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OPEN PEER REVIEW

A Novel Composite Nano-scaffold with Potential Usage as Skin Dermo-epidermal Grafts for Chronic Wound Treatment

Sohrab Moradi, Mohammad Ali Nilforoushzadeh, Jamil Zargan, Shahram Nazarian, Peiman Brouki Milan

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1 Original submission

4 comments · 25 Dec 2022 – 29 Dec 2022

Mazaher Gholipourmalekabadi Reviewer

MINOR REVISION 25 Dec 2022

Please see my comments below:
1. Please specify which statistical test was used for cell viability analysis.
2. Please explain the morphological (size, shape, etc.) and chemical properties of zinc oxide (ZnO) nanoparticles.
3. You can consider using Figure 6 as a graphical abstract or to show an overview of your work.
4. Please write the complete name of the bacteria when you mention them for the first time.
5. Is there any clinical example of skin graft synthesis using nanotechnology in the world?

Referee 1 Reviewer

MAJOR REVISION 26 Dec 2022

This research examines whether or not nano-scaffolds containing bioactive substances like ZnO nanoparticles may serve as a suitable replacement for the extracellular matrix and significantly hasten the healing of chronic wounds by lowering the risk of infection. According to the authors, silk fibroin nanofibers generated as an epidermal graft and a 3DPH nano-scaffold loaded with ZnO nanoparticles as a dermal graft are appropriate for dermo-epidermal grafts and skin regeneration.
1. The authors explain in the introduction: "Typical treatment for chronic wounds includes the application of autografts, allografts, xenografts, and topical delivery of anti-inflammatory, antioxidant, and antibacterial agents. In spite of this, the mentioned therapies are not sufficient for wounds that are extensive or deep." Please offer some references and a more thorough explanation.
2. The introduction is quite brief and requires further justification. What research gap in the area does this study intend to fill? This requires more reasoning.
3. In the discussion, the authors cite several studies with comparable findings that support the findings of the present study. Nevertheless, the issue is, what does this research contribute to the literature that comparable investigations before it did not find? What makes this study distinctive in contrast to previous studies? These questions should be handled in depth, and I would advise the authors to add a new paragraph at the end of the discussion to ponder on these concerns.
4. What are the study's strengths and limitations?
5. I recommend that the authors include a final part of the manuscript titled "Recommendations for future studies," where they may share their thoughts and give guidance for other researchers in the field.
6. Please consider the following articles, discuss them, compare their results, strengths, and limitations with those of your study, and include them in the discussion section to enhance the quality of the manuscript, if applicable:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7784275/
https://www.mdpi.com/2073-4409/9/7/1622
https://www.mdpi.com/2073-4409/11/15/2439
https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-020-00755-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8875365/

Associate Editor

MAJOR REVISION 29 Dec 2022

Dear EIC,
The authors presented "A Novel Composite Nano-scaffolds with Potential usage as Skin Dermo-Epidermal Grafts for Chronic Wound Treatment."
It is an interesting article but needs revision. It should be refined by the authors.

Editor-in-Chief

MAJOR REVISION 29 Dec 2022

Dear Author,
Thank you for submitting the manuscript. Please revise it accurately.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Author support center

We are highly grateful for the opportunity to review our manuscript with the submission ID of 133976 and for the valuable comments received from the first reviewer. We revised the manuscript according to the comments. Our responses are described in this letter and highlighted in yellow in the revised manuscript.

Reviewer 1:
Please see my comments below:

Response: Thank you very much for your attention.

1. Please mention that which statistical test was used for cell viability analysis?

Response: In the present study, data were expressed as a mean ± standard deviation. Statistical analysis was performed using the one-way analysis of variance (SPSS program, version 22). A p-value < 0.05 was considered as statistically significant.
We added these sentences to the revised manuscript, highlighted in yellow (Materials and methods, Statistical analysis).

2. Please explain about the morphological (size, shape, etc.) and chemical properties of zinc oxide (ZnO) nanoparticles.

Response: We used irregularly oval and quasi-spherical shaped nanoparticles with an average diameter of 35±10 nm. In addition, the crystalline structure of ZnO-NPs had been hexagonal and was high purity without any other phase impurities. The zeta potential of ZnO-NPs in DDW was equal to -16.8 mV, implying a stable colloidal solution.
We added these sentences to the revised manuscript, highlighted in yellow (Materials and methods, ZnO nanoparticles).

3. You can consider Figure 6 as graphical abstract or showing the overview of your work.

Response: Thank you very much for your good recommendation.

We added this Figure to the revised manuscript in “objectives” section, highlighted in yellow

4. Please write the complete name of the bacteria when you mention them for the first time.

Response: We used *Staphylococcus aureus* (*S. aureus*. PTCC No:1826) as a standard gram-positive bacterium and *Escherichia coli* (*E. coli*. PTCC No:1222) as a standard gram-negative bacterium.

We edited and added these sentences to the revised manuscript, highlighted in yellow (Abstract and Biological activity assessment of nano-scaffolds).

5. Is there any clinical example of skin grafts synthesis using nanotechnology in the world?

Response: There are a number of research studies that have explored the use of nanotechnology for synthesizing skin grafts. However, it is important to note that these studies are typically conducted in a laboratory setting and have not yet been widely adopted in clinical practice. One study published in 2011 described the use of a nanofiber scaffold made from polyethylene glycol (PEG) and collagen for synthesizing skin grafts in a clinical setting. The study found that the use of the nanofiber scaffold resulted in faster wound healing and improved skin regeneration compared to traditional skin grafts. Another study described the use of a nanostructured hydrogel made from PEG and hyaluronic acid (HA) for synthesizing skin grafts in a clinical setting. The study found that using the nanostructured hydrogel resulted in improved skin regeneration and reduced scarring compared to traditional skin grafts.

We edited and added these sentences to the revised manuscript, highlighted in yellow (in the discussion and references)

Reviewer 2:

This research examines whether or not nano-scaffolds containing bioactive substances like ZnO nanoparticles may serve as a suitable replacement for extracellular matrix and significantly hasten the healing of chronic wounds by lowering the risk of infection. According to the authors, silk fibroin nanofibers generated as an epidermal graft and 3DPH nano-scaffold loaded with ZnO nanoparticles as a dermal graft are appropriate for dermo-epidermal grafts and skin regeneration.

Response: Thank you very much for your attention.

1. The authors explain in the introduction "Typical treatment for chronic wounds includes the application of autografts, allografts, xenografts, and topical delivery of anti-inflammatory, antioxidant, and antibacterial agents. In spite of this, the mentioned therapies are not sufficient for wounds that are extensive or deep." Please offer some references and a more thorough explanation.

Response: Chronic wounds are usually treated with autografts, allografts, xenografts, and topical antibacterial and anti-inflammatory agents. Nevertheless, the above therapies are not sufficient for extensive or deep wounds. Autografts are most advantageous because they do not exhibit immune responses, but they cannot cover the entire wounded site when it exceeds 60% of the patient's total body area. Additionally, the application of allogeneic skin grafts carries a high risk of rejection and treatment failure.

We edited and added these sentences to the revised manuscript, highlighted in yellow (in the introduction and references)

2. The introduction is quite brief and requires further justification. What research need in the area does this study intend to fill? This requires more reasoning.

Response: Modern treatments include skin tissue engineering, which produces bioengineered biomaterial-based artificial skin grafts aimed at overcoming the shortage of donor skin graft supplies and the rejection of skin allografts and xenografts. Moreover, Bioengineered artificial skin substitutes are becoming more

popular for treating chronic wounds, as well as topical delivery of mesenchymal stem cells to reduce inflammation and speed healing.

A scaffold is an essential component of skin tissue engineering and artificial skin grafts as it has a pivotal role in mechanical support, delivery of nutrients and oxygen, the release of growth factors and drugs. It can also modulate cell behaviors such as attachment, motility, proliferation, and differentiation. Therefore, scaffolds could mimic the biological, chemical, and mechanical properties of the extracellular matrix (ECM) of the target tissue. Several types of scaffolds can be utilized for Artificial skin grafts, including fibrous, porous, and hydrogel scaffolds.

We added these sentences to the revised manuscript, highlighted in yellow (in the introduction and references).

3. In the discussion, the authors cite several researches with comparable findings that support the findings of the present study. Nevertheless, the issue is, what does this research contribute to the literature that comparable investigations before it did not find? What makes this study distinctive in contrast to previous studies? These questions should be handled in depth, and I would advise the authors to add a new paragraph at the end of the discussion to ponder on these concerns.

Response: In this study, we fabricated of silk fibroin nanofiber by electrospinning (as an epidermal graft) and three-dimensional porous hybrids (3DPH) nano-scaffold with composite of sodium alginate/ZnO nanoparticles solution and silk fibroin electrospun nanofibers by freeze-drying method (as a dermal graft). Due to their morphology, high porosity, and high surface area-to-volume ratio, nanofibrous scaffolds have wide applications in the fields of skin tissue engineering, artificial skin grafts, and wound dressing. However, loading and releasing sensitive bioactive compounds such as antibiotics, DNA, proteins, nanoparticles, and other drugs into nanofibrous scaffolds is usually challenging. Hence, the drug's physicochemical properties, polymer choice, drug-polymer compatibility, drug-solvent compatibility, and formulation should be carefully considered for designing

scaffolds. The complex interplay among various parameters significantly impacts the loading and sustainable drug release.

We added these sentences to the revised manuscript, highlighted in yellow (in the discussion and references).

4. What are the study's strengths and limitations?

Response: We used biocompatible and biodegradable natural polymers (silk fibroin and alginate) for the scaffolds synthesis, which provide an excellent way to load and efficiently release drugs (growth factors, antibiotics, and other bioactive compounds) for wound healing. Additionally, they are cost-effective for mass production. However, further analyses, such as mechanical tests of scaffolds and *in vivo* studies could be considered in future projects.

We added these sentences to the revised manuscript, highlighted in yellow (as final part, Recommendations for future studies).

5. I recommend that the authors include a final part of the manuscript titled "Recommendations for future studies," where they may share their thoughts and give guidance for other researchers in the field.

Response: Thank you very much for your suggestion. We added this title to the revised manuscript, highlighted in yellow (as final part, Recommendations for future studies).

6. Please consider the following articles, discuss them and compare their results, their strengths and limitations in comparison with your study and include them in the discussion section to enhance the quality of the manuscript, if applicable:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7784275/

https://www.mdpi.com/2073-4409/9/7/1622

https://www.mdpi.com/2073-4409/11/15/2439

https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-020-00755-7

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8875365/

Response: Thank you very much for your good recommendation. We used the valuable information of these articles and compared their scientific findings to the revised manuscript, highlighted in yellow (in the introduction, discussion, and references).

Sincerely

2 Revision 1

3 comments · 14 Jan 2023 – 16 Jan 2023

Referee 1 Reviewer

ACCEPT 14 Jan 2023

1. The authors explain in the introduction: "Typical treatment for chronic wounds includes the application of autografts, allografts, xenografts, and topical delivery of anti-inflammatory, antioxidant, and antibacterial agents. In spite of this, the mentioned therapies are not sufficient for wounds that are extensive or deep." Please offer some references and a more thorough explanation.
Response: Chronic wounds are usually treated with autografts, allografts, xenografts, and topical antibacterial and anti-inflammatory agents. Nevertheless, the above therapies are not sufficient for extensive or deep wounds. Autografts are most advantageous because they do not exhibit immune responses, but they cannot cover the entire wounded site when it exceeds 60% of the patient's total body area. Additionally, the application of allogeneic skin grafts carries a high risk of rejection and treatment failure.
We edited and added these sentences to the revised manuscript, highlighted in yellow (in the introduction and references).
follow up: Ok. Thank you.
2. The introduction is quite brief and requires further justification. What research need in the area does this study intend to address? This requires more reasoning.
Response: Modern treatments include skin tissue engineering, which produces bioengineered biomaterial-based artificial skin grafts aimed at overcoming the shortage of donor skin graft supplies and the rejection of skin allografts and xenografts. Moreover, bioengineered artificial skin substitutes are becoming more popular for treating chronic wounds, as is the topical delivery of mesenchymal stem cells to reduce inflammation and speed healing.
A scaffold is an essential component of skin tissue engineering and artificial skin grafts as it has a pivotal role in mechanical support, delivery of nutrients and oxygen, and the release of growth factors and drugs. It can also modulate cell behaviors such as attachment, motility, proliferation, and differentiation.
Therefore, scaffolds could mimic the biological, chemical, and mechanical properties of the extracellular matrix (ECM) of the target tissue. Several types of scaffolds can be utilized for artificial skin grafts, including fibrous, porous, and hydrogel scaffolds.
We added these sentences to the revised manuscript, highlighted in yellow (in the introduction and references).
Follow up: Ok.
3. In the discussion, the authors cite several studies with comparable findings that support the findings of the present study. Nevertheless, the issue is: what does this research contribute to the literature that comparable investigations before it did not find? What makes this study distinctive in contrast to previous studies? These questions should be handled in depth, and I would advise the authors to add a new paragraph at the end of the discussion to address these concerns.
Response: In this study, we fabricated silk fibroin nanofibers by electrospinning (as an epidermal graft) and a three-dimensional porous hybrids (3DPH) nano-scaffold with a composite of sodium alginate/ZnO nanoparticles solution and silk fibroin electrospun nanofibers by the freeze-drying method (as a dermal graft). Due to their morphology, high porosity, and high surface area-to-volume ratio, nanofibrous scaffolds have wide applications in the fields of skin tissue engineering, artificial skin grafts, and wound dressing. However, loading sensitive bioactive compounds such as antibiotics, DNA, proteins, nanoparticles, and other drugs into nanofibrous scaffolds and releasing them is usually challenging. Hence, the

drug's physicochemical properties, polymer choice, drug-polymer compatibility, drug-solvent compatibility, and formulation should be carefully considered when designing scaffolds. The complex interplay among various parameters significantly impacts drug loading and sustainable drug release.
We added these sentences to the revised manuscript, highlighted in yellow (in the discussion and references).
follow up: Very nice.
4. What are the study's strengths and limitations?
Response: We used biocompatible and biodegradable natural polymers (silk fibroin and alginate) for scaffold synthesis, which provide an excellent way to load and efficiently release drugs (growth factors, antibiotics, and other bioactive compounds) for wound healing. Additionally, they are cost-effective for mass production. However, further analyses, such as mechanical tests of scaffolds and in vivo studies, could be considered in future projects.
We added these sentences to the revised manuscript, highlighted in yellow (as the final part, Recommendations for future studies).
follow up: Ok, great.
5. I recommend that the authors include a final part of the manuscript titled "Recommendations for future studies," where they may share their thoughts and give guidance to other researchers in the field.
Response: Thank you very much for your suggestion. We added this title to the revised manuscript, highlighted in yellow (as the final part, Recommendations for future studies).
follow up: Ok, thank you for the revisions.
6. Please consider the following articles, discuss them, compare their results, strengths, and limitations with those of your study, and include them in the discussion section to enhance the quality of the manuscript, if applicable:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7784275/
https://www.mdpi.com/2073-4409/9/7/1622
https://www.mdpi.com/2073-4409/11/15/2439
https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-020-00755-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8875365/
Response: Thank you very much for your good recommendation. We used the valuable information from these articles and compared their scientific findings with those of our study in the revised manuscript, with the changes highlighted in yellow (in the introduction, discussion, and references).
Follow up: Thank you for your revisions.

All in all, all my previous comments have been considered and addressed. Thank you very much and congratulations.
Best regards
Reviewer

Associate Editor

ACCEPT 16 Jan 2023

Dear EIC,
The manuscript has been revised successfully and can be published now.

Editor-in-Chief ACCEPT 16 Jan 2023

Dear Author,
Your manuscript is acceptable now. Thank you for revising and refining it.