Electronic Supplementary Material (ESI) for Dalton Transactions

Supporting Information

Mangiferin Functionalized Radioactive Gold Nanoparticles (MGF-^{198}AuNPs) in Prostate Tumor Therapy: Green Nanotechnology of Production, *In Vivo* Tumor Retention and Evaluation of Therapeutic Efficacy

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Therapeutic effect of MGF-^{198}AuNPs and their effect on animal health

The results from antitumor studies demonstrate that the radioactive MGF-^{198}AuNPs have significant therapeutic effects as reflected in their ability to control and reduce the tumor volume in comparison to control groups during three weeks of treatment. Animals were monitored for their body weight to measure the toxic effect of MGF-^{198}AuNPs. As shown in figure SI 1A, bodyweight change curves indicate that MGF-^{198}AuNPs did not show any toxic effect on animal body weight. The overall body weight of treated groups of animals remained comparable with those of healthy normal control group. However, untreated control set of prostate tumor bearing animals showed significant reduction in the body weight by the end of the experiment (Figure SI 1A). These results indicate that MGF-^{198}AuNPs did not show any adverse toxic effects on animal health. It should be noted that, in the untreated control group of animals, due to the severe body weight loss and excess tumor growth, few animals were sacrificed. Therefore, 33.33% saline treated tumor bearing mice were left at the end of the last day of *in vivo* investigations (Figure SI 1B). However, in the MGF-^{198}AuNPs treated group, 50% animals survived till 24 days (Figure SI 1B). The results together corroborate that MGF-^{198}AuNPs inhibit the growth of tumor without any adverse effects.

Evaluation of radiotoxicity of MGF-^{198}AuNPs in blood samples of treated and untreated mice

In order to evaluate potential radiotoxicity effects post treatment with MGF-^{198}AuNPs, we have analyzed blood parameters of treated animals and compared with the control group which were not treated and not inoculated with tumor cells, and was assigned as the 4th group with baseline levels of SCID mice that had not been experimentally manipulated and served as control for this analysis. Comparisons included mean counts for white cells, red cells, lymphocytes, and platelets counts (Figure SI 2). The analysis showed that the mean white blood cell (WBC) count for MGF-^{198}AuNPs-treated groups was $2.96\pm0.88\times10^3$ WBC/µL, and for saline treated group was $3.56\pm2.04\times10^3$ WBC/µL. These data have revealed that (WBC) count in MGF-^{198}AuNPs-treated groups was slightly lower than (WBC) count in the normal control group.

Red blood cells count (RBCs) in MGF-^{198}AuNPs-treated group and control saline treated group was $11.97\times10^6\pm0.7$ /µL and $11.73\times10^6\pm1.6$ /µL respectively. The results indicated that the mean RBC count in treated group was slightly higher than RBC count in the normal control group ($10.81\times10^6\pm0.31$/µL). The lymphocytes count was found to be $0.57\times10^3\pm0.22$/µL in MGF-^{198}AuNPs-treated group and $0.52\times10^3\pm0.27$/µL in saline treated group. The lymphocytes count was found similar in both groups and in the normal control group ($0.66\times10^3\pm0.25$). Furthermore, the platelet counts for MGF-^{198}AuNPs treated groups was $1098\pm327\times10^3$/µL, and for the saline treated group was $981\pm260\times10^3$/µL. Platelets count for normal control group was 741± 293 10_3/µL. It can be observed that the platelets count for both treatment groups was higher than for the

normal control group. Overall, detailed analysis on blood parameters as outlined above have demonstrated MGF-^{198}AuNPs treatment did not cause any adverse radiotoxicity throughout the course of treatment regimen.

Control experiment for therapeutic study

The effect of radioactive MGF-^{198}AuNPs showed compelling evidence on the reduction of human prostate tumor in SCID mice. The biodistribution and tumor retention time point studies indicate the stability and tumor specificity of MGF-^{198}AuNPs. However, it was important to compare the antitumor efficacy with MGF alone to prove the effective and selective antitumor efficacy of MGF-^{198}AuNPs. Therefore, separate experiment was performed to evaluate the effect of MGF on human prostate tumor bearing SCID mice. PC-3 cells were inoculated into SCID mice and were allowed to grow prostate tumor for 3-4 weeks. Animals were monitored under experimental conditions for their body weight and tumor volume. After three weeks, animals were randomized based on their average tumor volume and body weight; and divided into two groups: group 1 saline treated and group 2 MGF treated. Mice were given single dose: group1 (saline 30µL) and group 2 (MGF 5.8 µg/30µL) intratumorally and were kept for another 3-4 weeks to monitor their tumor volume and body weight. The MGF dose was calucated and slelected based on the amount of MGF present in the MGF-^{198}AuNPs. Mice were sacrificed at the end of the study. The results presented in the figure SI 3 suggest that the tumor volume in the MGF treated group was smaller (0.12 cm^3) than the untreated control group (0.15 cm^3) on day 10. However, the tumor volumes appear to increase in the MGF treated (0.4 cm^3 on day 24^{th}) and untreated control groups (0.44 cm^3 on day 17^{th}). The results suggest that MGF alone does improve the life span of the animal but it does not inhibit the tumor growth. Therefore, the results from these control experiments suggest that MGF functionalized radioactive gold nanoparticles have definitive and selective antitumor efficacy in human prostate tumor induced SCID mice. These studies corroborate the importance of selectivity of MGF-^{198}AuNPs towards Lam-67R receptors which are overexpressed in prostate tumor cells and the overall therapeutic efficacy of this new radioactive mangiferin functionalized gold nanoparticulate.

Conjugation efficiency- Determination of number of MGF per nanoparticle: We have followed procedures reported in the literature for the calculation of conjugation efficacy of Mangiferin around gold nanoparticles. The concentration of MGF in MGF-AuNPs, as calculated by UV-visible spectrophotometry, was found to be 173 µg/mL. The number of MGF moles per ml is 2.463×10^{17}. Further, the number of MGF molecules per NPs was calculated using the[1, 2]-

MGF per nanoparticles= Number of MGF per ml/ number of NPs per ml

$= 2.463 \times 10^{17} \times 7.27368 \times 10^{11} = 338617.7463$

Therefore, using the above calculation, we have confirmed that the average number of Mangiferin molecules per nanoparticle to be 338618.

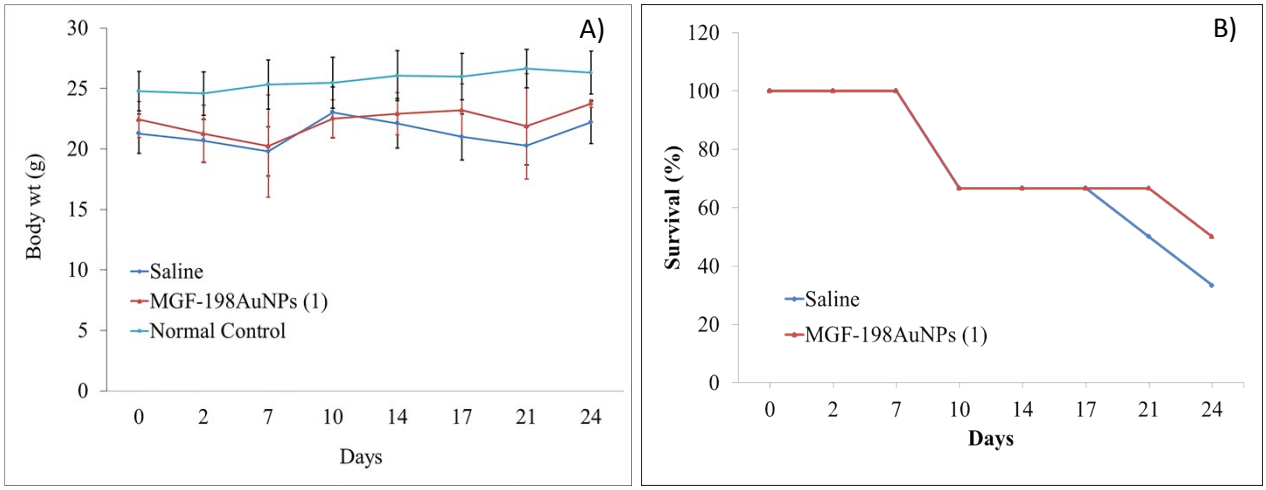

Figure SI 1. (A) Effect of MGF-^{198}AuNPs on body weight of SCID mice after a single dose intra tumoral administration in human prostate cancer bearing SCID mice (mean±SD). (B) Percent survival of mice after treatment with MGF-^{198}AuNPs and comparison with untreated control mice; N=5±SD.

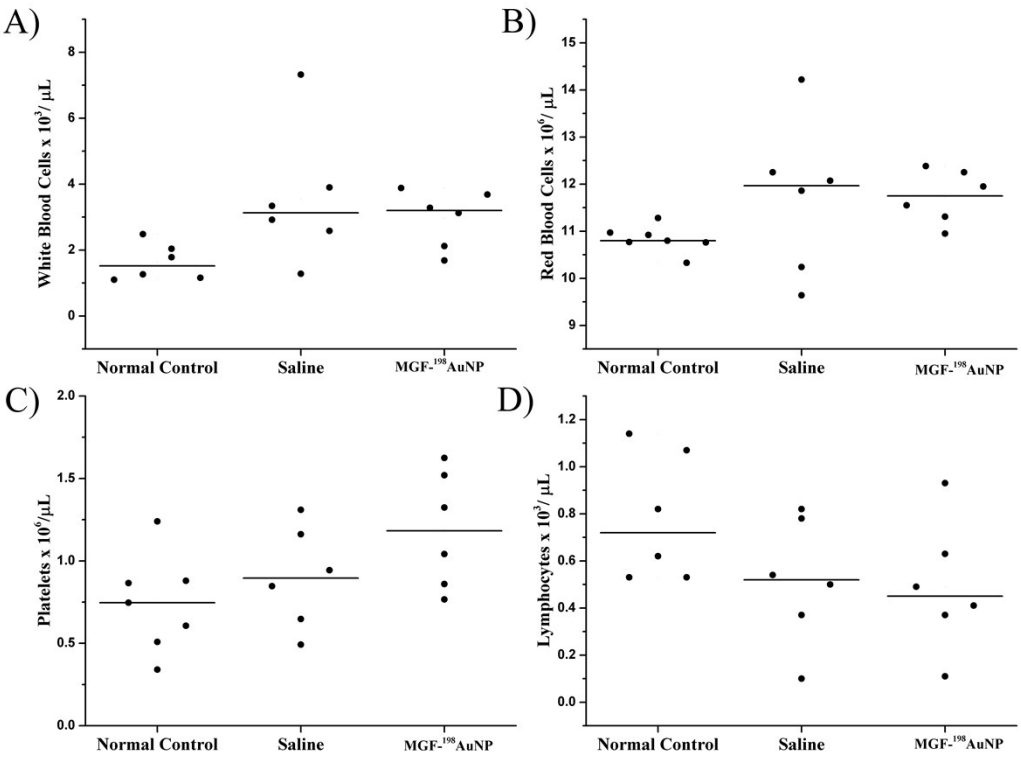

Figure SI 2. Comparison of blood parameters including (A) white blood cells, (B) red blood cells, (C) platelets, and (D) lymphocytes counts between the treatment and control groups with baseline levels obtained from a group of SCID mice that received no manipulations (normal control); N=5.

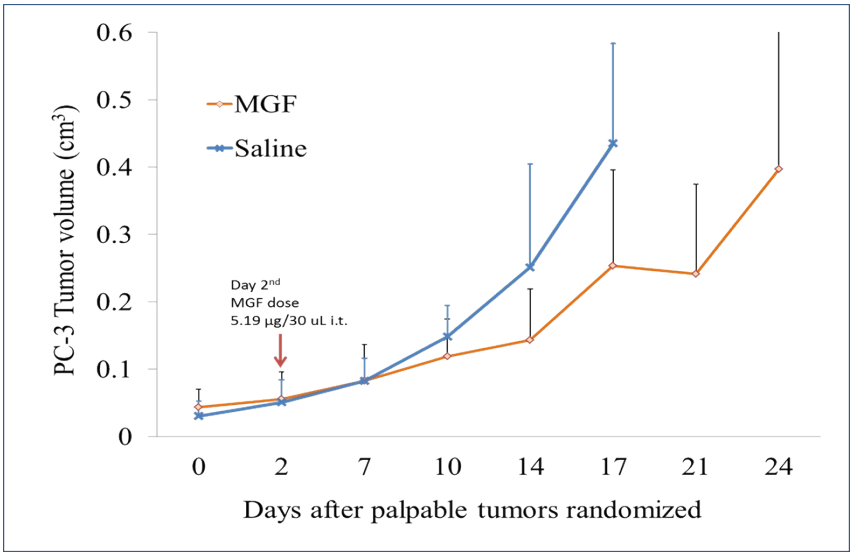

Figure SI 3. Therapeutic efficacy studies of free MGF after a single dose intra tumoral administration in human prostate cancer bearing SCID mice (N=5±SD). The therapeutic effect was maintained over a three weeks period.

References:

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2. X. O. Liu, M. Atwater, J. H. Wang and Q. Huo, **Extinction coefficient of gold nanoparticles with different sizes and different capping ligands**. *Colloid Surface B*. 2007;58:3-7