BUKTI KORESPONDENSI
JURNAL INTERNASIONAL BEREPUTASI TERAKREDITASI Q4

Judul Artikel : **(C11)** The Effect Of Scaffold Hydroxyapatite Derived From Portunus Pelagicus Shell On The Expression Of Fibroblast Growth Factor-2 (Fgf-2) And Bone Morphogenetic Proteins-2 (Bmp -2) In The Extraction Sockets Of Cavia Cobaya.

Jurnal : Journal of Oral Research
Penulis : ***Michael J. Kridanto Kamadjaja****, Sherman Salim, Gigih Gemiudeas.

No	Perihal	Tanggal	Halaman
1	Bukti submit dan artikel yang disubmit	30 Agustus 2021	1-3
2	Bukti Revisi 1	02 September 2021	4-5
3	Bukti Revisi 2	29 Oktober 2021	7
4	Bukti Revisi 3	07 Agustus 2022	13-22
5	Decision 1	08 September 2022	25-36
6	Decision 2		
7	Decision 3		
8	Decision 4		
9	Bukti accepted	09 September 2022	37
10	Bukti published	1 Februari 2021	38-54

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

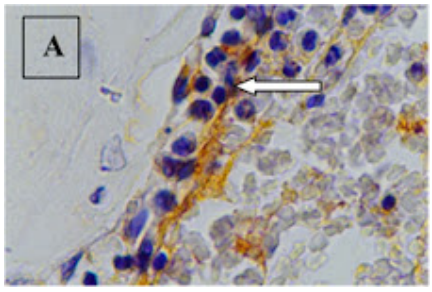
michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Mon, Aug 30, 2021 at 1:40 PM
To: journal@joralres.com

Dear Editor in Chief of Journal of Oral Research
Celia A. Lima,

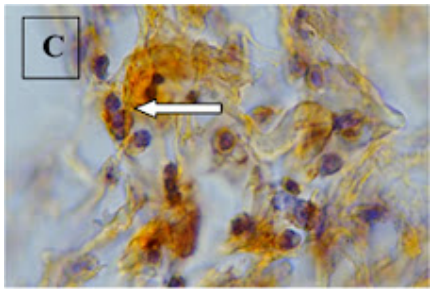
Please kindly allow me to introduce myself. I am Dr. Michael Josef Kridanto Kamadjaja, lecturer at Department of Prosthodontic, Faculty of Dental Medicine, Universitas Airlangga. Through this email, I would like to submit a manuscript entitled "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya" authored by me, Dr. Sherman, and Gigih. Hereby I also upload several files to encourage the manuscript.
If you have any questions regarding the manuscript, please let me know.

Sincerely,
Michael Josef Kridanto Kamadjaja

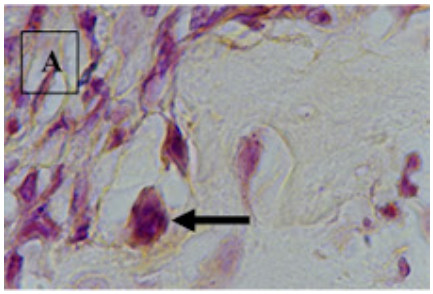
12 attachments



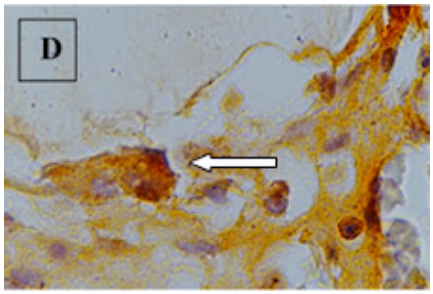
2a.jpg
274K



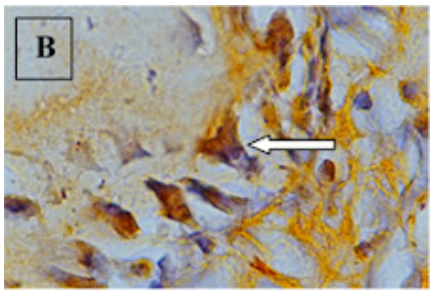
2c.jpg
256K



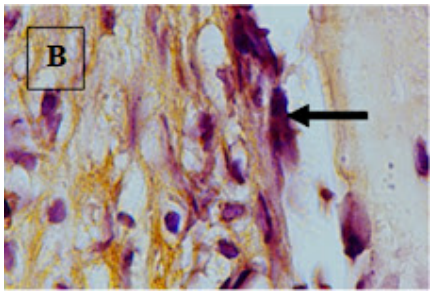
3a.jpg
253K



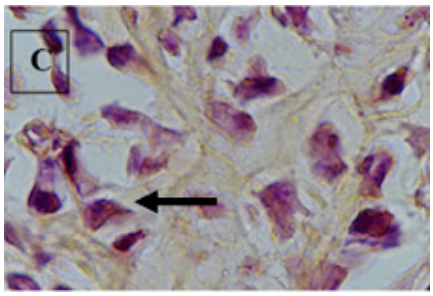
2d.jpg
267K



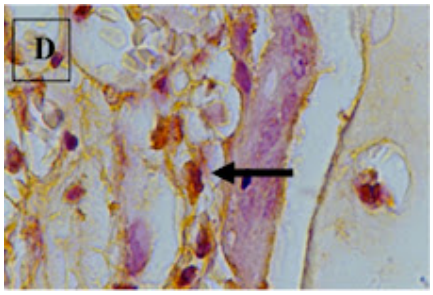
2b.jpg
293K



3b.jpg
296K



3c.jpg
264K



3d.jpg
277K

Abstract Gigih.docx
18K

Gigih Ready to Submit.docx
68K

Graph Table Captions.docx
26K

title page.docx
14K

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Mon, Aug 30, 2021 at 10:02 PM
To: Editor J Oral Res <journal@joralres.com>

Please kindly apologize for some errors of mistyping one of the author
in the manuscript. The revision one has been uploaded.
Thank you

On Mon, Aug 30, 2021 at 1:40 PM michael josef kridanto kamadjaja
<michael-j-k-k@fkg.unair.ac.id> wrote:
>
> Dear Editor in Chief of Journal of Oral Research
> Celia A. Lima,
>
> Please kindly allow me to introduce myself. I am Dr. Michael Josef
> Kridanto Kamadjaja, lecturer at Department of Prosthodontic, Faculty
> of Dental Medicine, Universitas Airlangga. Through this email, I would
> like to submit a manuscript entitled "The Effect of Scaffold
> Hydroxyapatite derived from Portunus pelagicus Shell on the Expression
> of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic
> Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya"
> authored by me, Dr. Sherman, and Gigih. Hereby I also upload several
> files to encourage the manuscript.
> If you have any questions regarding the manuscript, please let me know.
>
> Sincerely,
> Michael Josef Kridanto Kamadjaja

title page.docx
15K

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Thu, Sep 2, 2021 at 4:52 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear author,

Today, the 1st of September, manuscript submissions have opened. New manuscripts will start being processed today and a first editorial decision will be sent to you within the next four weeks. If you have not received an editorial decision by the end of September, please write to us via e-mail.

Best regards,

Estimado autor,

Hoy, 01 de Septiembre, se ha abierto la recepción de manuscritos. Los nuevos manuscritos comenzarán a procesarse hoy y se le enviará una primera decisión editorial dentro de las próximas cuatro semanas. Si no ha recibido una decisión editorial a finales de septiembre, escríbanos por correo electrónico.Saludos cordiales,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

El lun, 30 ago 2021 a las 2:40, michael josef kridanto kamadjaja (<michael-j-k-k@fkg.unair.ac.id>) escribió:

> Dear Editor in Chief of Journal of Oral Research
> Celia A. Lima,
>
> Please kindly allow me to introduce myself. I am Dr. Michael Josef Kridanto Kamadjaja, lecturer at Department of Prosthodontic, Faculty of Dental Medicine, Universitas Airlangga. Through this email, I would like to submit a manuscript entitled "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya" authored by me, Dr. Sherman, and Gigih. Hereby I also upload several files to encourage the manuscript.
> If you have any questions regarding the manuscript, please let me know.
>
> Sincerely,
> Michael Josef Kridanto Kamadjaja

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Thu, Sep 2, 2021 at 4:53 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Thank you for your information, I received the title page corrected.
Best wishes,
Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

El lun, 30 ago 2021 a las 11:02, michael josef kridanto kamadjaja (<michael-j-k-k@fkg.unair.ac.id>) escribió:

> Please kindly apologize for some errors of mistyping one of the author
> in the manuscript. The revision one has been uploaded.
> Thank you
>
> On Mon, Aug 30, 2021 at 1:40 PM michael josef kridanto kamadjaja
> <michael-j-k-k@fkg.unair.ac.id> wrote:
> >
> > Dear Editor in Chief of Journal of Oral Research
> > Celia A. Lima,
> >
> > Please kindly allow me to introduce myself. I am Dr. Michael Josef
> > Kridanto Kamadjaja, lecturer at Department of Prosthodontic, Faculty
> > of Dental Medicine, Universitas Airlangga. Through this email, I would
> > like to submit a manuscript entitled "The Effect of Scaffold
> > Hydroxyapatite derived from Portunus pelagicus Shell on the Expression
> > of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic
> > Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya"
> > authored by me, Dr. Sherman, and Gigih. Hereby I also upload several
> > files to encourage the manuscript.
> > If you have any questions regarding the manuscript, please let me know.
> >
> > Sincerely,
> > Michael Josef Kridanto Kamadjaja

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Tue, Oct 19, 2021 at 1:02 PM
To: Editor J Oral Res <journal@joralres.com>

Dear Editor in Chief of Journal of Oral Research
Celia A. Lima, PhD

I would like to ask about the current status of my manuscript entitled "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya". According to your latest email, by the end of September, the decision has been made. Could you please inform me the decision? Thank you very much

Regards,
Michael Josef Kridanto Kamadjaja

On Thu, Sep 2, 2021 at 4:52 AM Editor J Oral Res <journal@joralres.com> wrote:
>
> Dear author,
>
> Today, the 1st of September, manuscript submissions have opened. New manuscripts will start being processed today and a first editorial decision will be sent to you within the next four weeks. If you have not received an editorial decision by the end of September, please write to us via e-mail.
>
> Best regards,
>
>
>
> Estimado autor,
>
> Hoy, 01 de Septiembre, se ha abierto la recepción de manuscritos. Los nuevos manuscritos comenzarán a procesarse hoy y se le enviará una primera decisión editorial dentro de las próximas cuatro semanas. Si no ha recibido una decisión editorial a finales de septiembre, escríbanos por correo electrónico.Saludos cordiales,
>
> Celia A. Lima, PhD
> Editor-in-Chief
> Journal of Oral Research
>
>
> El lun, 30 ago 2021 a las 2:40, michael josef kridanto kamadjaja (<michael-j-k-k@fkg.unair.ac.id>) escribió:
>>
>> Dear Editor in Chief of Journal of Oral Research
>> Celia A. Lima,
>>
>> Please kindly allow me to introduce myself. I am Dr. Michael Josef
>> Kridanto Kamadjaja, lecturer at Department of Prosthodontic, Faculty
>> of Dental Medicine, Universitas Airlangga. Through this email, I would
>> like to submit a manuscript entitled "The Effect of Scaffold
>> Hydroxyapatite derived from Portunus pelagicus Shell on the Expression
>> of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic
>> Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya"
>> authored by me, Dr. Sherman, and Gigih. Hereby I also upload several
>> files to encourage the manuscript.
>> If you have any questions regarding the manuscript, please let me know.
>>
>> Sincerely,
>> Michael Josef Kridanto Kamadjaja

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Fri, Oct 29, 2021 at 5:03 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja
Hope you are doing well. I am very sorry for the long delay in getting back to you with an answer. We were overwhelmed with manuscripts during September, and are still processing them. I am sorry that you had to wait so long. I will have an editorial decision within a couple of days!
Best wishes,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Wed, Nov 3, 2021 at 1:57 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja
Hope you are doing well.
First, allow me to apologize for the delay in providing an editorial decision. We received many more manuscripts than expected during September.
The manuscript "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya" has been editorially approved to enter peer review.
We are a small and slow journal, so please expect at least 3 months for this process to take place. I appreciate your patience during this time, but I remain attentive to inquiries via email if you need any updates or additional information.
I am very sorry for the delay.
Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Mon, Feb 7, 2022 at 12:42 PM
To: Editor J Oral Res <journal@joralres.com>

Dear Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research,

I wonder if there are any updates regarding my manuscript. Three months have passed since your last email. Could you please give me the information update?
Thank you for your help.

Regards,
Michael Josef Kridanto Kamadjaja

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Mon, Feb 7, 2022 at 10:44 PM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja
The manuscript is still under peer review, I am aware that many months have passed and I appreciate your patience.
I hope to have a resolution soon for you, but I really cannot tell you right now how long it will take for peer review to complete.
As soon as we have enough reviews we will let you know, hoping to have better news soon.
Best wishes,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Tue, Jun 28, 2022 at 11:08 AM
To: Editor J Oral Res <journal@joralres.com>

Dear Editor in Chief,
It's been 4 months since your last email and almost one year since my first submission. Could you please give me information regarding the status of my manuscript?
Thank you for your time and consideration

Regards,
Michael Josef Kridanto Kamadjaja

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Tue, Jun 28, 2022 at 10:03 PM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja
Hope you are doing well. I absolutely can understand your frustration regarding this manuscript. It has been a long time under revision. So far there is one review ready, positive in tone. As soon as I have a second review we will be able to have a final resolution.
best regards,
Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Sun, Aug 7, 2022 at 5:17 PM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja,

I hope this email finds you well.

The manuscript "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast G rowth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya" has gone through the process of peer review of the journal, the reviewers comments are attached to this email.

In consideration of the Reviewers' appreciation, the manuscript has been recommended for publication conditional to minor changes, so we hope you make the suggested changes and / or justify your decision not to. The comments of the reviewers are few, with mostly a request to strengthen the introduction and justify why the study was conducted.

Starting today, you have 30 days to send the new version of the manuscript. Please highlight changes performed to the new version of the text in color, in order to ease tracking. If you need more time, please write to us. We appreciate your patience during this lengthy review process.

Along with your corrected manuscript, you must send a response letter to each reviewer comment. A detailed response to the reviewers' comments is crucial.

I take this opportunity to thank you for your patience and to apologize for the long period of time this process took.

Looking forward to a new version of this manuscript.

Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

2 attachments

rev 2 Kridanto Kamadjaja.docx
56K

rev 1 Kridanto Kamadjaja.docx
37K

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Sun, Aug 7, 2022 at 5:17 PM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja,

I hope this email finds you well.

The manuscript "The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast G rowth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya" has gone through the process of peer review of the journal, the reviewers comments are attached to this email.

In consideration of the Reviewers' appreciation, the manuscript has been recommended for publication conditional to minor changes, so we hope you make the suggested changes and / or justify your decision not to. The comments of the reviewers are few, with mostly a request to strengthen the introduction and justify why the study was conducted.

Starting today, you have 30 days to send the new version of the manuscript. Please highlight changes performed to the new version of the text in color, in order to ease tracking. If you need more time, please write to us. We appreciate your patience during this lengthy review process.

Along with your corrected manuscript, you must send a response letter to each reviewer comment. A detailed response to the reviewers' comments is crucial.

I take this opportunity to thank you for your patience and to apologize for the long period of time this process took.

Looking forward to a new version of this manuscript.

Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

2 attachments

rev 2 Kridanto Kamadjaja.docx
56K

rev 1 Kridanto Kamadjaja.docx
37K

J Oral Res
oral and craniofacial sciences

Dear Reviewer:

Thank you, once more, for accepting to act as Reviewer of the manuscript

The peer review process of our journal is double-blind, so your comments are anonymous and will be confidentially handled. From today you have 10 calendar days to make your assessment.

Review planning	**Date**
Request from the editor	
Review delivery date	

This account manuscript meets the following requirements (EDITOR use)	Yes	No
1) This manuscript has a statement of authorship by the author(s).	x	
2) Informed consent by the patient, if applicable.		
3) Approval from the Ethical Committee, if applicable.		
4) Does not present Conflicts of interest.	x	

Pertinence	Yes	No
1) Appropriate manuscript for the subject of the Journal of Oral Research	x	
2) This manuscript is of interest to the academic or professional community	x	
3) It has an innovative and current theme	x	
4) There is literature that supports the theme of this manuscript	x	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

J Oral Res
oral and craniofacial sciences

- Evaluation-

(Mark the most appropriate option, if it applies to the manuscript)

Title, Abstract and keywords	Yes	No
1) The title of the work, is concise and informative	x	
2) The purpose of the study or investigation are described	x	
3) The keywords help in the identification of the work	x	
4) The keywords are adequate	x	

Introduction	Yes	No
1) There is an adequate contextualization, either Clinical; Epidemiological or Scientific	x	
2) The interest or importance of this work is justified	x	

Material and method	Yes	No	n/a
1) Sociodemographic data, clinical characteristics are included	x		
2) Laboratory tests or histopathology are included	x		
3) Figures are included to facilitate its description	x		
4) It is properly described	x		

Result	Yes	No
1) The results are adequately described	x	
2) Tables are used to help better understand the results	x	
3) Figures are included that help better understand the study	x	

Discussion	Yes	No
1) New and important aspects are emphasized	x	
2) It is consistent with the existing literature	x	
3) It is easy to understand	x	
4) It is properly described	x	

Conclusion	Yes	No
1) It is necessary and pertinent	x	
2) It is appropriate and properly written	x	

TABLES	Yes	No
1) The results in the tables are adequately described	x	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

2) They are self-explanatory	x	
3) Tables have titles and legends	x	
4) Abbreviations or symbols conform to the international scientific nomenclature	x	

FIGURES (data plots, x-rays, CAT scans, ultrasounds, etc).	Yes	No
1) Each figure is cited within the text, in consecutive order	x	
2) Figures have titles and legends	x	
3) The figures help better compression of the work	x	
4) The abbreviations or symbols conform to the international scientific nomenclature	x	

COMMENTS ON THE MANUSCRIPT
Page (4): 2nd paragraph, (-800C), how you get it and where, its true or -80C, check it please.

FINAL RESOLUTION (please tick one option only)	
1) Acceptance of the Manuscript	
2) Acceptable with minor modifications	x
3) Major changes and second round of revisions	
4) Rejection	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

J Oral Res
oral and craniofacial sciences

Dear Reviewer:

Thank you, once more, for accepting to act as Reviewer of the manuscript

The peer review process of our journal is double-blind, so your comments are anonymous and will be confidentially handled. From today you have 10 calendar days to make your assessment.

Review planning	**Date**
Request from the editor	Jul 16, 2022
Review delivery date	Jul 30, 2022

This account manuscript meets the following requirements (EDITOR use)	Yes	No
1) This manuscript has a statement of authorship by the author(s).	x	
2) Informed consent by the patient, if applicable.		
3) Approval from the Ethical Committee, if applicable.		
4) Does not present Conflicts of interest.	x	

Pertinence	Yes	No
1) Appropriate manuscript for the subject of the Journal of Oral Research	x	
2) This manuscript is of interest to the academic or professional community	x	
3) It has an innovative and current theme		x
4) There is literature that supports the theme of this manuscript	x	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

J Oral Res
oral and craniofacial sciences

- Evaluation-

(Mark the most appropriate option, if it applies to the manuscript)

Title, Abstract and keywords	Yes	No
1) The title of the work, is concise and informative	x	
2) The purpose of the study or investigation are described	x	
3) The keywords help in the identification of the work	x	
4) The keywords are adequate	x	

Introduction	Yes	No
1) There is an adequate contextualization, either Clinical; Epidemiological or Scientific	x	
2) The interest or importance of this work is justified	x	

Material and method	Yes	No	n/a
1) Sociodemographic data, clinical characteristics are included			x
2) Laboratory tests or histopathology are included			x
3) Figures are included to facilitate its description			x
4) It is properly described	x		

Result	Yes	No
1) The results are adequately described	x	
2) Tables are used to help better understand the results	x	
3) Figures are included that help better understand the study	x	

Discussion	Yes	No
1) New and important aspects are emphasized	x	
2) It is consistent with the existing literature	x	
3) It is easy to understand	x	
4) It is properly described	x	

Conclusion	Yes	No
1) It is necessary and pertinent	x	
2) It is appropriate and properly written	x	

TABLES	Yes	No
1) The results in the tables are adequately described	x	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail journal@joralres.com
http://www.joralres.com

2) They are self-explanatory	x	
3) Tables have titles and legends	x	
4) Abbreviations or symbols conform to the international scientific nomenclature	x	

FIGURES (data plots, x-rays, CAT scans, ultrasounds, etc).	Yes	No
1) Each figure is cited within the text, in consecutive order	x	
2) Figures have titles and legends	x	
3) The figures help better compression of the work	x	
4) The abbreviations or symbols conform to the international scientific nomenclature	x	

COMMENTS ON THE MANUSCRIPT

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

J Oral Res
oral and craniofacial sciences

Comments for authors and editor

The scope of the manuscript presented entitled “The Effect of Scaffold Hydroxyapatite derived from Portunus pelagicus Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of Cavia cobaya” is well articulated by the authors and sufficient studies have been cited to support each section.
Moreover, there are some comments for consideration:

1. In addition to a brief summary of your work, the final paragraph of your introduction should include a statement of originality and a preview of your principal arguments and conclusions. These articles may also be used to complete the introduction section:
https://doi.org/10.1016/j.jhazmat.2019.121139
https://doi.org/10.29252/jcc.1.1.3
https://doi.org/10.1016/j.jddst.2019.101131
https://doi.org/10.3390/biom12020155
https://doi.org/10.29252/jcc.2.1.4
https://doi.org/10.1016/j.ceramint.2019.06.183
https://doi.org/10.1021/acsnano.9b04723
https://doi.org/10.1016/j.mseb.2020.114950
https://doi.org/10.1007/s42242-021-00130-x
https://doi.org/10.29252/jcc.2.2.7
https://doi.org/10.1016/j.ceramint.2019.10.057
https://doi.org/10.29252/jcc.2.3.1
https://doi.org/10.1007/s12010-019-03046-6
https://doi.org/10.1016/j.ceramint.2019.09.219
https://doi.org/10.29252/jcc.1.1.3
https://doi.org/10.1186/s40824-021-00203-z
https://doi.org/10.29252/jcc.2.1.2
2. Leave extra space between the paragraph and other paragraphs.
3. This article does not provide a specific and clear novelty. The introduction section is devoted to the purpose without a clear novelty.
4. There are grammar errors in the manuscript. For example page 1 line 12 “were observed to be”, page 1 line 5 “group should delete”, line 13 “than in”, and page 2 line 17 “calcium” page 3 line 12 “was applied” etc. The manuscript should be checked in this regard.
5. Avoid using abbreviations for the first time. Page 4, line 1
6. References need to be more up-to-date
7. JCC adheres to the JOURCC EndNote style (in the guide for author’s webpage).
Examples: Reference to a journal publication:
[1] M.S. Abd-Elwahed, A. Wagih, I.M.R. Najjar, Correlation between micro/nano-structure, mechanical and tribological properties of copper-zirconia nanocomposites, Ceramics International 46(1) (2020) 56-65. https://doi.org/10.17632/xxxxx1.
Reference to a journal publication with an article number:
[2] K.C.R. Kolan, J.A. Semon, A.C. Brombeutel, D.E. Day, M.C. Leu, Bioprinting with bioactive glass loaded polylactic acid composite and human adipose stem cells, Bioprinting 18 (2020) e00075. Reference to a book:
[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000. Reference to a chapter in an edited book:
[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
e-mail: journal@jcraniores.com

J Oral Res
oral and craniofacial sciences

FINAL RESOLUTION (please tick one option only)	
1) Acceptance of the Manuscript	
2) Acceptable with minor modifications	x
3) Major changes and second round of revisions	
4) Rejection	

Facultad de Odontología, Universidad de Concepción, Roosevelt 1550, Concepción, Chile.
Phone:(+56-41)2204386
Mailbox: 160-C, Concepción, Chile.
E-mail **journal@joralres.com**
http://www.joralres.com

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Wed, Aug 31, 2022 at 1:49 PM
To: Editor J Oral Res <journal@joralres.com>

Dear Editor in Chief Journal of Oral Research
Celia A. Lima, PhD

I am very pleased to receive your email regarding the status of my manuscript. Me and the other authors are still working on it. Could you please give me extended time to re-submit the revision?
Thank you for your understanding

Regards,
Michael Josef Kridanto Kamadjaja

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Wed, Aug 31, 2022 at 9:44 PM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja,
No problem, take the necessary time, I will rebook the deadline for 21th September, I hope if this is long enough for your needs, if not just let me.
Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id> Thu, Sep 8, 2022 at 10:52 PM
To: Editor J Oral Res <journal@joralres.com>

Dear Celia A. Lima, PhD
Editor-in-Chief of Journal of Oral Research

We just finished revising the manuscript according to the reviewers.
Please find the file of response letter and revised manuscript below.
If you have any questions, please let me know. Once again, thank you for this opportunity

Regards,
Michael Josef Kridanto Kamadjaja

[Quoted text hidden]

2 attachments

Response letter for mas Gigih JOR.docx
34K

Gigih Submit Revisi.docx
143K

The Effect of Scaffold Hydroxyapatite derived from *Portunus pelagicus* Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of *Cavia cobaya*

Introduction

Patients may frequently experience unpleasant consequences resulting from tooth loss, after which the redundant alveolar bone atrophies due to a lack of physiological stimulation.[1] Alveolar ridge resorption reduces patient comfort with dentures while simultaneously increasing the number of complaints about denture stability which is influenced by the vertical height of the residual ridge.[2] Resorption ridges will provide less stability than residual ridges with sufficient vertical height.[3] Following tooth extraction, efforts should be made to maintain the vertical dimension of the residual ridge.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic material with frequent biomedical applications due to its close resemblance to the primary mineral ingredient of bones and teeth. Numerous investigations have established that hydroxyapatite is biocompatible and possesses osteoconductive properties. It can prove an effective therapy in the prevention of post-extraction physiological bone loss, in both horizontal and vertical dimensions.[4] Hydroxyapatite synthesized from natural raw materials or natural waste can be more beneficial because it contains many valuable ions, also found in biological hydroxyapatite.[5] One of the sources of natural hydroxyapatite is the shell waste of Portunus Pelagicus, known as the Rajungan crab in Indonesia. A previous study revealed that the Calcium hydroxyapatite content of Rajungan crab shells can be as high as 66.62%.[6]

Hydroxyapatite scaffolds from waste bone from different species have been studied, such as in a study by Sharifianjazi (2021), which concluded that hydroxyapatite scaffolds from waste bone pigeon could be a promising and economically viable material for bone grafting.[7] According to Raya et al. (2015),

calcium hydroxyapatite from Rajungan crab were effective to inhibit demineralization of the tooth *in vitro*. Its effect as a scaffold after tooth extraction has not been studied. [8] In this study, administration of hydroxyapatite scaffold derived from Rajungan crab shell to the tooth extraction socket was expected to increase alveolar bone formation and reduce alveolar bone resorption, thereby enabling the bone replacement process in the extraction socket to be completed.

Fibroblast Growth Factor-2 (FGF-2) has been shown to activate the transcription factor Runx2 in osteoblasts by increasing the protein's stability and acetylation level via extracellular signal-regulated kinase mitogen-activated protein (ERK MAP Kinase). [9] A subsequent study conducted by Ai et al. (2014) reported that Bone Morphogenetic Proteins-2 (BMP-2) functions as an osteoinductive growth factor since it recruit mesenchymal progenitor cells and induce their differentiation to bone-forming osteobalsts. [10] FGF-2 and BMP-2 have also been found to work synergistically in the formation of bone and fibrous tissue.[11]

This study aimed to determine the expression of FGF-2 and BMP-2 in the post-extraction socket following application of hydroxyapatite scaffold from Rajungan crab shell in order to investigate bone forming ability and cellular activity.

Material and Methods

This study was conducted between April and October 2020. The Federer formula was used to calculate the number of *Cavia cobaya* participants of this study, resulting in a final research population of 28. The subjects had to meet certain criteria: healthy, 3-3.5 months old, weighing 300-350 grams, being lesion-free, and having complete use of their five senses. The *Cavia cobaya* were provided with standard pellets and water *ad libitum* during a one-week acclimatization period prior to being randomly assigned to one of two groups; control and treatment. The latter group was given hydroxyapatite scaffold of Rajungan crab shell. Both groups were observed after 7 and 14 days.

Soft tissue was removed from *Portunus pelagicus* crab shell waste using distilled water. The crab shells were then immersed in a chlorine solution at a ratio of 30 ml of chlorine to 5 liters of water. Immersion in 3% H_2O_2 was continued for another 24 hours before the shells were dried at room temperature. Shell calcination was carried out in a furnace at 1000°C. The initial heating temperature of approximately 50°C was increased at a rate of 5°C/min. On reaching 1000°C, it was kept constant for around two hours before being rapidly reduced to 100°C. SEM-EDX was used to characterize hydroxyapatite compounds. The powder sifting process was conducted using a sifting machine to obtain crab shell hydroxyapatite powder measuring less than 150 µm.[6]

The hydroxyapatite scaffold was produced by adding 0.5 grams of gelatin to distilled water and stirring it for one hour at 40°C. The scaffold was created by stirring 1.5 grams of hydroxyapatite powder into the gelatin solution for six hours. The gel was then centrifuged for ten minutes. Following extraction of excess water, the residue was poured in a cylindrical, 5 x 2 mm (2 mm high, 5 mm diameter) sized mold and, having cooled, freeze dried at -800C for 24 hours.[12]

Ketamine 20mg/300mg body weight was injected intramuscularly into the *Cavia cobaya* subjects for anesthetic/sedation and analgesic purposes. The region surrounding the left mandibular incisive tooth was cleansed of debris prior to the tooth being carefully extracted in a specified direction with a sterile needle holder. The socket was irrigated with sterile distilled water after removal and hydroxyapatite scaffold applied in accordance with the previously defined group. Simple suturing was subsequently performed using DS 12 3 / 8c, 12 mm, 6/10 met, 0.7 (Braun Aesculap) polyamide monofilament.[13]

After 7 and 14 days, the subjects in each group were sacrificed in order to observe FGF2 and BMP2 expression. The mandible was cut and removed with samples of the tooth subsequently being fixed in 10% formalin buffer for 24 hours

at room temperature and decalcified with ethylenediaminetetraacetic acid (EDTA). Dehydration by means of graded alcohol concentration was then completed, followed by clearing in xylol and embedding in paraffin. Finally, 4-micron thick paraffin blocks were cut and placed on the object glass.

Staining of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP-2) was performed on the 7th and 14th-day observation groups using single staining technique. The preparations were immersed in Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP-2) primary antibodies for 30 minutes, then washed three times with PBS. At that point, the preparation was immersed in a secondary antibody, namely; anti-mouse monoclonal antibody (Thermo Scientific, USA), for 30 minutes, washed twice with PBS, and immersed in a chromogen substrate for five minutes. After rinsing with distilled water, the incision was placed in a haematoxylin mayer for six minutes, washed with running water and, finally, mounted and placed under a cover glass to enable it to be viewed with a light microscope [14]. The area under observation was the apical third of the socket.

Statistical analysis was performed using the Statistical Package for the Social Sciences Software (SPSS) edition 24.0 (SPSS™, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) was used to analyze the data followed by a Tukey HSD comparison multiple test at a confidence level of 95%.

Results

The statistical analysis revealed significant differences between the control and treatment groups ($p < 0.05$) on both days 7 and 14 (Table 1). Compared to the other groups, group C7 had the lowest level of FGF-2. The control group, designated C7, was observed on day 7. On the other hand, Group HS14, which was treated with hydroxyapatite scaffolds made from crab shells and tested on the

14th day, had the highest level of FGF-2 (Figure 1). Brown staining was utilized to detect osteoblasts expressing FGF-2 (Figure 2).

Similarly, a statistically significant difference ($p < 0.05$) was found between the observations in the BMP-2 treatment group and the control group (Table 1). The levels of BMP-2 in each group demonstrated a similar pattern to the levels of FGF-2. The BMP-2 levels in the C7 group were the lowest. In a related manner, after 14 days of treatment with a hydroxyapatite scaffold produced from crab shells, the HS14 group had the highest BMP-2 concentrations compared to the other groups in the experiment (Figure 1). Brown staining was utilized to detect osteoblasts expressing BMP-2 (Figure 3).

Discussion

The findings showed that the group receiving the crab shell hydroxyapatite scaffold in the extraction socket expressed significantly more FGF-2 and BMP-2 than the group that was not provided with the crab shell hydroxyapatite scaffold. There was a significant difference in the expression of FGF-2 and BMP-2 between the two groups ($p < 0.05$). These findings demonstrate that placing a hydroxyapatite scaffold derived from the *Portunus pelagicus* shell into a tooth extraction socket increases the expression of FGF-2 and BMP-2 in *Cavia cobaya* alveolar bone. This significant difference is evident in the 7th and 14th-day groups.

The findings of this study are similar to those of research conducted by Ramadhani et al. (2016), namely; there is a significantly higher FGF-2 expression in the Wistar rats group to whose dextra femoral bone defect hydroxyapatite had been applied than in the control group which was not administered with hydroxyapatite. [15] In another study conducted by Fauzia et al. (2019), applying a hydroxyapatite xenograft to the extraction sockets of guinea pigs induced a higher BMP-2 expression than in the untreated control group. [16] Both studies indicate that the

administration of hydroxyapatite has a positive effect on the number of FGF-2 and BMP-2 expressions found.

The increase in FGF-2 and BMP-2 expression identified in this study could have occurred due to the osteoinduction properties of the gelatin-hydroxyapatite scaffold of crab shells applied to the extraction sockets of the *Cavia cobaya*. Previous studies asserted that the osteoconductive nature of hydroxyapatite crab shell stimulated stem cells and osteoblasts to proliferate and differentiate in the formation of new bone or the process of bone regeneration. [17] The content of hydroxyapatite crystals in the scaffold, which is homogeneously distributed throughout it, can result in an increase in mechanical properties and cellular activity on the surface of the scaffold.[18] Moreover, calcium phosphates such as HA are well known for their affinity for binding to various proteins, including BMPs, and their increase on a particular surface area may be required to accumulate sufficient amounts of BMPs to induce osteoinduction.[19]

During the bone formation phases and activation of osteogenesis, several growth factors are present in the early and intermediate stages, with some differences in each peak expression. FGF-2 and BMP-2 will reach their peak on day 14, at which point the amount will decrease.[20] The results of this study support this finding since they indicated that the highest FGF-2 and BMP-2 expression in both the treatment and control groups was found on day 14. This finding is also in line with the results of the research conducted by Huang et al. (2007), which revealed that the mRNA expression of FGF-2 and BMP-2 was at its highest point on day 14during the differentiation phase.[21]

Hydroxyapatite can be synthesized from various resources such as eggshells, bones of various animals, shells, and plants. Research conducted to date shows

that this natural resource can be a good source of biologically and thermally stable hydroxyapatite.[22] On this occasion, the researchers chose to use hydroxyapatite from natural sources, namely; from processed crab (Portunus pelagicus) shell. This action was intended to reduce crab shell waste that often produces an offensive odor and pollutes seawater. However, in reality, the amount of hydroxyapatite contained in crab shell is relatively high. Crab shell waste (Portunus pelagicus) has been used as raw material in the synthesis of calcium hydroxyapatite [Ca10(PO4)6(OH)2] because of its high calcium content of 66.62%.[6] Other research conducted by Wibisono et al. (2018) found that crab shells contain more calcium (93.78%) than fish scales (82.31%) making them suitable as raw material in the synthesis of hydroxyapatite.[23] It transpired that crab shells demonstrate high compatibility. Research conducted by Kamadjaja (2019) succeeded in revealing that grafts made from crab shells have strong biocompatibility in cell culture and have the optimum biocompatibility at a concentration of 25 ppm.[24]

In this study, gelatin was selected as the scaffold material because gelatin-based scaffold demonstrates excellent biocompatibility and biodegradability, while possessing a porous structure.[25] Microporosity is also an essential characteristic of a scaffold in a bone graft. It will increase the specific surface area, providing more protein adsorption sites, where cells have more numerous opportunities to interact with osteogenic-related proteins, thereby facilitating cellular osteogenic function to form new bone tissue.[26]

Finally, further development of this research in the future remains a strong possibility because its findings show that natural ingredients, especially hydroxyapatite derived from crab shells, have a positive effect on the increased expression of growth factors, especially FGF- 2 and BMP-2 which play an essential role in helping post-extraction bone regeneration. Furthermore, to identify the additional benefits of this material, a long-term study must be conducted until the

bone regeneration process is complete in order to enable comparisons with other bone grafting materials to be made.

Conclusion

The application of hydroxyapatite scaffold derived from Rajungan crab shell (Portunus pelagicus) in the tooth extraction socket affects the increased expression of FGF-2 and BMP-2 in the alveolar bone of Cavia cobaya. Furthermore, in order to identify the additional benefits of this material, a long-term study should be conducted until the bone regeneration process is complete to allow comparisons with other bone grafting materials to be undertaken.

References

KM S, Koli DK, Jain V, Pruthi G, Nanda A. Comparison of ridge resorption and patient satisfaction in single implant-supported mandibular overdentures with conventional complete dentures: A randomised pilot study. *J Oral Biol Craniofac Res*. 2021;11(1):71-77. doi:10.1016/J.JOBCR.2020.11.014

Kovacic I, Persic S, Kranjcic J, Lesic N, Celebic A. Rehabilitation of an Extremely Resorbed Edentulous Mandible by Short and Narrow Dental Implants. *Case Rep Dent*. 2018;2018:7597851.

Prakash V, Gupta R. *Concise Prosthodontics*. 2nd ed. New Delhi: Elsevier India; 2017.

Mishra R, Shubham S, Yadav D. Role of Alloplast in Alveolar Ridge Preservation Role of Alloplast in Alveolar Ridge Preservation. *Journal of Dental and Medical Sciences*. 2019;17(August 2019):53-58. doi:10.9790/0853-1709055358

Akram M, Ahmed R, Shakir I, Ibrahim WAW, Hussain R. Extracting hydroxyapatite and its precursors from natural resources. *J Mater Sci*. 2014;49(4):1461-1475. doi:10.1007/s10853-013-7864-x

Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. https://www.hindawi.com/journals/ijbm/2015/469176/. Accessed February 12, 2020.

Sharifianjazi F, Esmaeilkhanian A, Moradi M, et al. Biocompatibility and mechanical properties of pigeon bone waste extracted natural nano-hydroxyapatite for bone tissue engineering. *Materials Science and Engineering: B*. 2021;264:114950. doi:10.1016/J.MSEB.2020.114950

Raya I, Mayasari E, Yahya A, Syahrul M, Latunra AI. Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. *Int J Biomater*. 2015;2015. doi:10.1155/2015/469176

Park OJ, Kim HJ, Woo KM, Baek JH, Ryoo HM. FGF2-activated ERK mitogen-activated protein kinase enhances Runx2 acetylation and stabilization. *Journal of Biological Chemistry*. 2010;285(6):3568-3574. doi:10.1074/jbc.M109.055053

Zhang Y, Yang S, Zhou W, Fu H, Qian L, Miron RJ. Addition of a Synthetically Fabricated Osteoinductive Biphasic Calcium Phosphate Bone Graft to BMP2 Improves New Bone Formation. *Clin Implant Dent Relat Res*. 2016;18(6):1238-1247. doi:10.1111/CID.12384

Zhang C, Han DH. Synergistic Effect of BMP2 with Bone Grafts Covered by FGF2 Soaked Collagen Membrane on New Bone Formation in Mongrel Dogs Department of Dentistry. 2016.

Kamadjaja MJK. MEKANISME REGENERASI DEFEK TULANG CALVARIA KRANIUM TIKUS DENGAN SCAFFOLD CHITOSAN – CARBONATE APATITE / HIDROXY APATITE - HUMAN AMNIOTIC MESENCHYMAL STEM CELL. 2016.

Kresnoadi U, Rahmania PN, Caesar HU, Djulaeha E, Agustono B, Ari MDA. The role of the combination of Moringa oleifera leaf extract and demineralized freeze-dried bovine bone xenograft (xenograft) as tooth extraction socket preservation materials on osteocalcin

and transforming growth factor-beta 1 expressions in alveolar bone of Cavia cobaya. *J Indian Prosthodont Soc*. 2019;19(2):120-125. doi:10.4103/JIPS.JIPS_251_18

Kamadjaja MJK. MEKANISME REGENERASI DEFEK TULANG CALVARIA KRANIUM TIKUS DENGAN SCAFFOLD CHITOSAN – CARBONATE APATITE / HIDROXY APATITE - HUMAN AMNIOTIC MESENCHYMAL STEM CELL. 2016.

Ramadhani T, Sari RP, W W. Efektivitas Kombinasi Pemberian Minyak Ikan Lemuru (Sardinella longiceps) dan Aplikasi Hidroksiapatit terhadap Ekspresi FGF-2 pada Proses Bone Healing. *Denta*. 2016;10(1):20. doi:10.30649/denta.v10i1.24

Malianawati Fauzia, Poernomo Agoes Wibisono EM. Ekspresi Bmp-2 Pada Pemberian Hydroxiapatite Xenograft Dan Hydroxiapatite Tooth-Derived Bone Graft Material Pada Soket Marmut. 2019;3(47):225-231.

Dahlan A, Hidayati HE, Hardianti SP. Collagen fiber increase due to hydroxyapatite from crab shells (*Portunus pelagicus*) application in post tooth extraction in Wistar rats. *Eurasian J Biosci*. 2020;14(2):3785-3789.

Vachiraroj N, Damrongsakkul S, Kanokpanont S. Gelatin/hydroxyapatite scaffolds: Studies on adhesion, growth and differentiation of Mesenchymal stem cells. *Adv Mat Res*. 2010;93-94:121-124. doi:10.4028/www.scientific.net/AMR.93-94.121

Barradas AMC, Yuan H, van Blitterswijk CA, Habibovic P. Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. *Eur Cell Mater*. 2011;21:407-429. doi:10.22203/eCM.v021a31

Baldini N, Cenni E, Ciapetti G, Granchi D, Savarino L. *Bone Repair and Regeneration*. Woodhead Publishing Limited; 2009. doi:10.1533/9781845696610.1.69

Huang Z, Nelson ER, Smith RL, Goodman SB. The sequential expression profiles of growth factors from osteroprogenitors to osteoblasts in vitro. *Tissue Eng*. 2007;13(9):2311-2320. doi:10.1089/ten.2006.0423

278. Akram M, Ahmed R, Shakir I, Ibrahim WAW, Hussain R. Extracting hydroxyapatite and its precursors from natural resources. *J Mater Sci*. 2014;49(4):1461-1475. doi:10.1007/s10853-013-7864-x

281. Wibisono Y, Dwijaksara NLB, Widayatno WB, et al. Synthesis and sinterability of hydroxyapatite from fishery by-products. *Journal of the Korean Ceramic Society*. 2018;55(6):570-575. doi:10.4191/kcers.2018.55.6.03

284. Kamadjaja MJK, Abraham JF, Laksono H. Biocompatibility of Portunus Pelagicus Hydroxyapatite Graft on Human Gingival Fibroblast Cell Culture. *Med Arch*. 2019;73(5):303-306. doi:10.5455/medarh.2019.73.303-306

287. Ji L, Qiao W, Zhang Y, et al. A gelatin composite scaffold strengthened by drug-loaded halloysite nanotubes. *Mater Sci Eng C Mater Biol Appl*. 2017;78:362-369. doi:10.1016/J.MSEC.2017.04.070

290. Zhang K, Fan Y, Dunne N, Li X. Effect of microporosity on scaffolds for bone tissue engineering. *Regen Biomater*. 2018;5(2):115-124. doi:10.1093/rb/rby001

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Fri, Sep 9, 2022 at 5:12 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Josef Kridanto Kamadjaja,
I hope you are doing well. I accuse receipt of the new version of the manuscript and the answers to the authors comments.
The manuscript is accepted for publication and scheduled for issue 11(5). A few days before publication we will send you print proofs for your review and approval. This will probably happen at the end of November, as we are running late with the publication schedule.
Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

New Manuscript to Submit

Editor J Oral Res <journal@joralres.com> Wed, Feb 1, 2023 at 6:31 AM
To: michael josef kridanto kamadjaja <michael-j-k-k@fkg.unair.ac.id>

Dear Dr. Michael Kridanto Kamadjaja,
I hope you are doing well.
We are implementing a section with pre-publication manuscript drafts, before typesetting, without final format, as a Word document. This was a decision made to make the papers available sooner, with the proper DOI already assigned, especially considering the **huge** delay we face getting the final PDF document ready. They can be read and cited using the year of publication and the DOI number.
The press version of the article will be removed and the final version will appear when it is laid out and once again reviewed by you as a PDF (print proof).
I want to make sure there is as little difference as possible between this proof and the final typefaced PDF. As such, I ask that you review the attached document, which has been proofread by the copy editor and is now in the queue for layout.
You can make corrections to the manuscript using Word's track-changes function please, or leave comments in the document.
I remain attentive to your comments or queries and I apologize for the long, long delay in the editorial process of your manuscript.
Best regards,

Celia A. Lima, PhD
Editor-in-Chief
Journal of Oral Research

[Quoted text hidden]

DIAGRAMAR Kridanto.docx
1404K

The Effect of Scaffold Hydroxyapatite derived from *Portunus pelagicus* Shell on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP -2) in the Extraction Sockets of *Cavia cobaya*

El efecto del *scaffold* de hidroxiapatita derivada de *Portunus pelagicus* sobre la expresión del factor de crecimiento de fibroblastos-2 (FGF-2) y las proteínas morfogenéticas óseas-2 (BMP-2) en los alvéolos dentarios de *Cavia cobaya*

Michael Josef Kridanto Kamadjaja[1], Sherman Salim[1], Gigih Gemiudeas[1]

[1]Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Michael-j-k-k@fkg.unair.ac.id, ORCID ID: 0000-0003-3226-7781

Sherman-s@fkg.unair.ac.id, ORCID ID: 0000-0002-6015-586X

si_gegeh@yahoo.com, ORCID ID: 0000-0003-4031-6248

Corresponding author: Michael Josef Kridanto Kamadjaja, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjen. Prof. Dr. Moestopo 47, Surabaya 60132, Indonesia. Phone/Fax: +6231 5030255. Email: michael-j-k-k@fkg.unair.ac.id

Conflict of interest: The authors declare no conflict of interest.

Ethics approval: Ethical approval for this study was granted by the Ethics Committee of the Faculty of Dentistry, Universitas Airlangga (528/HRECC.FODM/XII/2020)

Funding: none

Authors Contribution:

Michael Josef Kridanto Kamadjaja: conceived and designed the analysis, contributed data, performed the analysis, edited the manuscript.

Sherman Salim: conceived and designed the analysis, contributed data, performed the analysis, edited the manuscript.

Gigih Gemiudeas: conceived and designed the analysis, collected the data, contributed data, performed the analysis, wrote the manuscript.

Acknowledgments: none

Abstract

Objective: To determine the expression of Fibroblast Growth Factor (FGF)-2 and Bone Morphogenetic Protein (BMP)-2 after application of scaffold hydroxyapatite from Rajungan crab shell (*Portunus pelagicus*) in the tooth extraction socket of *Cavia cobaya*. **Methods:** This study used a post-test only control group design with 28 *Cavia cobaya* separated into two groups, control and treatment group. The left mandibular incisor was extracted and socket preservation was conducted. A hydroxyapatite graft derived from crab shells was mixed with gelatin and eventually turned into a scaffold, which was afterwards put into the extraction socket. After 7 days and 14 days, each group was terminated and examined using

immunohistochemical staining to observe the expression of FGF-2 and BMP-2. One-Way Anova and Tukey HSD were used to examine the research data. **Results:** FGF-2 and BMP-2 expressions were observed higher in the group that received hydroxyapatite scaffold at the post-extraction socket than those in the group that did not receive hydroxyapatite scaffold. **Conclusion:** The application of a hydroxyapatite scaffold from Rajungan crab shell (*Portunus pelagicus*) to the tooth extraction socket can increase FGF-2 and BMP-2 expression.

Keywords: *Portunus pelagicus*; Hydroxyapatites; Fibroblast growth factor 2; Bone morphogenetic proteins; Tissue scaffolds; Tooth socket

Resumen

Objetivo: determinar la expresión del factor de crecimiento de fibroblastos (FGF)-2 y la proteína morfogenética ósea (BMP)-2 después de la aplicación de hidroxiapatita de andamio de caparazón de cangrejo Rajungan (*Portunus pelagicus*) en el alvéolo de extracción dental de *Cavia cobaya*.

Métodos: Este estudio utilizó un diseño de grupo de control solo posterior a la prueba con 28 *Cavia cobaya* separados en dos grupos, grupo de control y grupo de tratamiento. Se extrajo el incisivo mandibular izquierdo y se realizó la preservación del alvéolo. Un injerto de hidroxiapatita derivado de caparazones de cangrejo se mezcló con gelatina y se convirtió en un andamio, que luego se colocó en el alvéolo de extracción. Después de 7 días y 14 días, se terminó cada grupo y

se examinó mediante tinción inmunohistoquímica para observar la expresión de FGF-2 y BMP-2. Se utilizaron One-Way Anova y Tukey HSD para examinar los datos de la investigación.

Resultados: las expresiones de FGF-2 y BMP-2 se observaron más altas en el grupo que recibió la estructura de hidroxiapatita en el alvéolo posterior a la extracción que en el grupo que no recibió la estructura de hidroxiapatita. **Conclusión**: La aplicación de un andamio de hidroxiapatita de caparazón de cangrejo Rajungan (*Portunus pelagicus*) al alvéolo de extracción dental puede aumentar la expresión de FGF-2 y BMP-2.

Palabras clave: *Portunus pelagicus;* Hidroxiapatitas; Factor 2 de crecimiento de fibroblastos; Proteínas morfogenéticas óseas; Andamios del tejido; Alveolo dental

Introduction

Patients may frequently experience unpleasant consequences resulting from tooth loss, after which the redundant alveolar bone atrophies due to a lack of physiological stimulation.[1] Alveolar ridge resorption reduces patient comfort with dentures while simultaneously increasing the number of complaints about denture stability which is influenced by the vertical height of the residual ridge.[2] Resorption ridges will provide less stability than residual ridges with sufficient vertical height.[3] Following tooth extraction, efforts should be made to maintain the vertical dimension of the residual ridge.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic material with frequent biomedical applications due to its close resemblance to the primary mineral ingredient of bones and teeth. Numerous investigations have established that hydroxyapatite is

biocompatible and possesses osteoconductive properties. It can prove an effective therapy in the prevention of post-extraction physiological bone loss, in both horizontal and vertical dimensions.[4] Hydroxyapatite synthesized from natural raw materials or natural waste can be more beneficial because it contains many valuable ions, also found in biological hydroxyapatite.[5] One of the sources of natural hydroxyapatite is the shell waste of Portunus Pelagicus, known as the Rajungan crab in Indonesia. A previous study revealed that the Calcium hydroxyapatite content of Rajungan crab shells can be as high as 66.62%.[6]

Hydroxyapatite scaffolds from waste bone from different species have been studied, such as in a study by Sharifianjazi *et al.* in 2021, which concluded that hydroxyapatite scaffolds from waste bone pigeon could be a promising and economically viable material for bone grafting.[7] According to Raya *et al.*, calcium hydroxyapatite from the Rajungan crab was effective in inhibiting demineralization of the tooth *in vitro*. Its effect as a scaffold after tooth extraction has not been studied.[8] In this study, administration of hydroxyapatite scaffold derived from Rajungan crab shell to the tooth extraction socket was expected to increase alveolar bone formation and reduce alveolar bone resorption, thereby enabling the bone replacement process in the extraction socket to be completed.

Fibroblast Growth Factor-2 (FGF-2) has been shown to activate the transcription factor Runx2 in osteoblasts by increasing the protein's stability and acetylation level via extracellular signal-regulated kinase mitogen-activated protein (ERK MAP kinase).[9] A subsequent study conducted by Ai *et al.* reported that Bone Morphogenetic Proteins-2 (BMP-2) functions as an osteoinductive growth factor since it recruits mesenchymal progenitor cells and induces their differentiation to bone-forming osteoblasts.[10] FGF-2 and BMP-2 have also been found to work synergistically in the formation of bone and fibrous tissue.[11]

This study aimed to determine the expression of FGF-2 and BMP-2 in the post-extraction socket following application of hydroxyapatite scaffold from Rajungan crab shell in order to investigate bone forming ability and cellular activity.

Material and Methods

This study was conducted between April and October 2020. The Federer formula was used to calculate the number of *Cavia cobaya* for this study, resulting in a final research population of 28. The subjects had to meet certain criteria: healthy, 3-3.5 months old, weighing 300-350 grams, being lesion-free, and having complete use of their five senses. The *Cavia cobaya* were provided with standard pellets and water *ad libitum* during a one-week acclimatization period prior to being randomly assigned to one of two groups; control and treatment. The latter group was given a hydroxyapatite scaffold from Rajungan crab shell. Both groups were observed after 7 and 14 days.

Soft tissue was removed from *Portunus pelagicus* crab shell waste using distilled water. The crab shells were then immersed in a chlorine solution at a ratio of 30 ml of chlorine to 5 liters of water. Immersion in 3% H_2O_2 was continued for another 24 hours before the shells were dried at room temperature. Shell calcination was carried out in a furnace at 1000°C. The initial heating temperature of approximately 50°C was increased at a rate of 5°C/min. On reaching 1000°C, it was kept constant for around two hours before being rapidly reduced to 100°C; SEM-EDX was used to characterize hydroxyapatite compounds. The powder sifting process was conducted using a sifting machine to obtain crab shell hydroxyapatite powder measuring less than 150 μm.[6]

The hydroxyapatite scaffold was produced by adding 0.5 grams of gelatin to distilled water and stirring it for one hour at 40°C. The scaffold was created by stirring 1.5 grams of hydroxyapatite powder into the gelatin solution for six hours. The gel was then centrifuged for ten minutes. Following extraction of excess water,

the residue was poured in a cylindrical, 5 x 2 mm (2 mm high, 5 mm diameter) sized mold and, having cooled, freeze dried at -800C for 24 hours.[12]

Ketamine 20mg/300mg body weight was injected intramuscularly into the *Cavia cobaya* subjects for anesthetic/sedation and analgesic purposes. The region surrounding the left mandibular incisive tooth was cleansed of debris prior to the tooth being carefully extracted in a specified direction with a sterile needle holder. The socket was irrigated with sterile distilled water after removal and hydroxyapatite scaffold applied in accordance with the previously defined group. Simple suturing was subsequently performed using DS 12 3 / 8c, 12 mm, 6/10 met, 0.7 (Braun Aesculap) polyamide monofilament.[13]

After 7 and 14 days, the subjects in each group were sacrificed in order to observe FGF2 and BMP2 expression. The mandible was cut and removed with samples of the tooth subsequently being fixed in 10% formalin buffer for 24 hours at room temperature and decalcified with ethylenediaminetetraacetic acid (EDTA). Dehydration by means of graded alcohol concentration was then completed, followed by clearing in xylol and embedding in paraffin. Finally, 4-micron thick paraffin blocks were cut and placed on the object glass.

Staining of Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP-2) was performed on the 7th and 14th-day observation groups using single staining technique. The preparations were immersed in Fibroblast Growth Factor-2 (FGF-2) and Bone Morphogenetic Proteins-2 (BMP-2) primary antibodies for 30 minutes, then washed three times with PBS. At that point, the preparation was immersed in a secondary antibody, namely; anti-mouse monoclonal antibody (Thermo Scientific, USA), for 30 minutes, washed twice with PBS, and immersed in a chromogen substrate for five minutes. After rinsing with distilled water, the incision was placed in a haematoxylin mayer for six minutes, washed with running water and, finally, mounted and placed under a cover glass to

enable it to be viewed with a light microscope [14]. The area under observation was the apical third of the socket.

Statistical analysis was performed using the Statistical Package for the Social Sciences Software (SPSS) edition 24.0 (SPSS™, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) was used to analyze the data followed by a Tukey HSD comparison multiple test at a confidence level of 95%.

Results

The statistical analysis revealed significant differences between the control and treatment groups (p <0.05) on both days 7 and 14 (Table 1). Compared to the other groups, group C7 had the lowest level of FGF-2. The control group, designated C7, was observed on day 7. On the other hand, Group HS14, which was treated with hydroxyapatite scaffolds made from crab shells and tested on the 14th day, had the highest level of FGF-2 (Figure 1). Brown staining was utilized to detect osteoblasts expressing FGF-2 (Figure 2).

Similarly, a statistically significant difference (p <0.05) was found between the observations in the BMP-2 treatment group and the control group (Table 1). The levels of BMP-2 in each group demonstrated a similar pattern to the levels of FGF-2. The BMP-2 levels in the C7 group were the lowest. In a related manner, after 14 days of treatment with a hydroxyapatite scaffold produced from crab shells, the HS14 group had the highest BMP-2 concentrations compared to the other groups in the experiment (Figure 1). Brown staining was utilized to detect osteoblasts expressing BMP-2 (Figure 3).

Table 1: Tukey HSD comparison multiple test result between group

FGF-2						**BMP-2**				
Group	C7	HS7	C14	HS14		Group	C7	HS7	C14	HS14

C7		0,004*	0,190	0,000*		C7		0,002*	0,081	0,000*
HS7	0,004*		0,284	0,001*		HS7	0,002*		0,394	0,005*
C14	0,190	0,284		0,000*		C14	0,081	0,394		0,000*
HS14	0,000*	0,001*	0,000*			HS14	0,000*	0,005*	0,000*	

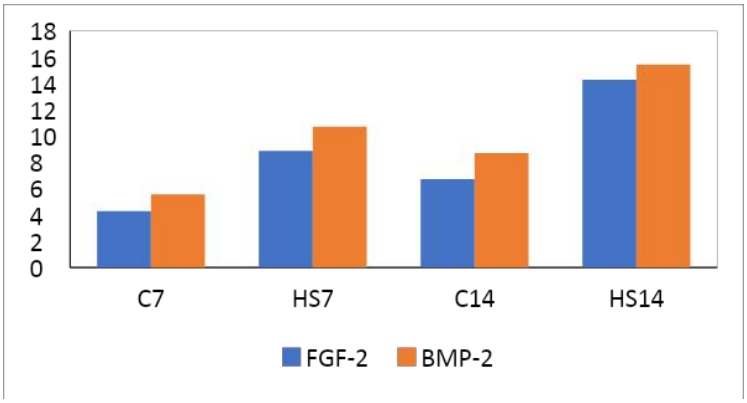


Figure 1: Mean number of osteoblast cells expressing FGF-2 and BMP-2 in each group

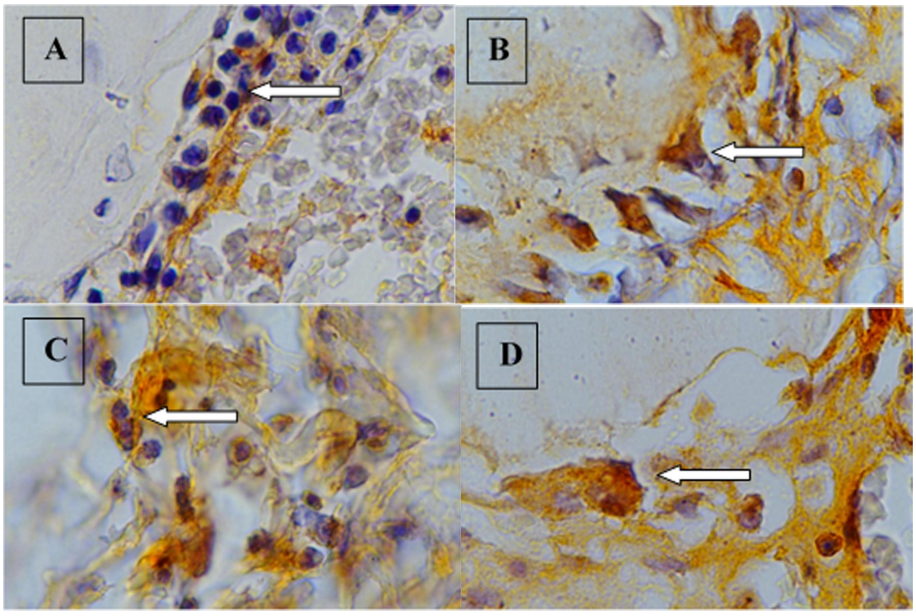


Figure 2: Expression of FGF-2 in osteoblasts located in the third apical after tooth extraction on day 7 (A, B) and day 14 (C, D) using a 1000x magnification light microscope is shown by the white arrows. A, C: The untreated control group. B, D: Extraction followed by administration of Hydroxyapatite scaffold.

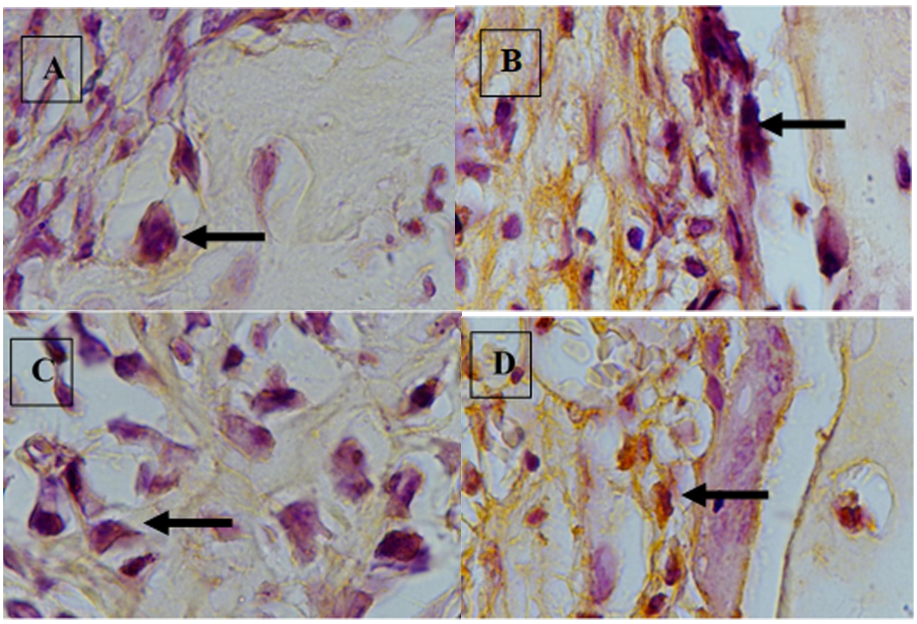


Figure 3: Expression of BMP-2 in osteoblasts located in the third apical after tooth extraction on day 7 (A, B) and day 14 (C, D) using a 1000x magnification light microscope is shown by the black arrows. A, C: The untreated control group. B, D: Extraction followed by administration of Hydroxyapatite scaffold.

Discussion

The findings showed that the group receiving the crab shell hydroxyapatite scaffold in the extraction socket expressed significantly more FGF-2 and BMP-2 than the group that was not provided with the crab shell hydroxyapatite scaffold. There was a significant difference in the expression of FGF-2 and BMP-2 between the two groups (p <0.05). These findings demonstrate that placing a hydroxyapatite scaffold derived from the *Portunus pelagicus* shell into a tooth extraction socket increases the expression of FGF-2 and BMP-2 in *Cavia cobaya* alveolar bone. This significant difference is evident in the 7th and 14th-day groups.

The findings of this study are similar to those of research conducted by Ramadhani et al., as there is a significantly higher FGF-2 expression in the Wistar rats group to whose femoral bone defect hydroxyapatite had been applied, compared to the control group which was not administered with hydroxyapatite.[15] In another study

conducted applying a hydroxyapatite xenograft to the extraction sockets of guinea pigs induced a higher BMP-2 expression than in the untreated control group.[16] Both studies indicate that the administration of hydroxyapatite has a positive effect on the number of FGF-2 and BMP-2 expressions found.

The increase in FGF-2 and BMP-2 expression identified in this study could have occurred due to the osteoinduction properties of the gelatin-hydroxyapatite scaffold of crab shells applied to the extraction sockets of the *Cavia cobaya*. Previous studies asserted that the osteoconductive nature of hydroxyapatite crab shell stimulated stem cells and osteoblasts to proliferate and differentiate in the formation of new bone or the process of bone regeneration.[17] The content of hydroxyapatite crystals in the scaffold, which is homogeneously distributed throughout it, can result in an increase in mechanical properties and cellular activity on the surface of the scaffold.[18] Moreover, calcium phosphates such as HA are well known for their affinity for binding to various proteins, including BMPs, and their increase on a particular surface area may be required to accumulate sufficient amounts of BMPs to induce osteoinduction.[19]

During the bone formation phases and activation of osteogenesis, several growth factors are present in the early and intermediate stages, with some differences in each peak expression. FGF-2 and BMP-2 will reach their peak on day 14, at which point the amount will decrease.[20] The results of this study support this finding since they indicated that the highest FGF-2 and BMP-2 expression in both the treatment and control groups was found on day 14. This finding is also in line with the results of the research conducted by Huang *et al.*, which revealed that the expression of mRNA expressed coding for FGF-2 and BMP-2 was at its highest point on day 14, during the differentiation phase.[21]

Hydroxyapatite can be synthesized from various resources such as eggshells, bones of various animals, shells, and plants. Research conducted to date shows that this natural resource can be a good source of biologically and thermally stable hydroxyapatite.[22] On this occasion, the researchers chose to use hydroxyapatite from natural sources, namely; from processed crab (*Portunus pelagicus*) shell. This action was intended to reduce crab shell waste that often produces an offensive odor and pollutes seawater. However, in reality, the amount of hydroxyapatite contained in crab shells is relatively high. Crab shell waste (*Portunus pelagicus*) has been used as raw material in the synthesis of calcium hydroxyapatite [Ca10(PO4)6(OH)2] because of its high calcium content of 66.62%.[6] Other research conducted by Wibisono et al. found that crab shells contain more calcium (93.78%) than fish scales (82.31%) making them suitable as raw material in the synthesis of hydroxyapatite.[23] Also, crab shells demonstrate high compatibility. Research conducted by Kamadjaja *et al.* revealed grafts made from crab shells have strong biocompatibility in cell culture and have the optimum biocompatibility at a concentration of 25 ppm.[24]

In this study, gelatin was selected as the scaffold material because gelatin-based scaffold demonstrates excellent biocompatibility and biodegradability, while possessing a porous structure.[25] Microporosity is also an essential characteristic of a scaffold in a bone graft. It will increase the specific surface area, providing more protein adsorption sites, where cells have more numerous opportunities to interact with osteogenic-related proteins, thereby facilitating cellular osteogenic function to form new bone tissue.[26]

Finally, further development of this research in the future remains a strong possibility because its findings show that natural ingredients, especially hydroxyapatite derived from crab shells, have a positive effect on the increased expression of growth factors, especially FGF- 2 and BMP-2 which play an essential role in helping post-extraction bone regeneration. Furthermore, to identify the

additional benefits of this material, a long-term study must be conducted until the bone regeneration process is complete in order to enable comparisons with other bone grafting materials to be made.

Conclusion

The application of hydroxyapatite scaffold derived from Rajungan crab shell (*Portunus pelagicus*) in the tooth extraction socket affects the increased expression of FGF-2 and BMP-2 in the alveolar bone of *Cavia cobaya*. Furthermore, in order to identify the additional benefits of this material, a long-term study should be conducted until the bone regeneration process is complete to allow comparisons with other bone grafting materials to be undertaken.

References

1. KM S, Koli DK, Jain V, Pruthi G, Nanda A. Comparison of ridge resorption and patient satisfaction in single implant-supported mandibular overdentures with conventional complete dentures: A randomised pilot study. *J Oral Biol Craniofac Res*. 2021;11(1):71-77. doi:10.1016/J.JOBCR.2020.11.014

2. Kovacic I, Persic S, Kranjcic J, Lesic N, Celebic A. Rehabilitation of an Extremely Resorbed Edentulous Mandible by Short and Narrow Dental Implants. *Case Rep Dent*. 2018;2018:7597851.

3. Prakash V, Gupta R. *Concise Prosthodontics*. 2nd ed. New Delhi: Elsevier India; 2017.

4. Mishra R, Shubham S, Yadav D. Role of Alloplast in Alveolar Ridge Preservation Role of Alloplast in Alveolar Ridge Preservation. *Journal of Dental and Medical Sciences*. 2019;17(August 2019):53-58. doi:10.9790/0853-1709055358

5. Akram M, Ahmed R, Shakir I, Ibrahim WAW, Hussain R. Extracting hydroxyapatite and its precursors from natural resources. *J Mater Sci*. 2014;49(4):1461-1475. doi:10.1007/s10853-013-7864-x

6. Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. https://www.hindawi.com/journals/ijbm/2015/469176/. Accessed February 12, 2020.

7. Sharifianjazi F, Esmaeilkhanian A, Moradi M, et al. Biocompatibility and mechanical properties of pigeon bone waste extracted natural nano-hydroxyapatite for bone tissue engineering. *Materials Science and Engineering: B*. 2021;264:114950. doi:10.1016/J.MSEB.2020.114950

8. Raya I, Mayasari E, Yahya A, Syahrul M, Latunra AI. Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. *Int J Biomater*. 2015;2015. doi:10.1155/2015/469176

9. Park OJ, Kim HJ, Woo KM, Baek JH, Ryoo HM. FGF2-activated ERK mitogen-activated protein kinase enhances Runx2 acetylation and stabilization. *Journal of Biological Chemistry*. 2010;285(6):3568-3574. doi:10.1074/jbc.M109.055053

10. Zhang Y, Yang S, Zhou W, Fu H, Qian L, Miron RJ. Addition of a Synthetically Fabricated Osteoinductive Biphasic Calcium Phosphate Bone Graft to BMP2 Improves New Bone Formation. *Clin Implant Dent Relat Res*. 2016;18(6):1238-1247. doi:10.1111/CID.12384

11. Zhang C, Han DH. Synergistic Effect of BMP2 with Bone Grafts Covered by FGF2 Soaked Collagen Membrane on New Bone Formation in Mongrel Dogs Department of Dentistry. 2016.

12. Kamadjaja MJK. MEKANISME REGENERASI DEFEK TULANG CALVARIA KRANIUM TIKUS DENGAN SCAFFOLD CHITOSAN – CARBONATE APATITE / HIDROXY APATITE - HUMAN AMNIOTIC MESENCHYMAL STEM CELL. 2016.

13. Kresnoadi U, Rahmania PN, Caesar HU, Djulaeha E, Agustono B, Ari MDA. The role of the combination of Moringa oleifera leaf extract and demineralized freeze-dried bovine bone xenograft (xenograft) as tooth extraction socket preservation materials on osteocalcin

and transforming growth factor-beta 1 expressions in alveolar bone of Cavia cobaya. *J Indian Prosthodont Soc*. 2019;19(2):120-125. doi:10.4103/JIPS.JIPS_251_18

14. Kamadjaja MJK. MEKANISME REGENERASI DEFEK TULANG CALVARIA KRANIUM TIKUS DENGAN SCAFFOLD CHITOSAN – CARBONATE APATITE / HIDROXY APATITE - HUMAN AMNIOTIC MESENCHYMAL STEM CELL. 2016.

15. Ramadhani T, Sari RP, W W. Efektivitas Kombinasi Pemberian Minyak Ikan Lemuru (Sardinella longiceps) dan Aplikasi Hidroksiapatit terhadap Ekspresi FGF-2 pada Proses Bone Healing. *Denta*. 2016;10(1):20. doi:10.30649/denta.v10i1.24

16. Malianawati Fauzia, Poernomo Agoes Wibisono EM. Ekspresi Bmp-2 Pada Pemberian Hydroxiapatite Xenograft Dan Hydroxiapatite Tooth-Derived Bone Graft Material Pada Soket Marmut. 2019;3(47):225-231.

17. Dahlan A, Hidayati HE, Hardianti SP. Collagen fiber increase due to hydroxyapatite from crab shells (*Portunus pelagicus*) application in post tooth extraction in Wistar rats. *Eurasian J Biosci*. 2020;14(2):3785-3789.

18. Vachiraroj N, Damrongsakkul S, Kanokpanont S. Gelatin/hydroxyapatite scaffolds: Studies on adhesion, growth and differentiation of Mesenchymal stem cells. *Adv Mat Res*. 2010;93-94:121-124. doi:10.4028/www.scientific.net/AMR.93-94.121

19. Barradas AMC, Yuan H, van Blitterswijk CA, Habibovic P. Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. *Eur Cell Mater*. 2011;21:407-429. doi:10.22203/eCM.v021a31

20. Baldini N, Cenni E, Ciapetti G, Granchi D, Savarino L. *Bone Repair and Regeneration*. Woodhead Publishing Limited; 2009. doi:10.1533/9781845696610.1.69

21. Huang Z, Nelson ER, Smith RL, Goodman SB. The sequential expression profiles of growth factors from osteroprogenitors to osteoblasts in vitro. *Tissue Eng*. 2007;13(9):2311-2320. doi:10.1089/ten.2006.0423

22. Akram M, Ahmed R, Shakir I, Ibrahim WAW, Hussain R. Extracting hydroxyapatite and its precursors from natural resources. *J Mater Sci*. 2014;49(4):1461-1475. doi:10.1007/s10853-013-7864-x

23. Wibisono Y, Dwijaksara NLB, Widayatno WB, et al. Synthesis and sinterability of hydroxyapatite from fishery by-products. *Journal of the Korean Ceramic Society*. 2018;55(6):570-575. doi:10.4191/kcers.2018.55.6.03

24. Kamadjaja MJK, Abraham JF, Laksono H. Biocompatibility of Portunus Pelagicus Hydroxyapatite Graft on Human Gingival Fibroblast Cell Culture. *Med Arch*. 2019;73(5):303-306. doi:10.5455/medarh.2019.73.303-306

25. Ji L, Qiao W, Zhang Y, et al. A gelatin composite scaffold strengthened by drug-loaded halloysite nanotubes. *Mater Sci Eng C Mater Biol Appl*. 2017;78:362-369. doi:10.1016/J.MSEC.2017.04.070

26. Zhang K, Fan Y, Dunne N, Li X. Effect of microporosity on scaffolds for bone tissue engineering. *Regen Biomater*. 2018;5(2):115-124. doi:10.1093/rb/rby001