For research article

Response to Reviewer 1 Comments

Comments:

The research showed in the article is of great importance, and based on the use of a microfluidic device previously designed by the authors aiming to obtain ultrasmall nanoliposomes for a deeper penetration into the skin. The experiments that were carried out are appropriate to demonstrate that the ultrasmall liposomes harboring CoQ10, are able to reduce significantly the UVB light damage on the skin.

Q1. My major concern is related to the quality of images. Some of them were provided to the reviewers, but not all of them. On the other hand, the labels on the figures are not clear, and thus made the revision of the figures quite difficult. Nevertheless, the explanation of the figures were generally clear, I would appreciate if the images on the pdf version of the article, can be of higher resolution for the second round of the review process.

Response: We are very sorry for this inconvenience. We have inserted high-resolution images in the revised manuscript and will additionally include high resolution images.

Q2. A minor concern is related to the abbreviations throughout all article sections. There are several abbreviations that were not explained the first time they were mentioned in the article. For instance, there is an important abbreviation that is key to understand the research carried out in this work and was not explained: TFR. To know what TFR stands for, I had to go to a reference of a previous work of the same group ("Wang, H., Lan, Z., Tian, R., Liang, X., Jia, F., Ma, M., & Chen, H. (2024). Combined helical-blade-strengthened co-flow focusing 612 and high-throughput screening for the synthesis of highly homogeneous nanoliposomes. Nano Today, 56, 102301"). In this case, the authors should specify that TFR stands for: "total flow rate", explaining that

it is the sum of the internal and external phase flow rates.

Response: We are very sorry for this inconvenience. We have carefully revised the manuscript and have defined abbreviations as they appeared for the first time in the manuscript. TFR represents the sum of the external and internal phase flow rates, while FRR refers to the ratio of the external phase flow rate to the internal phase flow rate. The explanations of TFR and FRR are provided in lines 75-78 of the revised manuscript for your reference.

Q3. Another abbreviation that it is not explained is DiI, which it is not DiI with the lowercase "l" (it is an "i" in capital letter, and in several parts of the article appears as an "l"). Please, correct this, and then also suggest that the first time this fluorophore dye is mentioned by the authors, the authors explain what DiI abbreviation stands for as well as specify that it is a fluorophore.

Response: Thank you very much for pointing out this error in our manuscript. We apologize for the oversight. We have carefully reviewed the manuscript for similar spelling errors and have highlighted in the revised manuscript.

Q4. When it comes to Collagen-1, the authors used the abbreviation, Col 1. Please maintain the same abbreviation throughout the whole article (and not use "Col").

Response: We are very sorry for this inconvenience. We have addressed this issue in the revised draft. The abbreviation "Col-I" has been used to refer to collagen type I and is highlighted in lines 487-496 of the revised manuscript.

Q5. The technique by which the conventional liposomes are prepared, should be specified (is it by CM?)

Response: We sincerely thank the reviewer for their kind enquiry. The conventional liposomes were synthesized at a lower TFR using a CF device. We have added this information to subsection 2.4 of the revised manuscript, as described below:

To visualize transdermal infiltration, 1 mL of FITC-labelled NLP_{US} (synthesized at a higher TFR by HBSCF device) or FITC-labelled NLP_{NM} (synthesized at a lower TFR by CF device) were added to the supply pool and uniformly applied to the pig skin.

Q6. Is there a reason by which the NLP_{US} were labeled with DiI instead of FITC to perform the experiments on HSF? Please, explain also this, if there is a justification

by which the authors did much work by also preparing DiI labeled nanoliposomes for another experiment and did not use the liposomes labeled with FITC that were already prepared.

Response: Thank you very much for pointing out this confusing issue. In the Franz diffusion cell transdermal assay, FITC was used as a carrier marker to observe the penetration of NLP_{US} and NLP_{NM} into the skin. In contrast, in the HSF cell uptake assay, DiI was used as a drug molecule to observe drug uptake by the cells. These two experiments aim to investigate different phenomena, which is why two distinct fluorescent probes, FITC and DiI, were employed. To differentiate the materials, they were referred to as FITC-labelled NLP_{US} and DiI@NLP_{US} in the initial manuscript.