THE ROYAL SOCIETY
PUBLISHING

ROYAL SOCIETY
OPEN SCIENCE

Optimization and characterization of Poly(Lactic-co-glycolic acid) nanoparticles loaded with astaxanthin and evaluation of anti-photodamage effect *in vitro*

Fangbin Hu, Weikang Liu, Liuliu Yan, Fanhui Kong and Kun Wei

Article citation details
R. Soc. open sci. **6**: 191184.
http://dx.doi.org/10.1098/rsos.191184

Review timeline
Original submission: 12 July 2019
Revised submission: 18 September 2019
Final acceptance: 25 September 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSOS-191184.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
I have no corrections to propose, except for a minor concern. I think that the authors have to briefly discuss in the conclusion the clinical potential of their results. This is a recent review on the role of astaxanthin in skin physiology and its clincal implications in dermatology. I think it will be useful to your topic and reference list (Nutrients. 2018 Apr 22;10(4).)

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
No

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
In this work, the authors used Box-Behnken experimental design and established appropriate condition for astaxanthin-loaded PLGA nanoparticle preparation. The physicochemical properties and cell uptake studies clearly showed that the nanoparticle possess appropriate properties for a drug carrier system. UVB induced photodamage model was successfully established in Hacat cells and cell viability assay was performed to study the antioxadative properties of the nanoparticle. The astaxanthin encapsulated PLGA nanoparticle secure the photodamage caused by UVB in Hacat cells by decreasing the ROS level. Nanoparticle treatment recovered the Hacat cells from the UVB damaged mitochondrial potential in dose dependent manner. Altogether the study indicated that astaxanthin-loaded PLGA nanoparticle treatment can recover Hacat cells from UVB damage and has potential to use in cosmetic. The novelty of the work is good enough for this Journal , but I still have some comments for improvement.
1) This work has rich content and describes the application and prospect of PLGA-loaded astaxanthin nanoparticles in Hacat UVB damage model. I suggest add some more latest literatures.
2) The experiment is well performed and clearly explained, but I recommend in English language correction.
3) The conclusion part is not appropriately written and need to be improved in detail.

Decision letter (RSOS-191184.R0)

08-Sep-2019

Dear Miss Hu

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-191184 entitled "Optimization and Characterization of PLGA nanoparticles loaded with Astaxanthin and evaluation of anti-photodamage effect in vitro" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link:
http://datadryad.org/submit?journalID=RSOS&manu=RSOS-191184

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors' contributions
All submissions, other than those with a single author, must include an Authors' Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:

AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Please ensure you have prepared your revision in accordance with the guidance at https://royalsociety.org/journals/authors/author-guidelines/ -- please note that we cannot publish your manuscript without the end statements. We have included a screenshot example of the end statements for reference. If you feel that a given heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 17-Sep-2019. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees. We strongly recommend uploading two versions of your revised manuscript:

1) Identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.

When uploading your revised files please make sure that you have:

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document";
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format);
3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account;
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi

within your manuscript. Make sure it is clear in your data accessibility statement how the data can be accessed;
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://rs.figshare.com/). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Please note that Royal Society Open Science charge article processing charges for all new submissions that are accepted for publication. Charges will also apply to papers transferred to Royal Society Open Science from other Royal Society Publishing journals, as well as papers submitted as part of our collaboration with the Royal Society of Chemistry (http://rsos.royalsocietypublishing.org/chemistry).

If your manuscript is newly submitted and subsequently accepted for publication, you will be asked to pay the article processing charge, unless you request a waiver and this is approved by Royal Society Publishing. You can find out more about the charges at http://rsos.royalsocietypublishing.org/page/charges. Should you have any queries, please contact openscience@royalsociety.org.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Andrew Dunn
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Shaked Ashkenazi (Associate Editor)
openscience@royalsociety.org

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
I have no corrections to propose, except for a minor concern. I think that the authors have to briefly discuss in the conclusion the clinical potential of their results. This is a recent review on the role of astaxanthin in skin physiology and its clincal implications in dermatology. I think it will be useful to your topic and reference list (Nutrients. 2018 Apr 22;10(4).)

Reviewer: 2

Comments to the Author(s)
In this work, the authors used Box-Behnken experimental design and established appropriate condition for astaxanthin-loaded PLGA nanoparticle preparation. The physicochemical properties and cell uptake studies clearly showed that the nanoparticle possess appropriate properties for a drug carrier system. UVB induced photodamage model was successfully established in Hacat cells and cell viability assay was performed to study the antioxadative properties of the nanoparticle. The astaxanthin encapsulated PLGA nanoparticle secure the photodamage caused by UVB in Hacat cells by decreasing the ROS level. Nanoparticle treatment recovered the Hacat cells from the UVB damaged mitochondrial potential in dose dependent manner. Altogether the study indicated that astaxanthin-loaded PLGA nanoparticle treatment can recover Hacat cells from UVB damage and has potential to use in cosmetic. The novelty of the work is good enough for this Journal , but I still have some comments for improvement.
1) This work has rich content and describes the application and prospect of PLGA-loaded astaxanthin nanoparticles in Hacat UVB damage model. I suggest add some more latest literatures.
2) The experiment is well performed and clearly explained, but I recommend in English language correction.
3) The conclusion part is not appropriately written and need to be improved in detail.

Author's Response to Decision Letter for (RSOS-191184.R0)

See Appendix A.

Decision letter (RSOS-191184.R1)

25-Sep-2019

Dear Dr Hu,

I am pleased to inform you that your manuscript entitled "Optimization and Characterization of PLGA nanoparticles loaded with Astaxanthin and evaluation of anti-photodamage effect in vitro" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org and openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (http://bit.ly/cpFAQ). Your article will be published straight into the next open issue and this

will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,

Lianne Parkhouse
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Shaked Ashkenazi (Associate Editor) and the Subject Editor
openscience@royalsociety.org

Appendix A

Response to Reviewer

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Optimization and Characterization of PLGA nanoparticles loaded with Astaxanthin and evaluation of anti-photodamage effect in vitro". Those comments are all valuable and very helpful for revising and improving our paper. And according to the comments, we have revised the manuscript as follows.

Reviewer comments to Author:
Reviewer: 1
Reviewer comment 1: I think that the authors have to briefly discuss in the conclusion the clinical potential of their results.

Author reply: As your great suggestion, we have discussed the clinical potential of AST-PLGA NP in Conclusion—"Therefore, these results may provide a new means to solve the problem such as high hydrophobicity and poor chemical stability of astaxanthin and demonstrate the therapeutic application of PLGA-encapsulated astaxanthin nanoparticles in skin diseases. All together, the AST-PLGA NP possessed the potential application in the field of cosmetics and might be considered as a potential therapeutic component for skin disease."

Reviewer comment 2: This is a recent review on the role of astaxanthin in skin physiology and its clincal implications in dermatology. I think it will be useful to your topic and reference list (Nutrients. 2018 Apr 22;10(4).)

Author reply: Thank you for your great suggestion. We have already added it in Introduction—"The anti-wrinkle and anti-oxidation effects of astaxanthin reflect its various health benefits and important nutritional health applications in dermatology[24]."

Reviewer:2
Reviewer comment 1: This work has rich content and describes the application and prospect of PLGA-loaded astaxanthin nanoparticles in Hacat UVB damage model. I suggest adding some more latest literatures.
Author reply: Thank you for your suggestion and comments. we have added more references in Introduction—"Skin, the largest organ in the human body, plays a major role as the protective barrier against harmful external agents such as ultraviolet (UV) radiation, dehydration, temperature changes, and pathogens[21]. Excessive exposure to UV radiation remains a major risk factor for melanoma and non-melanoma skin cancers, especially exposure to UVB radiation can generate excessive reactive oxygen species in cells, that can induce many deleterious effects, including DNA damage,

oxidative stress, photoaging, inflammation, and carcinogenesis[22, 23]. The anti-wrinkle and anti-oxidation effects of astaxanthin reflect its various health benefits and important nutritional health applications in dermatology[24]. Naoki et al. evaluated the effects of astaxanthin on UV-induced skin degradation in 23 healthy Japanese participants and demonstrated the protective and safe nature of astaxanthin[25]. Moreover, Hung et al. found that barrier defects caused by ultraviolet radiation may increase the skin penetration of polymer nanoparticles[26]."

Reviewer comment 2: The experiment is well performed and clearly explained, but I recommend in English language correction.
Author reply: We are sorry for several typos and grammatical errors. We have carefully corrected all typos and grammatical errors throughout the manuscript.

Reviewer comment 3: The conclusion part is not appropriately written and need to be improved in detail.
Author reply: Thank you for your kind comment. We have revised the conclusions in detail —"In this research, we had successfully developed the PLGA nano drug delivery system loaded with astaxanthin using the emulsion solvent evaporation technique. The four-factor and three-level Box-Behnken design optimized the parameters and obtained the optimal process conditions. With this, we synthesized the AST-PLGA NP with the concentration of PLGA 10 mg/mL, the concentration of astaxanthin 0.81 mg/mL, water volume 3 mL, and sonication time 0.5 min. This synthesized method optimized the AST-PLGA NP with an encapsulation efficiency of 96.42 ± 0.73%; drug loading capacity of 7.19 ± 0.12% and size of 154.4 ± 0.35 nm, indicating the good drug delivery capacity of AST-PLGA NP. In addition, the results of SEM and TEM showed that the NPs were discrete and spherical in shape, and displayed a good size distribution. At the same time, FT-IR studies confirmed that astaxanthin had been successfully loaded into PLGA nanoparticles. XRD and DSC studies demonstrated that astaxanthin existed in the form of dispersed amorphous or disordered crystals in the molecular state, while forms a solid solution state in the polymer matrix. PLGA nanoparticles were biocompatible and could be taken up by HaCaT cells in a time dependent manner. In particular, AST-PLGA NP showed better antioxidant activity compared to pure astaxanthin in the UVB radiation photodamage model of Hacat cell. Furthermore, AST-PLGA NP resists the photodamage in HaCaT cells by reducing ROS levels and restoring mitochondrial membrane potential. Therefore, these results may provide a new means to solve the problem such as high hydrophobicity and poor chemical stability of astaxanthin and demonstrate the therapeutic application of PLGA-encapsulated astaxanthin nanoparticles in skin diseases. All together, the AST-PLGA NP possessed the potential application in the field of cosmetics and might be considered as a potential therapeutic component for skin disease."

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of

the paper.And here we did not list the changes but marked in yellow in"Main Document-tracked changes". We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Best wishes!
Yours sincerely,
Hu Fangbin