

November 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format.

Title: The Preparation of MR Specific USPIO Probes Using One-Pot Method For in vitro Detection of Hepatocellular Carcinomas

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

REVIEWER EVALUATION (Reviewer No. 2348457)

1. Introduction: Not all HCCs express AFP and GPC3, the authors should provide some background knowledge about the molecular markers of HCC.
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The background knowledge about the molecular markers of HCC has been introduced.
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2. Introduction: Generally, SPIO nano particles are used for targeting probes, not USPIO, please clarify.

The superparamagnetic iron oxide particles have been classified into three categories: the standard superparamagnetic iron oxide particle (SPIO, 50-150 nm), the ultrasuperparamagnetic iron oxide particle (USPIO, 20-40 nm), and monocrySTALLine iron oxide nanoparticles (MION, 10 nm). The USPIO and the MION have been used commonly because the particles with a hydrodynamic diameter larger than 50nm were removed from the bloodstream rapidly when they were injected into the body, and usually accumulate in the liver and spleen.
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3. Introduction: There were many studies using other ligands to synthesis HCC nano probes, please add the related references.

The related references were added.

4. M&M, please move the Statistical Analysis section to the last paragraph.

Ok, thank you.

5. M&M, in vitro studies, please add another cell line without expression of AFP and GPC3 as the control.

This article was focused on the preparation of the probes. In order to confirm its effectiveness we did the experiment in vitro. The detailed information about using another cell line without expression of AFP and GPC3 as the control was introduced in reference 11(Li YW, Chen ZG, Li F, Wang JC, and Zhang ZM. Preparation and in vitro studies of MRI-specific superparamagnetic iron oxide antiGPC3 probe for hepatocellular carcinoma. *International Journal of Nanomedicine* 2012; 7: 4593-4611 [PMID: 22956868 doi: 10.2147/IJN.S32196]) .

6. Results, Measurement of antiAFP-USPIO, antiGPC3-USPIO and USPIO relaxivity, the authors did not provide a fitting curve of T1 and T2 relaxivity, please convert to a diagram to demonstrate relativity curves.

Figures 4,5 are the curve of T1 and T2 relaxivity which are the data of table 1 and table 2.

7. Results, the authors claimed that incorporation of the probes into intracellular organelles follows the endocytosis pathway. But no direct histologic micrographs were provided, such as endosome staining, etc.

The incorporation of the probes into intracellular organelles has two routes generally, one is the specific endocytosis pathway which ligand binding plays a key role and one the nonspecific endocytosis which differ depending on the type of USPIO nanoparticles or cells used. In this experiment we can see the probes binding the markers on the cell membrane and the probes entering the organelles through TEM.

8. Figures 4,5, please covert these figures to relaxvity curves.

9. The tittle, The Preparation of MR Specific USPIO Probes Using One-Pot Method For Early Detection of Hepatocellular Carcinomas, should be changed to The Preparation of MR Specific USPIO Probes Using One-Pot Method For in vitro Detection of Hepatocellular Carcinomas since this study is an in vitro experiment, no animal models were tested.

Thank you for your suggestion.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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