Bukti Korespondensi Artikel Jurnal

Accelerated Healing of Chronic Wounds under a Combinatorial Therapeutic Regimen Based on Cold Atmospheric Plasma Jet Using Contact and Noncontact Styles

Plasma Medicine, 11, 2, Mei 2021, Hal 1-18

(Sri Darmawati)

Link Jurnal: https://www.dl.begellhouse.com/download/article/36794c0929e42d00/PMED-39083.pdf

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Link Indeks Jurnal: https://www.scopus.com/sourceid/19700182808

No.	Tanggal	Aktivitas Korespondensi	Halaman
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Lampiran 1
Submission

Sri Darmawati <ciciekdarma@unimus.ac.id>

Begell House Online Submission System - new article has been created (PMED-39083)

1 message

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Wed, May 12, 2021 at 8:54 PM
To: ciciekdarma@unimus.ac.id

Dear Sri Darmawati,

You have just created a new article entitled
'Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet'
for 'Plasma Medicine'.
The article ID is PMED-39083.

For now the article is not visible for anyone in the system but you.
Please logon to the system and move the article further to make it available for other submission users:

- start from the login page - https://www.submission.begellhouse.com/usr/login.html?prod_code=journals
- open the article by clicking on its title
- choose an appropriate option on the tab "Main" to submit your article to a regular or a special issue and finish your submission.

Also, you have an option to completely delete the article from the system by choosing the option "Remove article".

Begell House
Online Submission.

Sri Darmawati <ciciekdarma@unimus.ac.id>

PMED-39083. Begell House Online Submission System - confirmation

1 message

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Wed, May 12, 2021 at 10:07 PM
To: ciciekdarma@unimus.ac.id

Dear Sri Darmawati.
This is a confirmation notice that your article "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" for the journal "Plasma Medicine" has been successfully submitted and sent to the Editor-in-Chief - Satoshi Hamaguchi.

Please use the submission site to track the status of your article. The article ID is "PMED-39083".

Begell House
Online Submission.

Lampiran 2
Review Requested

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'REVIEW_REQUESTED' to 'ON_CORRECT'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Tue, May 18, 2021 at 5:52 PM
To: ciciekdarma@unimus.ac.id

Dear Author - Sri Darmawati.

The status of article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has changed from REVIEW_REQUESTED to:
ON_CORRECT

The change in status of the article requires your attention.

The submission deadline is: June 01, 2021.

Please login https://www.submission.begellhouse.com/usr/login.html?prod_code=journals

Notes from Editor-in-Chief - Satoshi Hamaguchi:

Lampiran 3
Accepted for publication

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'REVIEW_REQUESTED' to 'ACCEPTED_FOR_PUBLICATION'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Thu, May 27, 2021 at 8:30 PM
To: ciciekdarma@unimus.ac.id

Dear author - Sri Darmawati,

The status of your article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has been changed from REVIEW_REQUESTED to:
ACCEPTED_FOR_PUBLICATION

Begell House
Online Submission.

Lampiran 4
Request changes for production

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'ACCEPTED_FOR_PUBLICATION' to 'REQUEST_CHANGES_FOR_PRODUCTION'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Fri, Jun 4, 2021 at 9:29 PM
To: ciciekdarma@unimus.ac.id

Dear Author - Sri Darmawati.

The status of article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has changed from ACCEPTED_FOR_PUBLICATION to:
REQUEST_CHANGES_FOR_PRODUCTION

The change in status of the article requires your attention.

Please login https://submission.begellhouse.com/usr/login.html?prod_code=journals

Notes from Production Assistant - Martha Link:
Dear Sri Darmawati,
Your article for PMED has been accepted for publication. However, before we can begin to process your article, we will need you to provide us with the following information:

1) We will need you to upload the program file(s) that was used to create the pdf for your text. This includes your text in Microsoft Word or LaTex (whichever applies). If you are providing LaTex files please also provide a pdf file that includes only your text, tables and figure captions (no figures), double-spaced and 12 point type. If providing a word document please include text, tables, and only figure captions, double spaced and 12 point type.

2) We will need you to upload the program files that were used to create your figures. Please provide your figures with no less than 300dpi-700dpi, in tiff, jpg, eps, or similar format, which will print in a much better quality. We do not accept figures embedded in an MS Word document, PowerPoint or PDF file as the quality/resolu
 tion is too poor for publication.

3) Please confirm that all figures, photos, and tables are your original work, have never been published before and that no permissions are required. If any figures or tables have been published before, you will need to contact the copyright holder (usually the publisher) and upload the permission documentation along with your other article files.

4) The callouts for your figures and tables are not listed in order or there are callouts missing in your manuscript. Figure 9 is not mentioned in the text. Please sent us a revised article with Figure 9 mentioned in the text. Figure/Table callouts must be in numerical order (Fig. 1 appears first, Fig. 2 second, etc.). Please make sure all callouts are listed in the correct order.

5) We also require that you submit a signed copyright form and fill out an electronic transmittal form. I have uploaded a new copyright form which can be found in your Files folder. Please download the file
 , fill out, and sign (please do not type) on the line under the word “CONTRIBUTOR.” Please print your name on the line under that (marked “Print Name”). Either scan and upload it onto the submission site, fax to 203-456-6167, or email to journals@begellhouse.com.

6) Please let us know if your research received funding from the NIH. If so, provide funding ID.

After uploading all your necessary files (figure files, etc.) for publication, click the SUBMIT TRANSMITTAL AND COPYRIGHT FORMS icon located on the “Main” tab. On the next screen you will be asked to confirm that you have uploaded all necessary files. After checking that all files are uploaded, click next. On the next screen you will see the TRANSMITTAL FORM. Fill out all necessary fields, then click SUBMIT TRANSMITTAL AND COPYRIGHT FORMS. The Production Dept. will then be notified that you have submitted your files.

All article files MUST be uploaded onto the submission site. Do not send via
 regular email.

Please return the requested files and information to us within 72 hours. Once all of the requested materials are received your article will be placed on-line as a forthcoming article for this journal and it will be assigned a DOI number for referencing and citing purposes.

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Please do not hesitate to contact journals@begellhouse.com if you have any questions or need assistance.

Best Regards,

Begell House Production Dept.

Lampiran 5
Request corrections

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'REQUEST_CHANGES_FILLED' to 'PM_REQUEST_CORRECTIONS'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Wed, Jun 9, 2021 at 10:21 PM
To: ciciekdarma@unimus.ac.id

Dear Author - Sri Darmawati.

The status of article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has changed from REQUEST_CHANGES_FILLED to:
PM_REQUEST_CORRECTIONS

The change in status of the article requires your attention.

Please login https://submission.begellhouse.com/usr/login.html?prod_code=journals

Notes from Production Assistant - Martha Link:
Dear Sri Darmawati,
Thank you for providing the files and information we previously requested. However, we will still need the following:

1) Please let us know if your research received funding from the NIH. If so, provide funding ID.

After uploading all your necessary files (figure files, etc.) for publication, click the SUBMIT TRANSMITTAL AND COPYRIGHT FORMS icon located on the “Main” tab. On the next screen you will be asked to confirm that you have uploaded all necessary files. After checking that all files are uploaded, click next. On the next screen you will see the TRANSMITTAL FORM. Fill out all necessary fields, then click SUBMIT TRANSMITTAL AND COPYRIGHT FORMS. The Production Dept. will then be notified that you have submitted your files.
All article files MUST be uploaded onto the submission site. Do not send via regular email.
Please return the requested files and information to us within 72 hours. Once all of the requested materials are receive
d your article will be placed on-line as a forthcoming article for this journal and it will be assigned a DOI number for referencing and citing purposes.
Please do not delete any files that were previously uploaded to the submission system.
Please do not hesitate to contact journals@begellhouse.com if you have any questions or need assistance.
Best Regards,
Begell House Production Dept.

Lampiran 6
Response to Reviewer

Manuscript ID: PMED-39083
Response to Reviewers

Dear, Editor Satoshi Hamaguchi

Thank you for giving us the opportunity to submit a revised draft of the manuscript "*Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet*". We appreciate the time and effort that you and the reviewers dedicated to providing feedback on our manuscript and are grateful for the insightful comments on and valuable improvements to our paper.

We have incorporated most of the suggestions made by the reviewers. Those changes are marked in red within the manuscript. Please see below, in blue, for a point-by-point response to the reviewers' comments and concerns. All page numbers refer to the revised manuscript file with tracked changes.

Reviewer ' Comments to the Authors

- Comment 1: *From the first look, it looks like the replica of work done by authors in the previous two articles Plasma Medicine, 10(4):259–271 (2020) and Clinical Plasma Medicine Volume 14, June 2019, 100085. Therefore, it is crucial that authors clearly explain the difference between the previous two manuscripts and how it was different from the current manuscript. It is better to show this through a diagram.*

  Response: Thank you for pointing this out. We have added about the differences between each study in [Line 1 in page 5] and appear in the diagram in Figure 1 [page 5].

- Comment 2: *On page 3, line 29 authors mentioned RONS, but it was used the first time, so the full form is required or authors noted "Reactive Oxygen Species (ROS) and Reactive Oxygen Species (RNS)" they can write reactive oxygen and nitrogen species (RONS).*

  Response: We have revised about that. "This ROS and RNS can be called Reactive Oxygen and Nitrogen Species (RONS)" has been corrected on [Line 29 in page 3].

- Comment 3: *Same page 3, line 31 authors mentioned, "RONS produced by plasma that suspicious to have effectiveness…" what is meaning by suspicious here? Try to write clear, simple English and avoid using words that changes the sentence meaning.*

  Response: We agree with the reviewer's suggestion. We have replaced the word "suspicious" to "potential". So the revised sentence "RONS produced by plasma that potential to have effectiveness are Hydrogen peroxide…." has been corrected on [Line 31 in page 3].

- Comment 4: *Page 4, lines 1-2 shows that in plasma, only these species are generated that are mentioned. It's better to write etc or many more.*

  Response: We agree with the reviewer's suggestion. We have revised the sentence on [Line 3 in page 4].

- Comment 5: *Again on Page 4 lines, 6-10 is not clear it shows only the mentioned species are generated; there are no other species generated in the system.*

  Response: Thank you for pointing this out but we write about the particular characteristics of the two main conditions the plasma jet refers to Lu, 2015 [9] and we write down the species that were generated referring on that report.

- Comment 6: *Authors should write the name of different drug resistance bacteria are killed by plasma and cite the articles. Especially drug resistance S. aureus as the current study also focused on that.*

  Response: Yes, we have added about the name of different drug resistance bacteria are killed by plasma on [Line 20 in page 4].

- Comment 7: *Discussion is not complete; there is no comparison with the other wound healing studies performed by the plasma. Also, there are details which reactive species are mainly responsible for wound healing effect based on the previous study.*

  Response: Thank you for the suggestions. We have added the topic such as reviewer's suggestion in discussions section on [Line 30 in page 21].

- Comment 8: *There are many typo mistakes. In many places, there is space between value and unit, while in other places not. In some places hours is written while in other places h. It should be consistent in the whole manuscript*

  Response: Yes, we have checked the manuscript again and found many typos. We have corrected the typos in the revised manuscript.

We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.

Sincerely

Author's team

Lampiran 7
Copyedit

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'REQUEST_CHANGES_FILLED' to 'ON_COPYEDIT'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Tue, Jun 22, 2021 at 12:23 AM
To: ciciekdarma@unimus.ac.id

Dear author - Sri Darmawati,

The status of your article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has been changed from REQUEST_CHANGES_FILLED to:
ON_COPYEDIT

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Online Submission.

Lampiran 8
Author proof

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'ON_TYPE_SETTING' to 'TYPESET'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Fri, Jul 2, 2021 at 8:50 PM
To: ciciekdarma@unimus.ac.id

Dear Author - Sri Darmawati.

The status of article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has changed from ON_TYPE_SETTING to:
TYPESET

The change in status of the article requires your attention.

Please login https://submission.begellhouse.com/usr/login.html?prod_code=journals

Notes from Typesetter - Angelito Infante:
Your author proof for the upcoming issue of the journal Plasma Medicine is now available on the Begell House submission site.

Here is the link to the site: http://submission.begellhouse.com/usr/login.html?prod_code=journals.

We ask that you please login to review your author proof and answer all inquiries. It is extremely important that you answer all queries so that we can avoid any delays in processing your article for publication. After carefully reviewing your article, please upload your corrections and submit them back within 48 hours.

The only way corrections can be received is through the Begell House submission website. If no corrections are needed please just submit the file back with a note to us with a response of no corrections are needed. Please advise if additional time is needed.

After uploading your files, please do not forget to click the “Submit” button from the ‘Main” tab of your article. The next screen you will see is a conf
irmation screen to make sure the files have been uploaded, please scroll down to the bottom of the screen and click on “Next,” then “OK” and follow the prompts.

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Manuscript ID: PMED-39083

Corrections for author proof

Dear, Begell House Production.

We will answer the copyeditor's queries in the margin in a list below.
Those changes are marked in blue.

No	Question	Line, pages	Answer/ Corrections
1.	This reference is not cited above. Eliminate and reorder or cite as originally intended. "g. Faculty of Medicine, Universitas Muhammadiyah Semarang, Semarang, Indonesia"	Page 1, Line 18	Yes, we will eliminate and reorder as originally intended. We had corrected in the below.

Sri Darmawati[1,2,3*], N Nasruddin[1,2,3], Gela Setya Ayu Putri[1,2], Arya Iswara[1,2], Putri Kurniasiwi[1,3,6], Eka Sakti Wahyuningtyas[3,4], Laela Hayu Nurani[5], Defi Nurul Hayati[1,2], Tatsuo Ishijima[7], Toshio Nakatani[8], Junko Sugama[8, 9]

[1]Department of Clinical Laboratory Science, Universitas Muhammadiyah Semarang, Semarang, Indonesia

[2]Interdisciplinary Research Laboratory for Experimental Plasma Medicine (iPlasmed), Universitas Muhammadiyah Semarang, Semarang, Indonesia

[3]Muhammadiyah Research Network for Plasma Medicine (M-Plasmed), Semarang, Indonesia

[4]Department of Nursing, Faculty of Health Sciences, Universitas Muhammadiyah Magelang, Indonesia

[5]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

[6]Department of Clinical Laboratory Technology, Universitas Aisyiyah Yogyakarta, Yogyakarta, Indonesia

[7]Faculty of Electrical, Information and Communication Engineering, Kanazawa University, Kakuma, Kanazawa-shi, Japan

[8]Division of Nursing, Faculty of Health Sciences, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Kanazawa-shi, Japan

[9]Advanced Health Care Science Research Unit, Institute for Frontier Science Initiative (InFiniti), Kanazawa University, Kanazawa-shi, Japan

2.	Please provide a new short running title (maximum of five words). "(Wound Healing Using Cold Atmospheric Plasma Jet Contact and Noncontact Styles)"	Page 3	We prefer short running title is "Wound Healing Using Combinative Treatment of Plasma Jet"
3.	Please clarify meaning of (a) and (A). "O_2 (a) and N_2 (A)."	Page 3, Line 4	We agreed to **delete** (a) and (A) to avoid misunderstanding.
4.	Please check. "Daeschlein et al [18]"	Page 3, Line 30	There was a mistake while entering the citation, the correct citation is Darwamati et al [20]
5.	Please check. "respectively."	Page 4, Line 17	We agreed to **delete** "respectively."
6.	Lower symbol key denotes "##" and "xx," but these double symbols do not appear in graph. Delete? FIG. 2:	Page 5, Line 43	Yes, we will delete the symbol key which not appear in graph. We will send you a new graph.
7.	Please check. Should this be NCP-NCP? If not, please define for reader. "NC–NC"	Page 9, Line 40	Yes, it should be NCP-NCP. " NCP-NCP"
8.	Please check edit. "α had the significance level of (1)"	Page 9, Line 42	It should be It is worth mentioning that (1) α the significance level of NCP–NCP vs. CP–CP,……
9.	Please see above query. NC–NC	Page 10, Line 33	It should be NCP-NCP. "NCP-NCP
10.	Please check. See Above. Please check. CPN–NCP	Page 10, Line 34	It should be CP-NCP. "CP-NCP
11.	Lower symbol key denotes "%," "%%," and "ε" but these symbols do not appear in graph. Delete? FIG. 6:	Page 11, Line 17	Yes, we will delete the symbol key which not appear in graph. We will send you a new graph.
12.	Please see above query. NC–NC	Page 12, Line 25	It should be NCP-NCP. "NCP-NCP
13.	Lower symbol key denotes single symbols but these do not appear in graph. Delete?	Page 13,	Yes, we agree to **delete** this.

		Line 19	
	Significance levels of *NCP–NCP vs. CP–CP; #, NCP–NCP vs. C; ×,NCP–NCP vs. CP–NCP; ^, CP CP vs CP–NC; @, C vs. CP–NC.		
14.	Please see above query. NC–NC	Page 13, Line 24	It should be NCP-NCP. "NCP-NCP
15.	This reference is repeated in Ref. 19. Please check which one has the correct title and renumber references here and in text. Daeschlein G.	Page 18, Line 5	There was a mistake when we input the citation. We had corrected in the below.
	See comment above for Ref. 15. Daeschlein G.	Page 18, Line 15	

Reference No 15 , Page 18 Line 5

Daeschlein G. Antimicrobial activity of plasma. In: Metelmann H-R, Woedtke TV, Weltmann K-D, editors. Comprehensive clinical plasma medicine. Berlin: Springer International; 2018. p. 113–25.

Reference No 19 , Page 18 Line 15

Daeschlein G, Scholz S, Arnold, A, von Podewils S, Haase H, Emmert S, Woedtke T, Weltmann K-D, Jünger M. In Vitro Susceptibility of Important Skin and Wound Pathogens Against Low Temperature Atmospheric Pressure Plasma Jet (APPJ) and Dielectric Barrier Discharge Plasma (DBD). Plasma Processes and Polymers; 2012;9(4):380–389.

Thank you for the time and effort of Begell House Production.

Best Regards

Author's team

Lampiran 9
Proofed by author

Sri Darmawati <ciciekdarma@unimus.ac.id>

Article PMED-39083. The status has been changed from 'TYPESETTING_CORRECTIONS' to 'PROOFED_BY_AUTHOR'

2 messages

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Wed, Jul 7, 2021 at 8:47 PM
To: ciciekdarma@unimus.ac.id

Dear author - Sri Darmawati,

The status of your article PMED-39083 "Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet" in journal "Plasma Medicine" has been changed from TYPESETTING_CORRECTIONS to:
PROOFED_BY_AUTHOR

Begell House
Online Submission.

Sri Darmawati <ciciekdarma@unimus.ac.id>

Begell House Submission System - Final Article File

1 message

journals@submission.begellhouse.com <journals@submission.begellhouse.com> Thu, Jul 15, 2021 at 10:40 PM
To: ciciekdarma@unimus.ac.id

Journal: Plasma Medicine
Article: PMED-39083. Accelerated healing of chronic wound under the combinatorial therapeutic regimen based on contact and non-contact styles of the cold atmospheric plasma jet

Dear Sri Darmawati,

Your article has been finalized and published. Congratulations. Attached is a complimentary pdf file of your published article.

Please note that this pdf file is provided for your own personal use and is not to be posted on any websites or distributed in any manner (electronic or print). Please follow all guidelines provided in the copyright agreement that was signed and included with your original manuscript files.

Any questions or concerns pertaining to this matter should be addressed to journals@begellhouse.com

Thank you for your contribution to our journal. We look forward to working with you again in the future.

Begell Hou se
Production Department.

2 attachments

PMED-39083.pdf
14925K

Cover_Letter.pdf
38K

Lampiran 10
Cover letter

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Fax: 1-203-456-6167
E-Mail: journals@begellhouse.com

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Dear Author,

Your article has been finalized and published. Congratulations. Attached is a complimentary pdf file of your published article.

Please note that this pdf file is provided for your own personal use and is not to be posted on any websites or distributed in any manner (electronic or print). Please follow all guidelines provided in the copyright agreement that was signed and included with your original manuscript files.

Any questions or concerns pertaining to this matter should be addressed to journals@begellhouse.com

Thank you for your contribution to our journal. We look forward to working with you again in the future.

Sincerely,
Brandon T. Bisceglia
Begell House Production Department

Lampiran 11
Published

Plasma Medicine, 11(2):1–18 (2021)

Accelerated Healing of Chronic Wounds under a Combinatorial Therapeutic Regimen Based on Cold Atmospheric Plasma Jet Using Contact and Noncontact Styles

Sri Darmawati,[a,b,c,*] N. Nasruddin,[a,b,c] Gela Setya Ayu Putri,[a,b] Arya Iswara,[a,b] Putri Kurniasiwi,[a,c,d] Eka Sakti Wahyuningtyas,[c,e] Laela Hayu Nurani,[f] Defi Nurul Hayati,[a,b] Tatsuo Ishijima,[g] Toshio Nakatani,[h] & Junko Sugama,[h,i]

[a]Department of Clinical Laboratory Science, Universitas Muhammadiyah Semarang, Semarang, Indonesia; [b]Interdisciplinary Research Laboratory for Experimental Plasma Medicine (iPlasmed), Universitas Muhammadiyah Semarang, Semarang, Indonesia; [c]Muhammadiyah Research Network for Plasma Medicine (M-Plasmed), Semarang, Indonesia; [d]Department of Clinical Laboratory Technology, Universitas Aisyiyah Yogyakarta, Yogyakarta, Indonesia; [e]Department of Nursing, Faculty of Health Sciences, Universitas Muhammadiyah Magelang, Indonesia; [f]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia; [g]Faculty of Electrical, Information, and Communication Engineering, Kanazawa University, Kakuma, Kanazawa-shi, Japan; [h]Division of Nursing, Faculty of Health Sciences, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Kanazawa-shi, Japan; [i]Advanced Health Care Science Research Unit, Institute for Frontier Science Initiative (InFiniti), Kanazawa University, Kanazawa-shi, Japan

*Address all correspondence to: Dr. Sri Darmawati, Department of Medical Laboratory Science, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Jl. Kedungmundu Raya No. 18, Semarang, 50273, Central Java, Indonesia; Tel.: +62-24-76740296, ext. 1102; Fax: +62-24-76740291, E-mail: ciciekdarma@unimus.ac.id

ABSTRACT: One critical element for applying atmospheric pressure plasma jet for medical purposes is that it is possible to construct a combinatorial therapeutic regimen based on contact and noncontact styles for the cold atmospheric plasma jet. This study evaluates plasma jet effectiveness for bacteria-infected wounds in a small animal model. In this investigation, we test a novel combinative treatment using contact and noncontact style for plasma jet that was generated at high voltage of ~ 9 kV. We use medical-grade argon gas as a single carrier gas. The object of plasma treatment is BALB/c mouse skin wounds that were infected with *Staphylococcus aureus*. We use four plasma jet treatments, namely, C (control), CP–CP (contact), NCP–NCP (noncontact), and CP–NCP (contact–noncontact). For CP–NCP, from days 0 to 7 we apply a contact style of plasma jet treatment to wounds to kill bacteria; from days 8 to 13, a noncontact style of plasma jet is applied to stimulate wound healing. Our results show that with CP–CP, contact plasma treatment can remove the biofilm layer, but after the biofilm layer disappears contact plasma treatment inhibits the wound-healing process. NCP–NCP is not effective in eliminating bacterial biofilms and impedes the wound-healing process. With CP–NCP, contact plasma exposure during days 0 to 7 is able to remove bacterial biofilms, and irradiation of noncontact plasma during days 8 to 14 accelerates wound healing. Finally, CP–NCP significantly accelerates healing. The combinatorial therapeutic regimen based on contact and noncontact styles of cold atmospheric plasma jet is recommended for chronic wound management, because it effectively removes bacterial biofilms and accelerates wound healing.

1947-5764/21/$35.00

KEY WORDS: animal model, chronic wound, plasma medicine, RONS, combination treatment

I. INTRODUCTION

It is well understood that chronic wounds have several levels of bacterial burden. Stotts[1] stated that bioburden is a microorganism presence on the surface of a wound. Generally, bacterial bioburden persistence on a wound can be divided into five microbial stages: contamination, colonization, critical colonization, biofilm, and infection. Contamination is the appearance of a nonduplicating microorganism on the wound surface without a host defend. Colonization is the appearance of duplicating microorganisms adherent to the wound surface without a host defend system. Critical colonization is the appearance of duplicating microorganisms on the wound and attached to the wound's cells and formations. Biofilm is a complex community of accumulated bacteria embedded in a self-secreted extracellular polysaccharide matrix. Infection is characterized by invading microorganisms into a wound's tissue and leads to local or systematic defense.

Topical management is positive wound care that restores the physiologic wound environment. A healthy environment's main features include adequate moisture level, temperature control, pH regulation, and bacterial burden control.[2] Recent methods that are known to control bacterial load include (1) debridement, (2) precise wound cleansing, (3) particular infection control precautions, (4) use of antimicrobials, and (5) use of moisture-retentive dressings.

Antimicrobial agent efficacy for eradicating bacterial burden in clinical care was known, but a lack still exists. For example, mupirocin 2% ointment is effective against *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus* (MRSA), but it cannot be used for a large burn. It also contains chemical materials, specifically, polyethylene glycol, which can damage kidneys if absorbed through the skin.[1] Therefore, an effort to find alternative antimicrobial agents is essential. For this point, atmospheric pressure plasma jet may provide a new avenue for bioburden eradication.

Plasma is well known as an ionized gas. In this context, plasma is not blood plasma but refers to physical plasma as a phase of the fourth state of matter after solid, liquid, and gas. Two parts exist in the plasma phase, namely, stable components (gases) and reactive components (ions and energetic and radical particles).[3] Conceptually, the main aspect of plasma's medical effectiveness is related to the capability of plasmas to generate biological molecules, namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS). Together, ROS and RNS is called reactive oxygen and nitrogen species (RONS). When accurately controlled and administered in appropriate doses RONS can be efficacious for medical treatment.[4–6] RONS that are produced by plasma and have potential for effectiveness include hydrogen peroxide (H_2O_2), superoxide ($O_2^{-}\bullet$), singlet oxygen ($^1O_2$), ozone (O_3), hydroxyl radical ($^{\bullet}OH$), organic radicals ($RO^{\bullet}$, $RO_2^{\bullet}$), nitric oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$), peroxynitrite ($ONOO^{-}$), and many others.[7]

Plasma jet is one of the most exciting sources for medical application, and a variety of human-made plasma sources can be developed under the environment of atmospheric pressure. The jet can be lengthened to approach a specific area that is not limited by

electrodes.[8] Conceptually, plasma jet has two main conditions with particular characteristics. A plasma condition in comprised of radicals with relatively shorter lifetimes, such as N_2*, O_2*, OH, and N_2^+. An afterglow condition contains radicals with relatively longer lifetimes, such as OH, O, O_3, NO, and some metastable molecules such as O_2 and N_2.[9] Differentiation in plasma jet treatment style is contact and noncontact, occurring perhaps as a manifestation of the two conditions.

In our previous study, we reported that the noncontact treatment style of plasma jet (afterglow condition) in animal models was capable of stimulating skin-wound healing by accelerating re-epithelialization, mimicking modern medical settings.[10,11] However, the contact style of plasma jet treatment (the plasma condition) has a destructive effect on normal mouse skin.[11,12] Abnormal epidermal tissue in skin wounds was found to be due to the contact style of plasma jet treatment.[13] However, applying plasma jet treatment in the contact style may effectively eradicate chronic wound-related bacteria.[14–16]

Plasma jet treatment is significantly effective in killing bacteria including *S. aureus*, *Pseudomonas aeruginosa*, MRSA, and methicillin-resistant *Staphylococcus epidermidis* (MRSE).[17–19] Furthermore, Darmawati et al.[17] reported that the contact style of plasma jet treatment was significantly effective in killing bacteria but also has a destructive effect on skin and wounds. On the other hand, noncontact style carries a reduced effect in killing bacteria but effectively stimulates wound healing. Finally, these reseachers[17] concluded that the noncontact style of plasma jet is more effective than the contact style. However, a lack of studies exist. In the human body, a chronic wound is clinically impossible to separate out the components of bacteria and wound. To combat this problem, it is important to evaluate plasma jet treatment effectiveness using a chronic wound model, namely, a bacteria-infected wound. Thus, the purpose of this study is to evaluate plasma jet effectiveness for bacteria-infected wounds.

This research was conducted as a development of our previous study.[17,20] Study differences are shown in Fig. 1. The Darmawati et al.[17] evaluation was carried out separately on two biological materials (bacteria in a media plate and acute wounds), whereas in the present study the evaluation was carried out on mice with chronic wounds using a bacteria-infected wound model. Darmawati et al.[20] used different styles based on treatment day. In this study, we introduced a novel treatment style of the plasma jet, namely, the combinative treatment of contact and noncontact styles. This means that from days 0 to 7, we applied the contact style of plasma jet treatment to the wound to kill bacteria, and from days 8 to 13 we used the noncontact style of plasma jet on wounds to stimulate healing.

II. MATERIALS AND METHODS

A. Atmospheric Pressure Plasma Jet

The atmospheric pressure plasma jet system that is used in this experiment was developed on the basis of Teschke et al.[21] and is explained previously.[17] We made a modification in the dimension of the capillary quartz tube. Tube inner and outer diameters

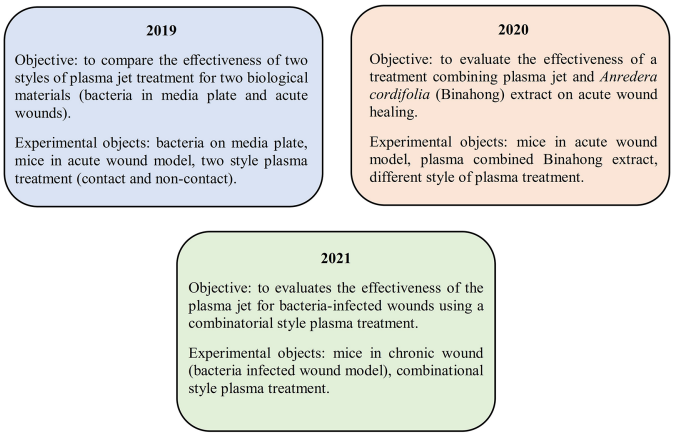

FIG. 1: Development from previous research that occurred in 2019[17] and 2020[20]

in this experiment were 1.55 and 0.65 mm. Around the quartz tube, we applied two ring-like electrodes. A nonconductor material (clay) was used to isolate the two electrodes.

We identified H_2O_2 and NO_2 using the Kyoritsu chemical method, which was reported previously.[17] Plasma jet effectiveness against bacteria on plate media has already been reported, and that study referred to previous studies by Darmawati et al.[17]

B. Microorganisms

The bacteria strains of *S. aureus* (American Type Culture Collection [ATCC] 6538) were obtained from the Laboratory of Microbiology, Department of Medical Laboratory Science, Universitas Muhammadiyah Semarang, Indonesia. Bacteria suspension was done by inoculating a colony of *S. aureus* into brain heart infusion broth and incubating for 24 h at 37°C. The suspension was plated on a blood agar plate (Oxoid Ltd., Hampshire, UK) medium and incubated for 24 h at 37°C. The colony was suspended in saline solutions (0.85%), and turbidity was adjusted to the standard of McFarland 7 solution (21×10^8 colony-forming units [CFU]/mL).

C. Animals and Experimental Protocol

All experimental protocols followed animal welfare guidelines and were approved by the ethics committee for preclinical investigation in Laboratorium Penelitian dan Pengujian Terpadu/Integrated Research and Testing Laboratory (LPPT UGM), Gadjah Mada University, Yogyakarta, Indonesia (certificate approval number 00003/04/LPPT/III/2020). LPPT UGM is accredited by ISO/IEC 17025 and the National Accreditation Committee of Indonesia (Komite Akreditasi Nasional/KAN, Indonesia). We used a total of 72 BALB/c male mice that were aged 8 wk and weighed 35.0–40.0 g. We purchased

the mice from LPPT UGM Indonesia. Mice weights during the observation period are shown in Fig. 2. Mice were individually caged under controlled conditions in an air-conditioned room at 28.0°C ± 2.0°C, with light–dark cycle of light from 08:00 am to 8:00 pm, and under ad libitum feeding conditions. All experiments were carried out under anesthesia, using ketamine–xylazine of 50 mg/kg + 5 mg/kg, respectively. Every effort was made to minimize suffering.[22]

D. Bacteria-Infected Wound Model

Bacteria-infected wounds developed by infecting *S. aureus* ATCC 6538 in acute wounds of mice that Davis et al.[23] previously explained. Male BALB/c mice were acclimatized for 1 wk, and then a full-thickness acute wound was made on the dorsal part of mice with a punch biopsy that was 4 mm in diameter (Kai Industries Co. Ltd., Gifu, Japan). A 50-µL bacterial suspension, equivalent to the turbidity of a McFarland 7 standard solution (21×10^8 CFU/mL), was inoculated into the acute wounds and rubbed into each wound for 10 s using a sterile loop. The wounds were covered with a dressing (Tegaderm Hydrocolloid Dressing; 3M Health Care, St. Paul, MN) for 72 h to allow wounds to become colonized. Dressings were secured in place with a bandage. This method creates a suitable environment for biofilm formation.

E. Plasma Jet Treatment on Wounds

Evaluations of plasma jet treatment on bacteria-infected wounds were conducted in the Laboratory Plasma Medicine for Experimental Wound Healing, Universitas

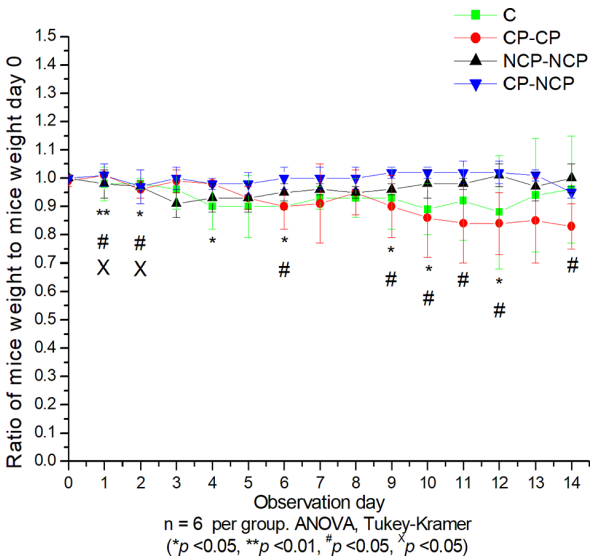

FIG. 2: Weight of mice during observation on days 0–13. ANOVA, analysis of variance.

Muhammadiyah Semarang, Indonesia. Mice were anaesthetized via ketamine–xylazine injection using intraperitoneal administration.[22] Three days after wounds were infected with bacteria, plasma jet treatment began (day 0). The experimental procedure after day 0 is explained in Fig. 3. Plasma jet treatment was carried out once daily for 3 min during 14 d. Mice were randomly divided into four groups, with three mice or six wound samples for every group as follows:

A. Control or untreated group (C): Bacteria-infected wounds were allowed to heal daily under Tegaderm Hydrocolloid Dressing (3M Health Care).
B. Plasma treatment group with contact style treatment (CP–CP): Bacteria-infected wounds were given plasma jet treatment in contact style for 3 min. In this context,

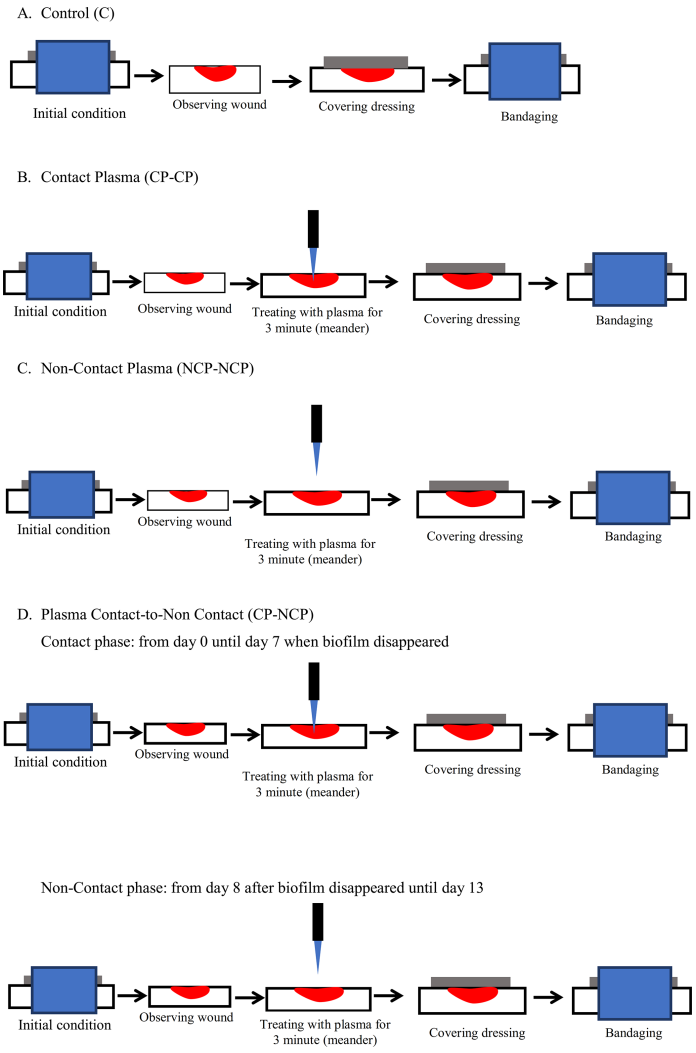

FIG. 3: (A−D) Experiment protocol during days 0–13

the distance from the nozzle of the plasma jet reactor tube to wound surface was 5 mm. In this condition, the plasma jet was visually contacted to the wound surface.

C. Plasma treatment group with noncontact style treatment (NCP–NCP): Bacteria-infected wounds were given plasma jet treatment in noncontact style for 3 min. In this context, the distance from the nozzle of the plasma jet reactor tube to wound surface was fixed at 20 mm. In this condition, plasma jet was visually not contacted to the wound surface.

D. Plasma treatment group with contact and noncontact style treatment (CP–NCP): Bacteria-infected wounds were given plasma jet treatment in contact style for 3 min from days 0 to 7. The following day, from days 8 to 13, noncontact styles of plasma jet treatment were applied for 3 min. In this context, the distance from the nozzle of the plasma jet reactor tube to wound surface for contact style was 5 mm and for noncontact style distance, 20 mm.

During the 14-d experimental period, the bandages and wound dressings in all groups were removed daily and renewed for plasma treatment or wound evaluation.

F. Thermal Distribution Evaluation

We measured temperature distribution of the treated wound skin and its surroundings using an infrared (IR) digital camera (FLIR C series; Teledyne FLIR LLC, Thousand Oaks, CA). Approximately five images were taken for each sample. We used FLIR Tools computer software to analyze image data. A schematic of a plasma medicine system with an infrared camera's position is shown in Fig. 4.

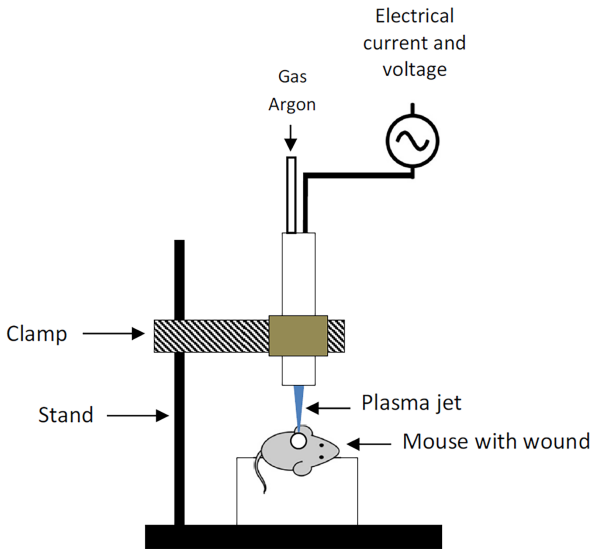

FIG. 4: Experimental setup

G. Macroscopic Evaluation of Wound

First, macroscopic evaluation of the wound was manually conducted, followed by computational processes based on the procedure, as elucidated previously.[17] This evaluation was conducted daily for 14 d. Day 0 is when plasma jet treatment was started. We used a digital camera to document observed wound conditions.

H. New Epithelial Evaluation

On experiment days 7, 11, and 14, the mice were euthanized via excessive ketamine–xylazine injection. The wound and surrounding skin were collected and then bisected at the center of the wound. Tissue processing and hematoxylin and eosin (HE) staining were performed based on the previously elucidated procedure.[17] On the basis of HE staining results, the percentage of re-epithelialization was calculated using the formula 100% × (length of new epithelium/length of wound between wound edges).

I. Total Bacteria Evaluation

We evaluated microorganisms by examining total bacteria periodically on days 0, 3, 7, 11, and 14 during the experiments period; this method is referenced in Liang et al.[24] Wound exudate was taken using sterile swabs and placed into 5 mL of saline solution. A serial dilution was performed until 10^{-3}, and then 100 μL of suspension were plated on Plate Count agar medium (Oxoid) and incubated overnight at 37°C. After incubation, we counted the number of colonies.

J. Total Neutrophil Evaluation

The hematology evaluation to count the number of neutrophils on days 3, 7, 11, and 14 was conducted using a Hematology Analyzer (Mindray BC-2600, Shenzhen, China). First, mouse blood samples were collected by cardiac puncture. Blood was placed in a microtube with 10% ethylenediaminetetraacetic acid anticoagulant. The neutrophil number was checked automatically with the hematology analyzer.

K. Tumor Necrosis Factor–α level

We conducted tumor necrosis factor (TNF)-α evaluations of the wound skin using an enzyme-linked immunosorbent assay (ELISA) (TNF-α rat ELISA kit; Sigma-Aldrich, St. Louis, MO) method. As mentioned above, on days 7 and 14 of the experimental period, mice were euthanized via injection of excessive ketamine–xylazine. The wound and surrounding skin was collected and weighed to be ± 10 mg. The wounds were then frozen with liquid nitrogen. We pounded the sample on a mortar until it was smooth, added 2 mL of phosphate-buffered saline, and centrifuged at 16,000*g* for 15 min at 4°C. The separated supernatant was used for the ELISA test. Absorbance

was immediately read at 450 nm, and sample concentration was calculated based on a standard curve.

L. Malondialdehyde Levels

We conducted a wound skin malondialdehyde (MDA) evaluation using a thiobarbituric acid reactive substance method. As explained above, on days 7 and 14 of the experimental period mice were euthanized via injection of excessive ketamine–xylazine and we collected cells of the wound and surrounding skin and blood. Wounds were weighed to be ± 10 mg, and blood was collected using cardiac puncture. Determination of the MDA level was established using the Lipid Peroxidation (MDA) (Sigma-Aldrich), according to Kapusta et al.'s procedure.[25]

M. Statistical Analysis

Data were subjected to statistical analyses using the Statistical Package for the Social Sciences (IBM, Armonk, NY), ver. 16.0. We evaluated the ratio of wound area to original wound area, percentage of re-epithelialization, amount of total bacteria and total neutrophils, and TNF-α and MDA levels by analysis of variance followed by the Tukey–Kramer method; p values of < 0.05 were considered to be significant.

III. RESULTS

A. Macroscopic Evaluation of Wounds

For every group, we inspected bacteria-infected wounds on mice skin daily from days −3 to 14, as shown in Fig. 5. On day −3, the wound was created and inoculated with bacteria. Bacterial biofilms as a yellowish layer on the wound's surface began to appear on day 0 for all groups. The wound areas of C and NCP–NCP groups increased from day 0 to 14 and a bacterial biofilm grew on the wound surface.

The wound areas of CP–CP and CP–NCP from days 0 to 3 increased, as did the biofilm layer. During days 7 to 14, the biofilm layer of CP–CP slowly disappeared, but the wound area did not decrease. In contrast, in those of CP–NCP during days 7 to 14 the biofilm layer slowly disappeared, followed by decreased wound area. For the CP–NCP condition at the end of the observational period, the wound surface area looked healthy, almost like normal skin. This result suggests that contact–noncontact plasma treatment is most effective for chronic wound treatment. In contrast to the CP–CP condition for which the wound surface area looked unhealthy, the surface (yellowish red) indicated that contact–contact plasma treatment impairs wound healing.

From days 5 to 14, the area of NCP–NCP was significantly larger than that of CP–CP and CP–NCP. At day 14, CPN–NCP was notably smaller than other groups. It is worth mentioning that (1) α the significance level of NCP–NCP vs. CP–CP, (2) the β significance level of NCP–NCP vs. CP–NCP, (3) the χ significance level of C vs. CP–CP,

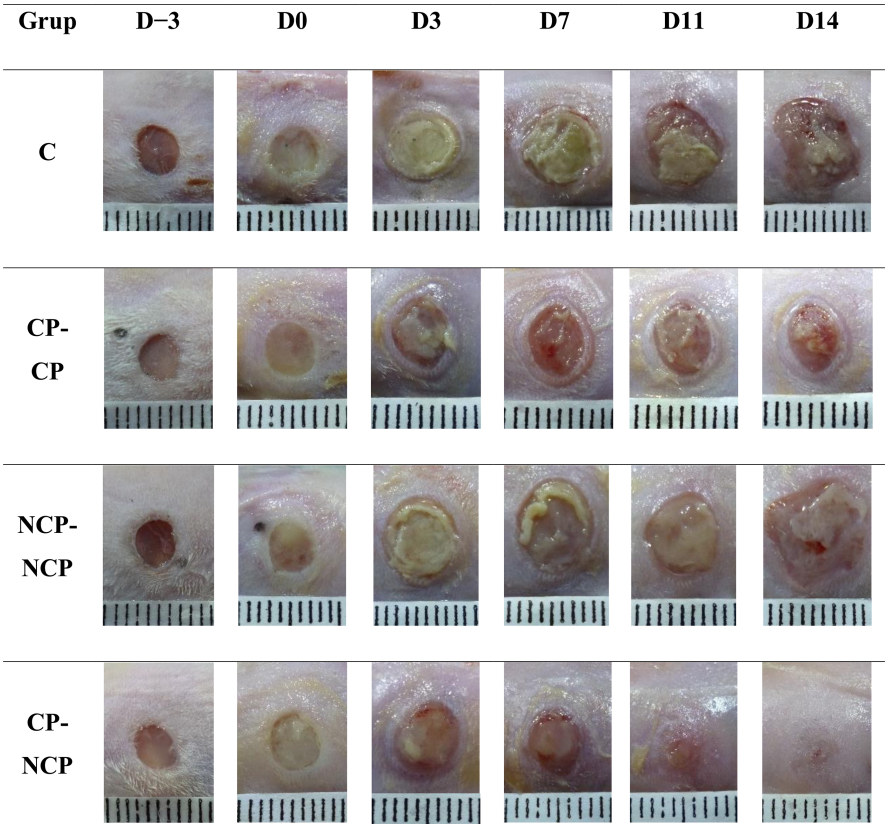

FIG. 5: Wound appearance on days −3, 0, 3, 7, 11, and 14

(4) the δ significance level C vs. CP–NCP, and (5) the ε level of significance of CP–CP vs. CP–NCP.

As mentioned above, macroscopic observation was performed by documenting with a digital camera and tracing the wound area to determine the ratio between wound area to initial wound area during the treatment period. Tracing data are shown in Fig. 6. From days 5 to 14, the area for NCP–NCP was significantly larger than that for CP–CP and CP–NCP (NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$). CP–NCP at day 14 was significantly smaller than other groups (CP–NCP vs. C: $p < 0.01$; CP–NCP vs. CP–CP: $p < 0.01$; CP–NCP vs. NCP–NCP: $p < 0.01$). From macroscopic evaluation, it can be concluded that CP–NCP is the most effective treatment for chronic wounds.

B. New Epithelial Evaluation

As shown in Fig. 7, the percentage of re-epithelialization with CP–NCP at days 7, 11, and 14 was significantly higher than C, CP–CP, and NCP–NCP (CP–NCP vs. C: $p < 0.01$; CP–NCP vs. CP–CP: $p < 0.01$; CP–NCP vs. NCP–NCP: $p < 0.01$). The percentage

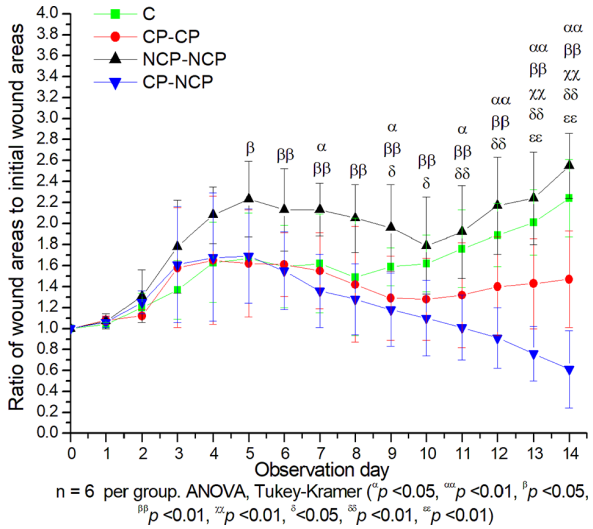

FIG. 6: Ratio of wound area to initial wound area during the 14-d treatment period. ANOVA, analysis of variance.

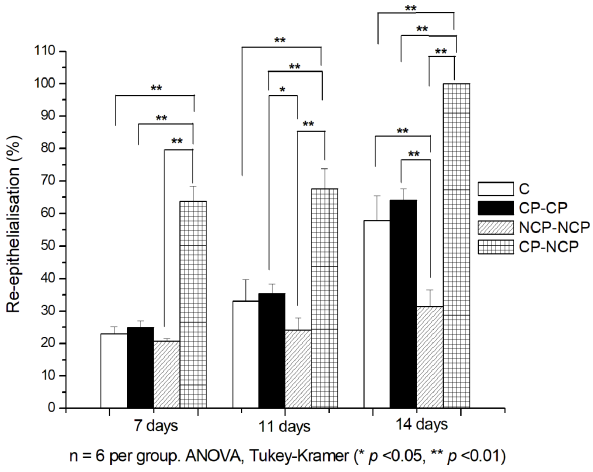

FIG. 7: Percentage of wound tissue re-epithelialization. Percentages of CP–NCP at days 7, 11, and 14 were significantly higher than those of the other groups. Percentages of NCP–NCP at day 14 were significantly lower than those of the other groups. ANOVA, analysis of variance.

of re-epithelialization at day 7 for C, CP–CP, and NCP–NCP did not differ significantly (C vs. CP–CP: $p > 0.05$; C vs. CP–NCP: $p > 0.05$; CP–CP vs. CP– NCP: $p > 0.05$), but NCP–NCP on day 14 was significantly less than for C and CP–CP (NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–CP: $p < 0.01$). Observation at day 14 revealed that the new epithelium had completely covered the wound in CP–NCP. The lowest re-epithelialization

percentage was found in NCP–NCP, whereas the percentage of re-epithelialization in groups C and CP–CP did not differ significantly (C vs. CP–CP: $p > 0.05$).

C. Total Bacteria Evaluation

Total bacteria on days 0, 3, 7, 11, and 14 is shown in Fig. 8. Starting from day 5, a notable difference in total bacteria among groups could be seen. Total bacteria of NCP–NCP and C from days 0 to 14 continued to increase, but CP–CP and CP–NCP continued to decrease. NCP–NCP at days 7 to 14 were significantly higher than for groups C, CP–CP, and CP–NCP (NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$). Observations for C during days 7 to 14 were significantly higher than CP–CP and CP–NCP (C vs. CP–CP: $p < 0.01$; C vs. CP–NCP: $p < 0.01$). Starting from day 7, CP–NCP's total bacteria was significantly lower than for the CP–CP and NCP–NCP groups (NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$).

1. Total Neutrophil Evaluation

Blood neutrophil was evaluated for systemic inflammatory response, with results shown in Fig. 9. NCP–NCP's number of neutrophils on day 7 was significantly higher than that for C, CP–CP, and CP–NCP (NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$), whereas in CP–CP total neutrophils were significantly higher than for C and CP–NCP (CP–CP vs. C: $p < 0.01$; CP–CP vs. CP–NCP: $p < 0.01$). Groups C and CP–NCP did not differ significantly (C vs. CP–NCP: $p > 0.05$).

Observation on days 11 and 14 showed a decreased number of neutrophils in NCP–NCP compared to the count on day 7. Total neutrophils in NCP–NCP, CP–CP, and C

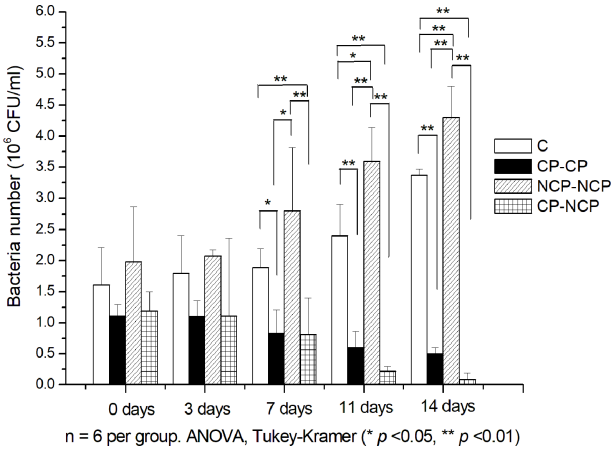

FIG. 8: Total bacteria histogram for days 3, 7, 11, and 14. Starting at day 7, a significant difference in total bacteria between groups occurred. NCP–NCPs during days 7 to 14 were significantly higher than for other groups. From day 7, CP–NCP continued to decline significantly.

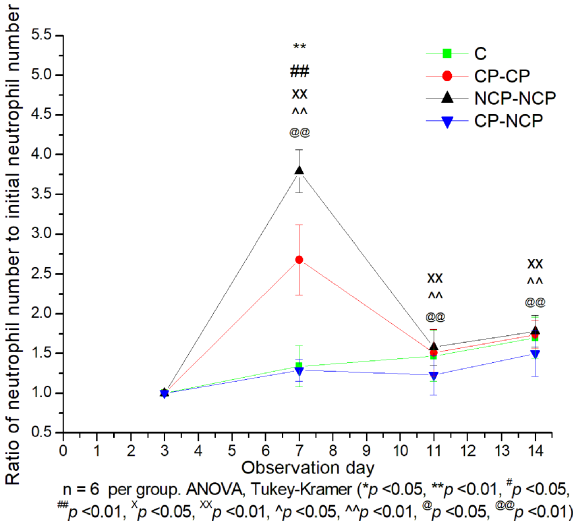

FIG. 9: Total neutrophil histograms for days 3, 7, 11, and 14. Total neutrophils in NCP–NCP on day 7 were significantly higher than in the other groups. CP–NCP on days 11 to 14 was significantly lower than in the other groups.

did not differ significantly (NCP–NCP vs. CP–CP: $p > 0.05$; NCP–NCP vs. C: $p > 0.05$; NCP–NCP vs. CP–NCP: $p > 0.01$). The number of neutrophils in CP–NCP was significantly less than NCP–NCP, CP–CP, and C (NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$).

2. *TNF-α Levels*

TNF-α levels for each group are shown in Fig. 10. TNF-α levels of NCP–NCP at days 7 and 14 were significantly higher than those of other groups (NCP–NCP vs. CP–CP: $p < 0.01$; NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$). C was significantly higher than CP–CP and CP–NCP (C vs. CP–CP: $p < 0.01$; C vs. CP–NCP: $p < 0.05$). CP–CP and CP–NCP did not differ significantly (C vs. CP–NCP: $p > 0.05$).

Observation on day14 showed decreased TNF-α levels in C, NCP–NCP, and CP–NCP groups; except for CP–CP, no significant difference was found. High levels of TNF-α in the NCP–NCP group indicated that bacterial infection was a factor in increased TNF-α levels.

3. *MDA Levels*

MDA levels for each group are shown in Fig. 11. On day 7, highest MDA levels occurred in the CP–CP and CP–NCP groups. Group C was significantly higher than NCP–NCP (C vs. CP–NCP: $p < 0.01$). Day 14 showed decreased MDA levels for groups C, CP–CP,

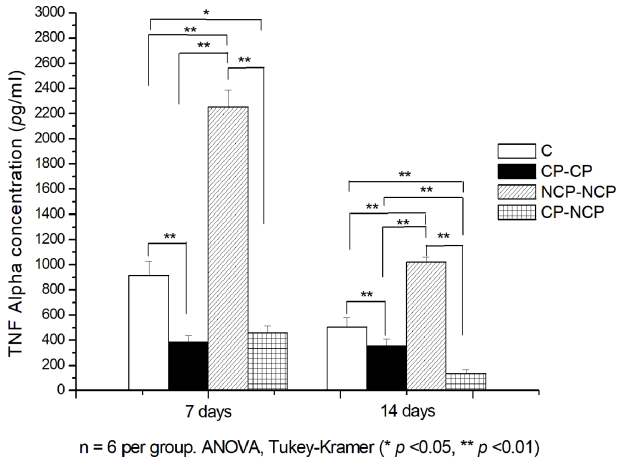

FIG. 10: TNF-α level histogram for days 7 and 14. From day 7, a significant difference is found in TNF-α levels among groups. NCP–NCP on days 7 and 14 were significantly higher than other groups. NCP–NCP, C, and CP–NCP decreased significantly by day 14, whereas CP–CP showed no significant difference between days 7 and 14. ANOVA, analysis of variance; TNF, tumor necrosis factor.

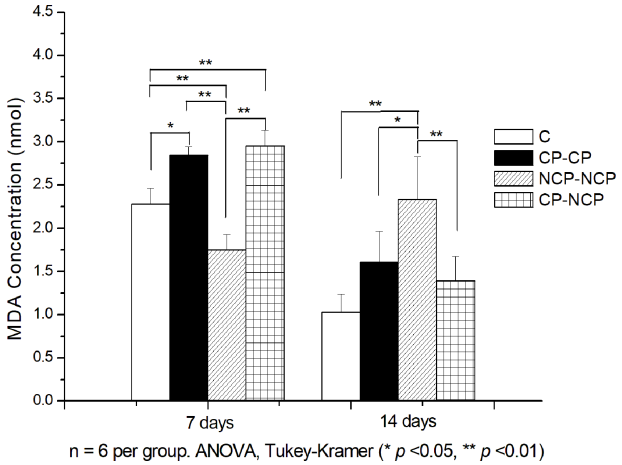

FIG. 11: MDA level histogram for days 7 and 14. On day 7, MDA levels were highest in CP–CP and CP–NCP. A decrease occurred from day 7 to 14 in groups C, CP–CP, and CP–NCP, whereas an increase was present in NCP–NCP. MDA, malondialdehyde.

and CP–NCP. At day 14, an increase occurred in the NCP–NCP group, and MDA levels in NCP–NCP were significantly higher than other groups (NCP–NCP vs. CP–CP: $p < 0.05$; NCP–NCP vs. C: $p < 0.01$; NCP–NCP vs. CP–NCP: $p < 0.01$).

4. Thermal Distribution Evaluation

Thermal images were analyzed using FLIR Software Tools; we used the color palette labeled medical to express the image. Temperature range was set between 20°C and 40°C. T_{max} is the point of highest skin temperature under plasma treatment.

Visually, distribution of wound temperatures among groups differs under plasma treatment, shown in Fig. 12. T_{max} in CP–CP and CP–NCP on day 3 was higher than NCP–NCP. T_{max} of plasma contact treatment was ± 33.4°C–34.7°C. Furthermore, T_{max} of CP–CP on days 7, 8, and 13 was uniform, at ~ 50.1°C–51.3°C. T_{max} of CP–NCP on day 7 tended to be the same as that of CP–CP, ~ 50°C; at days 8 and 13, T_{max} decreased to ~ 30.1°C–34°C. The NCP–NCP group generally had the lowest T_{max} of all groups on all observation days, ~ 28.2°C–32.9°C.

IV. DISCUSSION

It is well known that it is simple to switch plasma jet treatment style from contact to noncontact (and vice versa) in the clinical setting. Darmawati et al.[17] reported that contact style for plasma jet treatment could impede acute wound healing, whereas noncontact style can stimulate acute wound healing. Darmawati et al.[17] stated this conclusion on the

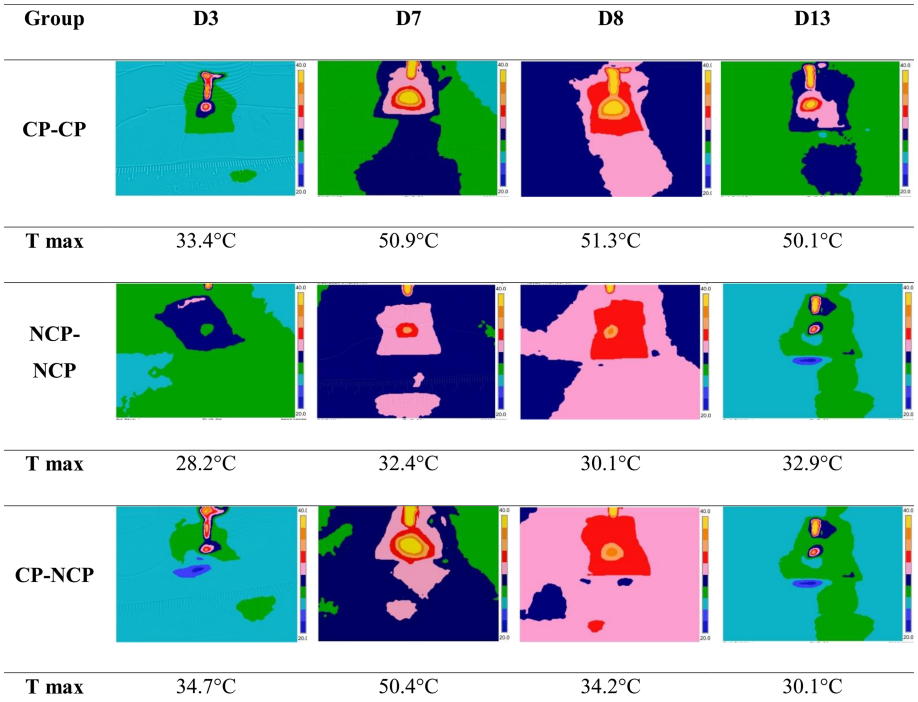

FIG. 12: Distribution of temperature and T_{max} in skin area under plasma treatment

basis of two data sources, namely, the effect of plasma jet on bacteria that is inoculated on the surface of an agar medium and on an acute wound in a small animal model. However, this investigation introduced a new style of plasma jet treatment: a combination of contact and noncontact treatment style (CP–NCP). We used a model of chronic wound or bacteria-infected wound for treatment. Results showed that the combination of contact and noncontact treatment style significantly accelerated healing compared to the control group and other styles of plasma jet treatment. In CP–PNC from days 0 to 7, the contact style was effective in killing bacteria on the wound, and from days 8 to 14 the noncontact style was effective in stimulating wound repair by promoting re-epithelialization. In CP–CP, contact style was also effective in killing bacteria on the wound from days 0 to 14; however, from days 8 to 14 it may damage wound tissue and impede re-epithelialization.

We reported on efficacy of noncontact style plasma jet treatment (NCP–NCP) for acute wounds based on 14 observation days.[17] However, its efficacy for treating chronic wounds in the present investigation was unproven. NCP–NCP tended to fertilize the bacteria burden and impede wound healing. This indicates that noncontact style is not effective in removing bacterial biofilms. Lu reported that noncontact plasma exposure produces radicals with relatively long lifetimes, such as OH, O, O_3, NO, and several diffuse molecules, including O_2 and N_2.[9] Nitrogen (N_2) in this context may have a role in providing nutrients needed for energy in the growth and formation of bacterial cells.[26]

Inflammation is a crucial phase in chronic wound healing. A chronic wound is characterized by a longer inflammation phase. We evaluated two biomarkers, neutrophil and TNF-α. It is well known that high levels of neutrophil and TNF-α elevate inflammation. The present study revealed that at the end of observation day 14, the two in the CP–NCP group were significantly lower than in other groups. Contact and noncontact style plasma jet suppressed the inflammation phase of chronic wound healing.

The thermal effect is also an essential parameter of plasma jet. It is commonly accepted that plasma jet should be fixed at a temperature below 40°C for medical therapy. Contact style of plasma jet elevated local skin temperature by > 40°C or caused ΔT by more than 4°C,[11,17] possibly damaging skin.[17] However, the present investigation indicated that when the bacterial biofilm still visually appears on the wound surface, it is possible to effectively use contact style to kill such bacteria. In addition, contact style must be switched to noncontact style when such bacterial biofilms disappear. Noncontact style produces radicals such as NO with a relatively long lifetime.[9] Thana et al. stated that the NO that was produced by plasma had a stimulating effect on wound healing and tissue regeneration.[27] NO is an important cellular signaling molecule in humans that can affect the immune system, increase growth factors, and stimulate cell proliferation, angiogenesis, and collagen synthesis, resulting in reconstructing damaged skin.[27]

This study used RONS that was produced by plasma jets to remove biofilms and stimulate wound healing. The dose of RONS produced by the plasma jet can be controlled by regulating plasma treatment time and distance. For comparison, a study by Alkawareek et al.[28] used time variations to evaluate use of atmospheric pressure plasma for eradicating bacterial biofilms. The present study used distance

combinations to evaluate use of plasma to remove biofilms and stimulate wound healing. RONS, especially H_2O_2 and NO_2^-, has an important role in the process of removing biofilms and stimulating wound healing. H_2O_2 and NO_2^- that are produced by contact style can kill cells (including bacteria) and normal tissue. H_2O_2 works by producing destructive hydroxyl free radicals that can attack membrane lipids and DNA.[29] On the other hand, H_2O_2 and NO_2^- that are produced by noncontact style can stimulate wound healing. The combinatorial therapeutic regimen based on contact and noncontact styles of cold atmospheric plasma jet is recommended for chronic wound management, because it effectively removes bacterial biofilms and can accelerate wound healing.

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