Dear Editor. Thank you for all the comments. We have changed the manuscript according to all the comments, below are answers for the questions in italic.

Answers are in italic.

1. Protocol language: change to the imperative tense.

*Protocol language has been changed to the imperative tense.*

1. What are the concentrator parameters/settings? Mention centrifugation speed (in g), filter size etc.

*The concentration is not done by centrifugation. A more accurate description of the process has been added.*

1. How much of each is added?

*The exacts amount have been added, although the final concentrations needed were mentioned in 1.5.*

1. Is this under a fume hood? Please add a note of caution from chloroform usage.

*Caution notes for chloroform and other organic solvents usage have been added were needed.*

1. Unclear how the cleaning is performed. Mention care to be taken. Is this done under a hood?

*A more detailed description of the cleaning procedure has been added.*

1. Please add citations to the previous studies. Incubate under same conditions as in 3.1?

*The citation has been added. It is a not published yet manuscript. And same conditions has been specified.*

1. What is the temperature for storage? For how long can they be stored?

*The information has been added.*

1. Protocol highlight:

*Our protocol is no longer than 3 pages, therefore no text has been highlighted, everything should be included.*

1. Discussion:

*The different parts have been clarified, and some information has been added.*

* 1. *Modifications and troubleshooting: adding lipid coat.*
  2. *Limitations of the technique:*
  3. *Significance with respect to existing methods*
  4. *Future applications: approach to encapsulate cancer drugs*
  5. *Critical steps within the protocol: organic phase injection, time of preparation of nanoparticles and time of seeding cells. All the steps are critical as they either affect the size of the nanoparticles or final concentration of the active ingredient or coating lipids. Considering ‘concentration’ as important parameter, step 1.1, 1.3, 1.5, 1.13 and 1.14 are critical. Considering ‘nanoparticle size,’ step 1.6, 1.9 and 1.10 are critical steps.*

1. Commercial language

*It has been deleted. Abbreviations have been defined, all figures are original.*

Answers to all peer review comments follow:

1. It is not clear whether this protocol is extended from a previous publication validating the efficacy of falcarindiol-coated nanoparticles or this manuscript is their first report describing this particular nanoparticle.

*Yes, there are two publications that involve the nanoparticle fabrication method mentioned in the current paper. (References 5 and 6).*

1. Critical steps or potential technical issues of the described technique have not been addressed through the protocol section.

*Organic phase injection, time of preparation of nanoparticles and time of seeding cells. A part from these, there are no other critical steps. All the steps are critical as they either affect the size of the nanoparticles or final concentration of the active ingredient or coating lipids. Considering ‘concentration’ as important parameter, step 1.1, 1.3, 1.5, 1.13 and 1.14 are critical. Considering ‘nanoparticle size,’ step 1.6, 1.9 and 1.10 are critical steps.*

1. Additional intro or discussion should be included to explain why human stem cells (MSC) instead of cancer cell is chosen for in vitro study. The author has mentioned that falcarindiol is an anticancer drug, and lipid coated nanoparticles can target cancer cells specifically due to the need of cancer cells for cholesterol. Therefore, it is not quite logical to use human stem cells as in vitro system to test how falcarindiol-coated nanoparticle can be taken by cells.

*They were chosen because they are large and suitable for microscopy.*

1. Falcarindiol is not the common anticancer drug, why would you choose it?

*It is the drug that the group was working with, it is used as an example anticancer drug to fabricate the nanoparticles.*

1. Why used hMSC to verify the delivery of anticancer drugs?

*They were chosen because they are large and suitable for microscopy.*

1. Please explain the reason that 833μL/s was selected as the injection flow rate.

*Optimization of the protocol in Prasad Walke’s PhD thesis.* *Its the highest speed that can be achieved with eVol XR® system. We tested the effect of all the speeds that can be achieved using this system and the finest particles with narrow particle size distribution were obtained at the highest speed, which is 833 µL/s.*

1. According to the protocol of cell treatment, I suggest the time point of stability experiment in vitro should be extended to the 24h at least.

*This time point was also checked and no aggregation was observed. Data is shown in another manuscript under preparation.*

1. In Figure 3, Please pay attention to the difference between drawing DSPE-PEG and PEG.

*It has been changed.*

1. In Figure 4, I can't clearly distinguish between cells and nanoparticles. It is recommended that the nucleus be stained with DAPI, and the Dil should emit orange-red fluorescence after being stimulated.

*Figure 4 now has the nucleus DAPI stain and the DiI nanoparticles separate images and the overlay of the two.*