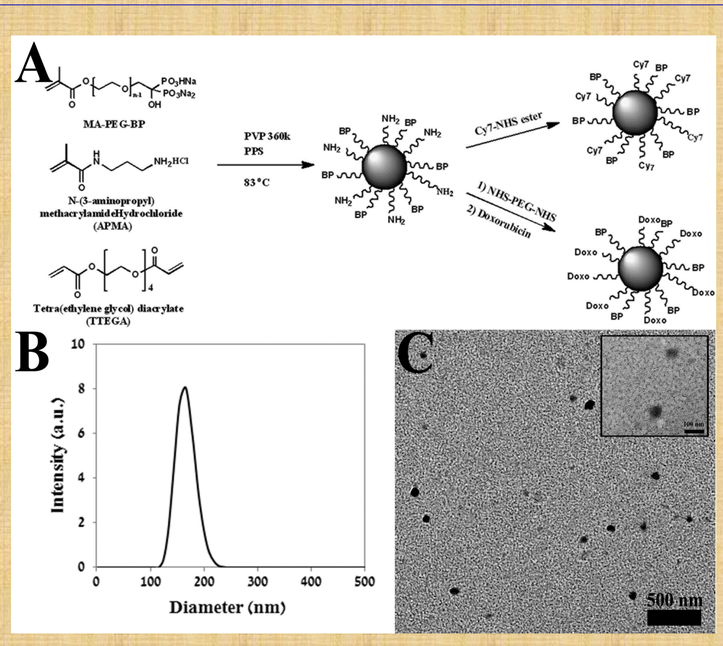
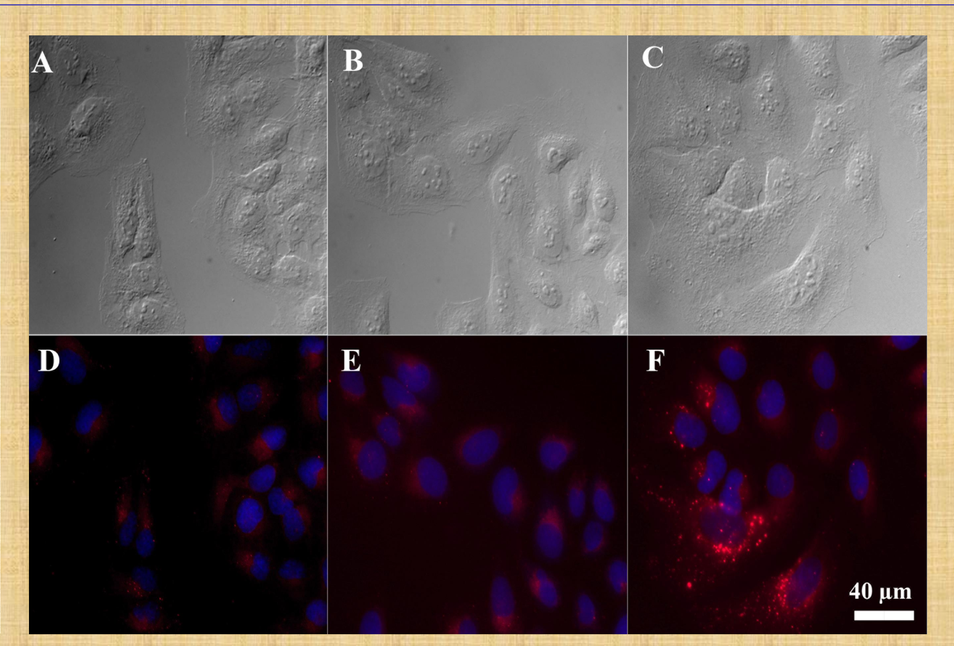
Poly (ethylene glycol) bisphosphonate nanoparticles for diagnosis and therapy of primary and metastatic bone cancer

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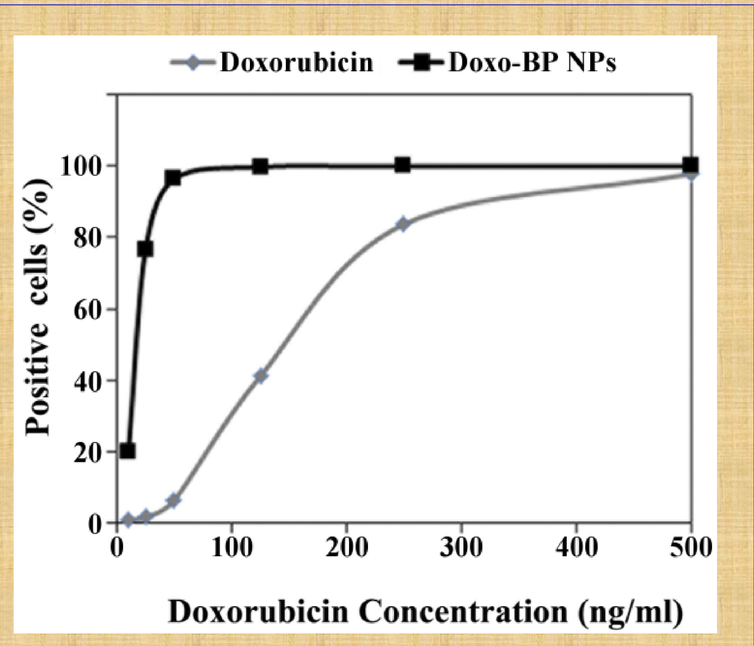
Abstract:
Most primary and metastatic bone tumors demonstrate increased osteoclast activity and bone resorption. Current treatment is based on a combination of surgery, radiotherapy and chemotherapy. Severe side effects are associated with chemotherapy due to use of high dosage and nonspecific uptake. Bisphosphonates have a strong affinity to Ca^{2+} ions and are widely used in the treatment of bone disorders. The purpose of this study is to evaluate the efficacy of our biodegradable bisphosphonate nanoparticle (NPs) for diagnosis and therapy of osteosarcoma (OS).



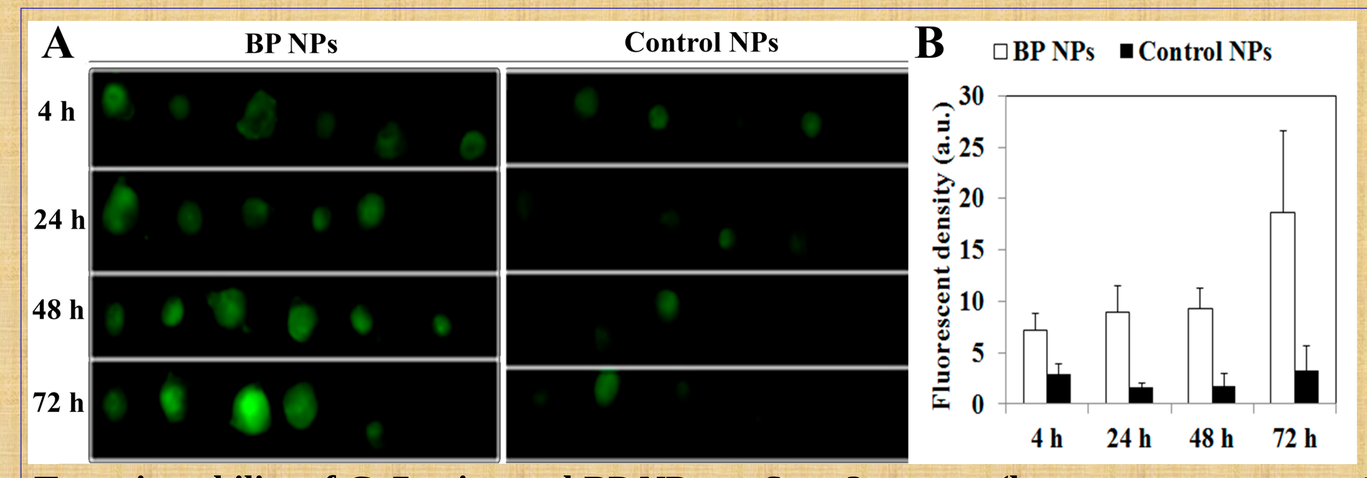
Synthesis scheme of BP NPs and conjugation of either Cy 7 or doxorubicin (A), size histogram (B) and TEM image (C) of BP NPs.



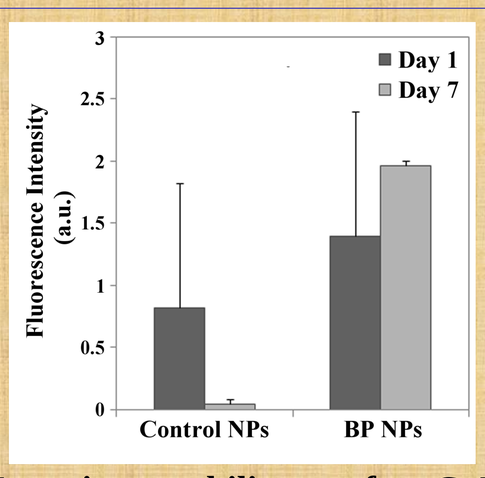
Uptake of Cy3-conjugated BP NPs in U-2OS cells (Microscope). U-2OS cells were incubated for 1 (A, D), 4 (B, E) and 24 (C, F) h with Cy3 conjugated BP NPs (0.16 mg/ml) Images were taken in bright field (A-C) and merged fluorescent images (D-F) red represents Cy3-conjugated BP NPs and nucleus is seen in blue (Hoecsht).



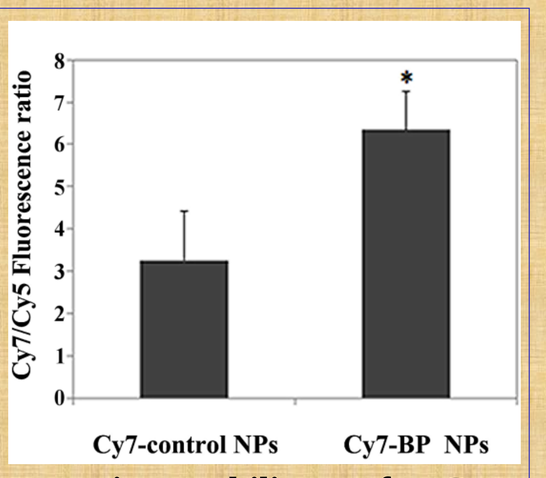
Uptake of doxo-conjugated BP NPs in Saos-2 cells (FACS). Graph comparing positive cell uptake of free and conjugated doxorubicin as a function of drug concentration.



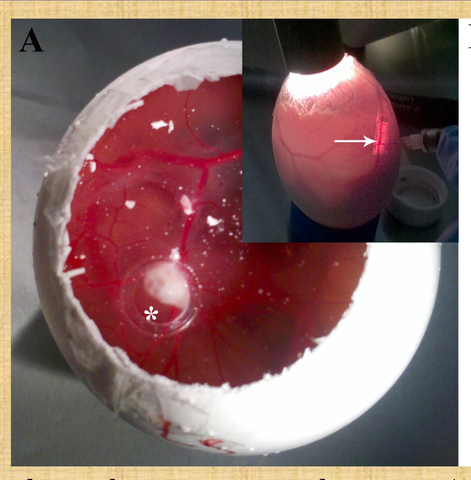
Targeting ability of Cy7-cojugated BP NPs on Saos-2 tumors (human osteosarcoma cells) in chicken embryo model. A photomicrograph (A) and histogram (B) showing the kinetics of the tumor marking by the NIR fluorescent BP and the control NPs in a chicken embryo model. On E8 of incubation, a human osteosarcoma Saos-2 cells were implanted in a plastic ring placed on the CAM. On E13, a total of 100 µL of 0.1 mg/mL of the Cy7-conjugated BP NPs or the Cy7-conjugated control NPs dispersed in PBS were IV injected into a large CAM blood vessel. The chicken embryos were then returned to incubation for different time periods (4, 24 48 and 72 h).

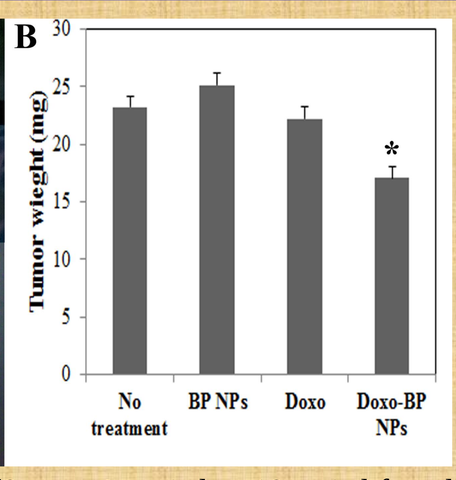


Targeting ability of Cy7-cojugated BP NPs on s.c. Saos-2 xenograft model. In order to evaluate the ability of the Cy7-conjugated BP NPs to target a bone tumor, Saos-2 cells were injected subcutaneously into nude mice to induce an osteosarcoma xenograft. Histogram of the difference in fluorescence between day 1 and day 7 of each NP.

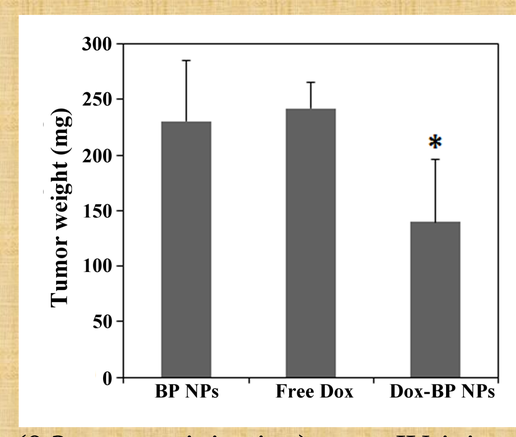


Targeting ability of Cy7-cojugated BP NPs in breast cancer metastasis model. Balb/c mice were injected intra-tibia mCherry-labeled 4T1 cells. One week post injection the mice were treated with 0.1 mg/ml of either Cy7-conjugated BP nanoparticles or Cy7-conjugated control nanoparticles.





Therapy of Saos-2 tumors in chicken embryo model. Topical view of the tumor and IV administration of NPs (A) and the therapeutic activity of the doxorubicin-conjugated BP NPs in a CAM model (B). On E8 of incubation, a Saos-2 cells were implanted in a plastic ring placed on the CAM (*). On E13, a total of 100 µL PBS containing 0.1 mg/mL of BP NPs or 0.1 mg/ml doxorubicin-conjugated BP NPs (0.5 µg Doxo/ml or 10 µg/ml free doxorubicin were IV injected (as pointed out by the arrow). (B) After 96 h the doxorubicin conjugated BP NPs have prevented the tumor growth significantly ($p < 0.05$), contrarily to the BP NPs and free doxorubicin which did not show any therapeutic effect.(Error bars represent standard error).



Therapy of Saos-2 subcutaneous xenograft tumors in nude mouse model. Doxorubicin-conjugated BP NPs (1 µg doxorubicin per injection), free doxorubicin (1 µg per injection) and non-conjugated BP NPs (0.2 mg per injection) were IV injected. *t-test: $p < 0.05$ and error bars represent standard deviation

Conclusion:

In this research we have shown the potential use of doxorubicin-conjugated BP NPs for the targeting and treatment of primary and metastatic bone tumors. The targeted delivery of doxorubicin to the tumor significantly increased the efficacy of the anti-cancer drug, thus enabling the effective use of a lower concentration of doxorubicin.