Therapeutic Delivery

Home

Author

Author Dashboard

7 Manuscripts with Decisions

1 Manuscripts I Have Co-Authored

Start New Submission

Legacy Instructions

5 Most Recent E-mails

English Language Editing Service

Manuscripts with Decisions

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	ADM: Brooks, Campbell • Immediate Accept (24-Aug-2021) *Archiving completed on 25-Aug-2022* view decision letter Contact Journal	TDE-2021-0044.R2	Ultradeformable vesicle: Concepts and applications relating to the delivery of skin cosmetics *Files Archived*	20-Aug-2021	24-Aug-2021
a revision has been submitted (TDE-2021-0044.R2)	ADM: Brooks, Campbell • Immediate Major Revision (16-Aug-2021)	TDE-2021-0044.R1	Ultradeformable vesicle: Concepts and applications relating to the delivery of skin cosmetics *Files Archived*	12-Aug-2021	16-Aug-2021

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	• a revision has been submitted *Archiving completed on 25-Aug-2022* view decision letter Contact Journal				
a revision has been submitted (TDE-2021-0044.R1)	ADM: Brooks, Campbell • Major Revision (19-Jul-2021) • a revision has been submitted *Archiving completed on 25-Aug-2022* view decision letter Contact Journal	TDE-2021-0044	INSIGHTS AND FUTURE PROSPECTS OF TRANSFERSOMES FOR COSMECEUTICS *Files Archived*	14-Jun-2021	19-Jul-2021
	ADM: Finnie, Rhiannon ADM: Lovesey, Kate • Reject (21-May-2020) *Archiving completed on 21-May-2021* view decision letter Contact Journal	TDE-2020-0041	Characterization and tumor distribution of cisplatin loaded in surfactant modified-hybrid nanoparticles *Files Archived*	16-Apr-2020	21-May-2020
	ADM: Finnie, Rhiannon • Accept (05-Jul-2019) *Archiving completed on 06-Jul-2020* view decision letter Contact Journal	TDE-2019-0015.R2	Enhancing skin penetration of epigallocatechin gallate by modifying partition-coefficient using reverse micelle method *Files Archived*	04-Jul-2019	05-Jul-2019
a revision has been submitted (TDE-2019-0015.R2)	ADM: Finnie, Rhiannon • Minor Revision (01-Jul-2019)	TDE-2019-0015.R1	Enhancing skin penetration of epigallocatechin gallate by modifying partition-coefficient using reverse micelle method	25-Jun-2019	01-Jul-2019 1

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
	• a revision has been submitted *Archiving completed on 06-Jul-2020* view decision letter Contact Journal		*Files Archived*		
a revision has been submitted (TDE-2019-0015.R1)	ADM: Finnie, Rhiannon • Major Revision (06-Jun-2019) • a revision has been submitted *Archiving completed on 06-Jul-2020* view decision letter Contact Journal	TDE-2019-0015	Enhancing skin penetration of epigallocatechin gallate by modifying partition-coefficient using reverse micelle method *Files Archived*	12-Mar-2019	06-Jun-2019

andang miatmoko <andang-m@ff.unair.ac.id>

Therapeutic Delivery - Decision on Manuscript ID TDE-2021-0044

5 messages

Campbell Brooks <onbehalfof@manuscriptcentral.com> Mon, Jul 19, 2021 at 6:25 PM
Reply-To: c.brooks@future-science.com
To: andang-m@ff.unair.ac.id, andangmiatmoko@gmail.com

19-Jul-2021

Dear Dr. Miatmoko,

Manuscript ID TDE-2021-0044 entitled "INSIGHTS AND FUTURE PROSPECTS OF TRANSFERSOMES FOR COSMECEUTICS" which you submitted to Therapeutic Delivery, has been reviewed.

The reviewers have requested revisions to your manuscript. Therefore, I invite you to respond to the reviewers' comments and revise your manuscript. Instructions on how to do this can be found at the bottom of this email. Please ensure that responses to all reviewer comments are included in the text of the manuscript as well as the Author Response document.

Because we are trying to facilitate timely publication of manuscripts submitted to Therapeutic Delivery, your revised manuscript should be submitted by 29th July 2021. If it is not possible for you to submit your revision by this date, then please let us know so that we can discuss rescheduling.

Once again, thank you for submitting your manuscript to Therapeutic Delivery and I look forward to receiving your revision.

Sincerely,
Mr. Campbell Brooks
Commissioning Editor, Therapeutic Delivery
c.brooks@future-science.com

Instructions for revision
To revise your manuscript, log into https://mc04.manuscriptcentral.com/fs-tde and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.

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https://mc04.manuscriptcentral.com/fs-tde?URL_MASK=a40929cdbece4e75bb3d6531569d8628

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, please also submit your responses to the reviewers' comments. Please provide an "Author response" underneath each comment, giving as much detail as possible, including line numbers, in order to expedite the process.

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Reviewer Comments to Author:

Reviewer: 1

Comments to the Author
The Review “INSIGHTS AND FUTURE PROSPECTS OF TRANSFERSOMES FOR COSMECEUTICS” is interesting as focuses on the use of transfersomes specifically tailored for cosmeceutical applications. In particular the paper describe all the events that are connected with skin ageing and the possible treatments.

1. The paper needs a careful check of the English language as different mistakes can be detected throughout the manuscript.
Editor comment: Please have the manuscript proof-read by a native English speaker to identify and correct any language or grammatical mistakes.
Author response and action taken:
Page/line numbers:

2. I also suggest to add especially in the introduction section the other innovative vesicles, formulated aiming at protecting the skin from external injuries and damages. I refer to glycersomes, hyalurosomes and hybrid vesicles: see papers published from 2013 to 2019 by Manca et al.
Editor comment: Reference recommendations may be included or not at the authors' discretion.
Author response and action taken:
Page/line numbers:

3. Moreover, another concern about this Review is connected to the cosmeceutical application also reported in the title. I know that cosmeceutical is an hybrid word obtained by the combination of cosmetics and pharmaceutics… but the authors should check the low that control the commercialization of these products… it is the same of cosmetics, so that even this products cannot be described as pharmaceutical products. I mean that authors should be careful in describing the use of synthetic drug especially proteins or hormones as they cannot be considered cosmeceutical products. Please check in the manuscript and modify accordingly.
Editor comment: Please ensure the distinction is made between pharmaceutical uses of these technologies and their cosmetic uses. If delivered for cosmetic purposes, an 'API' is not strictly a 'pharmaceutical' ingredient and this term must be used carefully, as the reviewer says, to ensure the activity of proposed active ingredients is strongly evidenced. As cosmetic products are not subjected to as rigorous study as pharmaceutical products, a distinction must be made between them to ensure no undue endorsement is given to cosmetic ingredients whose activity is supported by lower quality evidence.
Author response and action taken:
Page/line numbers:

Given that, I suggest minor revision before the acceptance of this paper for publication in this Journal.

Reviewer: 2

Comments to the Author
This article intends to review the Transfersome concept and applications in drug delivery and cosmetics. However, the article practically revises therapeutic application of such carriers. Proliposomes were also considered as alternative to transfersomes but the differences between the 2 systems were not properly explained. Many important references are missing. Studies regarding the cosmeceutical application of Transfersomes were not included. The paper needs major revisions before being published.

1. Title should be changed as it does not reflect the results presented.
Author response and action taken:
Page/line numbers:

2. Transfersomes is a plural form, please, use the verbs in accordance.
Author response and action taken:
Page/line numbers:

3. P1, ln 34-35: “cosmetics; for example”, use a comma instead of semi-colon
Author response and action taken:
Page/line numbers:

4. Stratum corneum: italicize the first time it appears and abbreviate thereafter as SC
Author response and action taken:
Page/line numbers:

5. P2, ln 52-53: “ingredients which can be natural products, vitamins, and proteins”. This phrase is extremely reductive of what the ingredients of anti-ageing cosmetics are. Please rephrase it.
Author response and action taken:
Page/line numbers:

6. P3, ln 27-28: “Thus, liposome can dissolve or carry hydrophobic, hydrophilic…”. Please, be rigorous on technological terminology. Liposomes do not “dissolve”. They are versatile systems but drug-carrier interaction is not

as solute-solvent interaction.
Author response and action taken:
Page/line numbers:

7. P3, ln 44-46: “These vesicles are 105 times more deformable than conventional liposomes.” If so, please insert the reference.
Author response and action taken:
Page/line numbers:

8. P3, ln 46: “However, because the stability of transfersomes remains low…”. This is not true. These systems are quite stable. Please revisit the work of Cevc and co-workers, Simões and Ascenso. Transfersomes are controllably destabilized systems to be deformable. The development of protransfersomes may be justified by other reasons but not by stability issues.
Author response and action taken:
Page/line numbers:

9. P4, ln 4-7: “…sedimentation, aggregation, fusion, leakage of trapped drugs, or hydrolysis of encapsulated drugs.” The corresponding references are missing.
Author response and action taken:
Page/line numbers:

10. Please, uniformize Protransfersomes or pro-transfersomes. Several typos are present: transferomal, Transfersomess, transfersom, sometimes capitalized some not, lack of spaces between words and commas, more than once “namely;”
Author response and action taken:
Page/line numbers:

11. Figure 3 – What articles fundament the transcellular route of intact vesicles? If it is an assisted transport, the figure should resemble that.
Author response and action taken:
Page/line numbers:

12. “Transfersomes acts as encapsulating agents for drug molecules” section: there is a big confusion between therapeutic use of transfersomes and cosmetic use of transfersomes in the initial paragraphs of this section. The introductory part of this section needs to be improved.
Author response and action taken:
Page/line numbers:

13. P8, ln 9-14: “The encapsulation of active cosmeceutical ingredients with large molecular weight can be based on several studies of transfersomes formulation for delivery of proteins, such as hormones, stem cells, and ribonucleic acids (RNAs).” Several studies: where are they? Several references are missing on the proper site where they should be cited.
Author response and action taken:
Page/line numbers:

14. P21, ln 25: “The lipid in the stratum corneum is negatively charged.” What lipid? Lack of rigor in scientific terminology should be avoided.
Author response and action taken:
Page/line numbers:

15. Why protransfersomes have no in vitro evaluation?
Author response and action taken:
Page/line numbers:

16. Results present only therapeutic applications and not cosmetic applications
Author response and action taken:
Page/line numbers:

17. P26, ln. 43-44: “…of edge activators, known as surfactants…”, isn´t it the other way around?
Author response and action taken:
Page/line numbers:

18. p27, ln. 16-18 “It is in consideration with cosmeceutical active agents can be locally or systemically delivered to target sites. Nonsense phrase. Please rephrase it.
Author response and action taken:
Page/line numbers:

Reviewer: 3

Comments to the Author
1. The inclusion & exclusion criteria in consort diagram needs improvement & clarity
Author response and action taken:
Page/line numbers:

2. During inclusion of papers, kindly ensure you are including more cosmetic related papers rather than therapeutic papers.
Author response and action taken:
Page/line numbers:

3. Discussion on mechanism by which transferosomes can enhance anti-aging efficacy is missing and which type of anti-aging molecule can be used in transferosomes also missing
Author response and action taken:
Page/line numbers:

4. Evaluation also kindly try to include more cosmetic/ topical related papers
Author response and action taken:
Page/line numbers:

5. The entire paper needed to be re-written with improved clarity
Author response and action taken:
Page/line numbers:

Comments from the Editor:

1. As identified by the reviewers, the structure and focus of this review could be greatly improved. Currently, the authors give some background on transfersomes, liposomes, and protransfersomes, and describe the mechanisms for skin penetration of these nanocarriers, then describe research pertaining to transfersomes. Much of the research described concerns therapeutic applications rather than cosmetic and therefore confuses the purpose of the work. Therefore, the editors suggest this article is reclassified as a Perspective rather than a Review (please make this change in ScholarOne when uploading the revised manuscript). The authors can then review all instances of transfersomes being used in the literature (therapeutic or otherwise), and then discuss what this research could mean in each case for the future of cosmetic transfersome delivery. While we welcome the speculative nature of this work, the speculation must be supported by thorough discussion of available evidence.
Author response and action taken:
Page/line numbers:

2. Few, if any, specific examples of cosmetic ingredients and their mechanisms of action are given (e.g. in the 'Result' section where the authors give an overview of ingredients). For example, insulin is presented as an example of a protein which can be delivered by transfersomes, however the cosmetic uses of insulin are not described, nor are any current technologies for the delivery of insulin for cosmetic purposes given. The editors recommend giving examples of specific cosmetic ingredients (and brief mechanisms of action) which may be delivered using transfersomes, and explain why tranfersome delivery would be an improvement compared to existing technologies.
Author response and action taken:
Page/line numbers:

3. In discussing specific research, please be clear in identifying oral versus topical delivery and systemic versus targeted delivery. The route of administration and the target of the active ingredient should be clarified and related back to the topic of cosmetics.
Author response and action taken:
Page/line numbers:

4. The relevance of the encapsulated ingredients to cosmetics should be discussed. For example, are there any cosmetic applications of Levonogestrel or is this simply presented as a hormone which has proved deliverable by transfersomes and therefore other, cosmetically active hormones could also be delivered this way. Equally with cisplatin, what relevance does this have to cosmetics? Please ensure that each ingredient mentioned is evaluated with respect to its relevance to the delivery of cosmetic ingredients using transfersomes.
Author response and action taken:
Page/line numbers:

5. In the introduction, please ensure that the aims of the article are clearly stated and that the structure of the discussion is outlined so that the readers are fully informed on the purpose of the work. Additionally, please ensure the novelty and significance of the work is justified with respect to existing literature.
Author response and action taken:
Page/line numbers:

6. Please review the manuscript to ensure that all statements are appropriately referenced. References that pertain to a particular sentence should be included at the end of that sentence.
Author response and action taken:

Page/line numbers:

7. In the Publishing Agreement form, the authors state that they are government employees. For the purposes of this form, employees of a university which receives government funding are not classed as government employees. Therefore, please recomplete the Publishing Agreement form with Section A filled in, rather than Section B.
Author response and action taken:

8. Please have the entire manuscript proof read by a native English speaker prior to resubmission to ensure all spelling and grammatical errors are corrected. Please note that Future Science partners with Enago to provide pre-submission editing services for our authors. Editing services include language check, copyediting and substantive editing. For more information, please visit the website here: www.enago.com/futurescience/
Author response:

9. Please make sure that all tables and boxes are clearly titled and cited in the text.
Author response:

10. Please include reference annotations: up to 8 references should be chosen from your bibliography and highlighted as being '*' – of interest, or "**" – of considerable interest, with a brief sentence explaining why in each case.
Author response:

11. Therapeutic Delivery offers a selection of post-acceptance services for authors; please indicate when you re-submit if you would be interested in any of the following:

a. Open access publication: Therapeutic Delivery offers an open access option, whereby for a fee, articles can be made freely available for all to read. Pricing varies by article type; for peer-reviewed content (i.e., Original Research or Review articles), the fee is $2800; for content not reviewed externally (i.e., Editorials or Commentaries), the fee is $980. For more information, visit our website here: https://www.future-science.com/authorguide/openaccess. If you are interested in taking this option, please let me know. Please note, if you would like to opt for Open Access, the OA form (available here: https://www.future-science.com/authorguide/openaccess) will be required in place of the Copyright Assignment form. If you have any concerns about your institution being able to pay the Open Access fee due to the current global situation, please do let me know and we can discuss appropriate arrangements.

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d. Video Journal of Biomedicine: The Video Journal of Biomedicine (https://www.biomedicine.video/) is a new platform from the Future Science Group publishing videos that can be freely accessed and watched. The aim is to provide a platform for authors to present their latest research in a short, high-quality video. Animated videos provide a ~90 second overview of a research paper where the article is converted into a bespoke animation with an engaging voiceover script. Publication Perspective videos are professionally produced videos, ~8 minutes in length that provide an opportunity for authors to discuss their publication in full. Videos are produced in chapter-style format allowing the viewer to navigate between segments as required. By watching the video, viewers gain a comprehensive understanding of the subject. If you are interested developing in a video alongside your article, please email the Publisher of the Video Journal of Biomedicine, Joanne Walker (j.walker@biomedicine.video).

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2 attachments

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Publishing%20Agreement_NP.pdf
576K

Andang MIATMOKO <andang-m@ff.unair.ac.id> Thu, Jul 15, 2021 at 1:08 AM
To: c.brooks@future-science.com

Dear Editor,
Many thanks for your email. However, due to many revisions that we have to do, could you please give us about 2-3 weeks more to give our best effort in responding to reviewers' comments?,
many thanks for your help
[Quoted text hidden]
--

Salam,

Andang Miatmoko, PhD., Apt.
Department of Pharmaceutical Sciences
Faculty of Pharmacy, Airlangga University
Nanizar Zaman Joenoes Building
Campus C Airlangga University, Mulyorejo, 60115
Surabaya

Campbell Brooks <c.brooks@future-science.com> Mon, Jul 19, 2021 at 9:46 PM
To: Andang MIATMOKO <andang-m@ff.unair.ac.id>

Dear Dr. Miatmoko,

Thank you for your email. Would a new submission deadline of 9th August 2021 be suitable?

Sincerely,
Campbell

Campbell Brooks
Commissioning Editor
(he/him)
Newlands Press | Future Medicine
part of Future Science Group
Unitec House, 2 Albert Place, London, N3 1QB, UK
+44 (0)20 3770 8971 | www.future-science-group.com

From: Andang MIATMOKO <andang-m@ff.unair.ac.id>
Sent: 14 July 2021 19:08
To: Campbell Brooks <c.brooks@future-science.com>
Subject: Re: Therapeutic Delivery - Decision on Manuscript ID TDE-2021-0044

[Quoted text hidden]

Andang MIATMOKO <andang-m@ff.unair.ac.id> Tue, Jul 20, 2021 at 10:47 AM
To: Campbell Brooks <c.brooks@future-science.com>

Dear Editor,
That would be great, thank you.
[Quoted text hidden]

Campbell Brooks <c.brooks@future-science.com> Tue, Jul 20, 2021 at 3:49 PM
To: Andang MIATMOKO <andang-m@ff.unair.ac.id>

Dear Dr. Miatmoko,

Great, I have updated the submission date now. Please let me know if I can be of any further assistance.

I look forward to receiving your revision soon.

Sincerely,
Campbell

Campbell Brooks
Commissioning Editor
(he/him)
Newlands Press | Future Medicine
part of Future Science Group
Unitec House, 2 Albert Place, London, N3 1QB, UK
+44 (0)20 3770 8971 | www.future-science-group.com

From: Andang MIATMOKO <andang-m@ff.unair.ac.id>
Sent: 20 July 2021 04:47
[Quoted text hidden]

[Quoted text hidden]

andang miatmoko <andang-m@ff.unair.ac.id>

Therapeutic Delivery - Decision on Manuscript ID TDE-2021-0044.R1

1 message

Campbell Brooks <onbehalfof@manuscriptcentral.com> Mon, Aug 16, 2021 at 10:15 PM
Reply-To: c.brooks@future-science.com
To: andang-m@ff.unair.ac.id, andangmiatmoko@gmail.com

16-Aug-2021

Dear Dr. Miatmoko,

Manuscript ID TDE-2021-0044.R1 entitled "Ultradeformable vesicle: Concepts and applications relating to the delivery of skin cosmetics", which you submitted to Therapeutic Delivery, has been evaluated.

Thank you for your revisions and your responses to reviewer and editor comments. The aims and structure of the article are now much clearer; however, I would like to recommend some further revisions to your manuscript. Therefore, I invite you to respond to my comments listed below and revise your manuscript.

1. The authors make clear that the purpose of this article is to illustrate the potential use of transfersomes and protransfersomes in cosmetic formulations using published research where these technologies are used to encapsulate non-cosmetic active ingredients. The editors think the article could be further improved by augmenting the discussion of these non-cosmetic active ingredients with comments on how these examples may be significant for future research into cosmetic transfersome technologies; for example, if the drugs mentioned have cosmetic applications or which have molecular structures or properties similar to other molecules which do have cosmetic applications. Please see the specific comments below:
a. Section 'Transfersomes act as encapsulating carriers for various active ingredients': the authors say 'protein is known as an active ingredient with a large molecular weight that has been used in skin care'. Please include some examples of proteins with cosmetic applications (and references) to contextualise why the discussion of encapsulation of therapeutic proteins is relevant.
Author response and action taken:
Page/line numbers:

b. Section 'Transfersomes act as encapsulating carriers for various active ingredients': the authors discuss 'the use of Small interfering RNA (siRNA) and microRNA'. Please include some examples of RNAs with cosmetic applications (and references) to contextualise why the discussion of encapsulation of therapeutic RNAs is relevant.
Author response and action taken:
Page/line numbers:

c. Section 'Active substances with low solubility and high permeability': the authors discuss Resveratrol, Quercetin, Glimepiride, Diclofenac, Ketoprofen, Rifampicin, Nifedipine, Raloxifene, and Retinyl Palmitate. For each of these drugs, please provide discussion of whether they have any cosmetic applications, if similar drugs/classes of molecule may have cosmetic applications, or how otherwise the encapsulation of these drugs may inform future cosmetic transfersome research.
Author response and action taken:
Page/line numbers:

d. Section 'Active substances with low solubility and high permeability': the authors discuss 5-Fluorouracil and methotrexate. For each of these drugs, please provide discussion of whether they have any cosmetic applications, if similar drugs/classes of molecule may have cosmetic applications, or how otherwise the encapsulation of these drugs may inform future cosmetic transfersome research.
Author response and action taken:
Page/line numbers:

e. Section 'Active substances with low solubility and low permeability': the authors discuss Curcumin, Psoralen, Cisplatin, Paclitaxel, and Ketorolac. For each of these drugs, please provide discussion of whether they have any cosmetic applications, if similar drugs/classes of molecule may have cosmetic applications, or how otherwise the encapsulation of these drugs may inform future cosmetic transfersome research.
Author response and action taken:
Page/line numbers:

2. Thank you for providing a certificate of English proofreading. Was this service paid for, and if so, who provided the funds to pay for it? Please include this information in the title page document and fill in Section 4b of the Author Disclosure form to disclose receipt of funded writing assistance.

Author response:

3. One paragraph of text in the revised document was found to be similar to previously published text. I have attached an image of the similarity report with the similar text highlighted in brown; please rephrase this in the authors' own words to avoid any possible plagiarism.
Author response:

To revise your manuscript, log into https://mc04.manuscriptcentral.com/fs-tde and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.

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You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, please also submit your responses to the reviewers' comments. Please provide an "Author response" underneath each comment, giving as much detail as possible, including line numbers, in order to expedite the process.

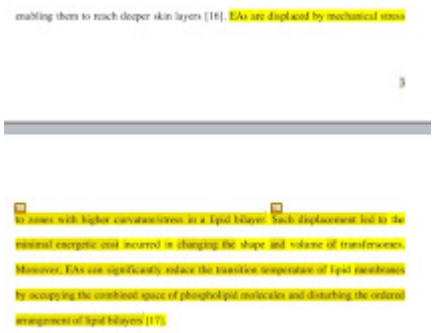
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to Therapeutic Delivery, your revised manuscript should be submitted by 24 August 2021. If it is not possible for you to submit your revision by this date, please let me know and we can discuss a more suitable submission date.

Once again, thank you for submitting your manuscript to Therapeutic Delivery and I look forward to receiving your revision.

Sincerely,
Mr. Campbell Brooks
Commissioning Editor, Therapeutic Delivery
c.brooks@future-science.com

3 attachments



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53K

Author-disclosure-form-FSG.docx
581K

Author-disclosure-form_FSG.pdf
744K

ANSWER FOR REVIEWER COMMENTS:

Dear Editor,
Many thanks for comments and correction to our manuscript. It is very helpful for us to improve the article. We have revised all section of the manuscript as peer reviewer's suggestions. We hope that the result would be fulfilling the correction and satisfying.
Many thanks

REVIEWER: 1

Comments to the Author
The Review "INSIGHTS AND FUTURE PROSPECTS OF TRANSFERSOMES FOR COSMECEUTICS" is interesting as focuses on the use of transfersomes specifically tailored for cosmeceutical applications. In particular the paper describes all the events that are connected with skin ageing and the possible treatments.

1. The paper needs a careful check of the English language as different mistakes can be detected throughout the manuscript.
Editor comment: Please have the manuscript proof-read by a native English speaker to identify and correct any language or grammatical mistakes.
Author response and action taken:
Many thanks for the comments. We have proofread the manuscript, please see the attached certificate.
Page/line numbers:-

2. I also suggest to add especially in the introduction section the other innovative vesicles, formulated aiming at protecting the skin from external injuries and damages. I refer to glycersomes, hyalurosomes and hybrid vesicles: see papers published from 2013 to 2019 by Manca et al.
Editor comment: Reference recommendations may be included or not at the authors' discretion.
Author response and action taken:
Many thanks for the comment. In this paper, we focus on the use of phospholipid as lipid component of vesicular drug carriers, in addition to the use of softening bilayer in its structure, which this combination is called transfersome, to produce deformability during skin penetration. This study aims to give prospective use in cosmetics for skin treatment, by focus on using trasnfersome and protransfersome.
Page 6 line 17-18: We have added some sentence regarding the type of nanoparticulate carriers used to deliver active ingredient for topical administration as the following: "Certain nanocarriers such as liposome, transfersome, glycerosome, ufosome, and hybrid vesicle have been developed to improve skin drug delivery. Liposome is a lipid-based vesicular carrier consisting of an inner water phase surrounded by lipid bilayer membranes [13]. The addition of softening bilayers such as surfactants to liposome can produce transfersome [14], while the use of glycerol as the edge activator of liposomal bilayer membrane generates glycerosome [15]."
"

3. Moreover, another concern about this Review is connected to the cosmeceutical application also reported in the title. I know that cosmeceutical is an hybrid word obtained by the combination of cosmetics and pharmaceutics… but the authors should check the low that control the commercialization of these products… it is the same of cosmetics, so that even this products cannot be described as pharmaceutical products. I mean that authors should be careful in describing the use of synthetic drug especially proteins or hormones as they cannot be considered cosmeceutical products. Please check in the manuscript and modify accordingly.
Editor comment: Please ensure the distinction is made between pharmaceutical uses of these technologies and their cosmetic uses. If delivered for cosmetic purposes, an 'API' is not strictly a 'pharmaceutical' ingredient and this term must be used carefully, as the reviewer says, to ensure the activity of proposed active ingredients is strongly evidenced. As cosmetic products are not subjected to as rigorous study as pharmaceutical products, a distinction must be made between them to ensure no undue endorsement is given to cosmetic ingredients whose activity is supported by lower quality evidence.
Author response and action taken:
Many thanks for the suggestion. This review is intended for prospective use of ultradeformable vesicle for improving delivery of cosmetic active ingredients, not only for decorative purposes, but we also refer to active ingredients used in the formula of cosmetics that have local biological effects on the skin tissue, improving its appearance. We have changed the term of active ingredients to cosmetic active ingredient. However, the study about the use of ultradeformable vesicle is still limited. Therefore, considering the similar physicochemical properties of such active pharmaceutical ingredients such as insulin, hormones, and other as stated in this paper is aimed to give analogies of successful delivery of these substances using vesicles, making it prospective also for cosmetic active ingredients.

The abstract section
Page 1 line 10: we have revised the use of “cosmeceutical agents” with “cosmetics”
Page 1 line 17: we have revised the use of “cosmeceutical active substances” with “active cosmetic ingredients”
Page 1 line 34: we have revised the use of “active substances” with “active cosmetic ingredients”
Page 1 line 45: we have revised the use of “cosmeceutics” with “active cosmetic ingredients”
Page 2 line 37-38: we have revised the sentence of “Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of active pharmaceutical ingredients… ” with “Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of local biological active cosmetic ingredients
Page 2 line 41: we have revised the use of “anti-ageing substances” with “anti-ageing ingredients”
Page 2 line 50-51: we have revised the sentence of “Anti-ageing cosmetics are formulated from various types of active pharmaceutical ingredients which can be natural products, vitamins, and proteins” with “*The active ingredients that have been used as antiaging cosmetics include extracts from natural ingredients, vitamins and proteins”*
Page 3 line 11: we have revised the use of “active cosmeceutical ingredients” with “active cosmetic ingredients”

Page 3 line 23: we have revised the use of "for the drugs" with "for substances"
Page 3 line 30, 34: we have revised the use of "drugs" with "active cosmetic ingredients"
Page 3 line 48: we have deleted the word of "drugs"
Page 4 line 7: we have revised the use of "leakage of trapped drugs, or hydrolysis of encapsulated drugs" with "leakage of trapped substances, or hydrolysis of encapsulated active ingredients

Method section
Page 4 line 26-27, we have added sentences as the following: "The existing research into the use of transfersome in cosmetics is limited. As stated in this paper, considering the similar physicochemical properties of active pharmaceutical ingredients such as insulin and hormones is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients**."**

Results Section
Page 4 line 11: we have deleted the word of "drugs"
Page 4 line 29-30: we have revised the words of "the active cosmeceutical ingredient" with "active cosmetic ingredients*"*
Page 4 line 36,41: we have revised the words of "drug" with "active ingredient*"*
Page 7 line 46: we have deleted the words of "drug molecule"
Page 7 line 50: we have revised the words of "drugs" with "active ingredient*"*
Page 7 line 55: we have revised the words of "lipophilic drugs" with "lipophilic substances*"*
Page Page 10 line 57: we have deleted the words of "drug"
Page 8 line 4: we have revised the words of "drug" with "encapsulated substances"
Page 8 line 6: we have deleted the words of "drug"
Page 8 line 9: we have revised the words of "the active cosmeceutical ingredient" with "active cosmetic ingredients*"*
Page 8 line 9: we have revised the words of "various types of active pharmaceutical ingredients" with "various types of active ingredients*"*
Page 8 line 32, 33: we have revised the sentence of "The encapsulation methods of drug in transfersomes" into "The encapsulation methods of active ingredients in transfersomes"
Page 11 line 7: we have revised the sentence of "Transfersomes, loading drugs characterized by low solubility and high permeability" with "which can load active ingredients characterized by low solubility and high permeability*"*
Page 11 line 11-12: we have revised the words of "Active pharmaceutical ingredients belonging to the drug group include…" with "Active ingredients belonging to this group include…*."*
Page 11 line 23: we have deleted the words of "pharmaceutical"
Page 11 line 32: we have replaced the words of "of both drugs" with "of both active ingredients"
Page 11 line 41: we have deleted the words of "of drugs"
Page 13 line 14: we have replaced the words of "drug precipitation" with "precipitation of active ingredients"
Page 16 line 7,9,11: we have replaced the words of "the drug" with "the active ingredients"
Page 16 line 11,27,36,46: we have deleted the words of "drug" in "drug carriers" , "drug loading", "gradual drug release","drug entrapping efficiency"
Page 18 line 7,46: we have replaced the words of "drug" with "ingredients"
Page 19 line 13: we have replaced the words of "drug" with "ingredients"

Page 19 line 23: we have replaced the words of “active cosmeceutical ingredients” with “active cosmetic ingredients”
Page 19 line 29: we have deleted the words of “drug” in “free drug solutions”
Page 21 line 4,11,43: we have replaced the words of “drug” with “active ingredients”
Page 21 line 13: we have replaced the words of “drug penetration” with “skin penetration”

Discussion and conclusion section
Page 22 line 36: we have replaced the words of “active cosmeceutical ingredients” with “active cosmetic ingredients”
Page 22 line 48: we have replaced the words of “drugs” with “active ingredients”
Page 23 line 11: we have replaced the words of “cosmeceutics fields” with “cosmetics field with local biological effects”
Page 23 line 13,18,37,46: we have replaced the words of “cosmeceutical ” with “cosmetic”
Page 23 line 20,55: we have replaced the words of “cosmeceutics ” with “cosmetics”
Page 24 line 16,48: we have replaced the words of “cosmeceutics ” with “cosmetics”
Page 24 line 20,44: we have replaced the words of “cosmeceuticals ” with “cosmetics”
Page 24 line 39,41: we have replaced the words of “drugs ” with “active cosmetic ingredients”
Page 25 line 9: we have replaced the words of “cosmeceuticals substances” with “cosmetic ingredient”
Page 25 line 14: we have replaced the words of “cosmeceuticals” with “cosmetic”

Given that, I suggest minor revision before the acceptance of this paper for publication in this Journal.

REVIEWER: 2

Comments to the Author
This article intends to review the Transfersome concept and applications in drug delivery and cosmetics. However, the article practically revises therapeutic application of such carriers. Proliposomes were also considered as alternative to transfersomes but the differences between the 2 systems were not properly explained. Many important references are missing. Studies regarding the cosmeceutical application of Transfersomes were not included. The paper needs major revisions before being published.

1. Title should be changed as it does not reflect the results presented.
Author response and action taken:
Many thanks for the correction. We reconsidered and changed the title from “ Insight and future prospects of transfersomes for cosmeceutics” into “Ultradeformable vesicle: Concepts and applications relating to the delivery of skin cosmetics”
Page/line numbers:Title page

2. Transfersomes is a plural form, please, use the verbs in accordance.
Author response and action taken:
Many thanks. We have corrected the use of “transfersomes” as plural nouns

because the stability of transfersomes remain
Page 1 line 20: we have corrected the use of transfersome into “Transfersome and its..”
Page 1 line 24: we have corrected the use of transfersome into “Transfersome as an ultradeformable vesicle is….”
Page 1 line 31: we have corrected the use of transfersome into “Transfersome increases….”
Page 1 line 34: we have corrected the use of transfersome into “Transfersome has….”
Page 1 line 45: we have corrected the keyword as the following: transfersome, vesicle, cosmetic
Page 5 line 7: we have corrected the sentence of “Characteristics of protransfersomes and transfersomes as vesicular carrier” into “Characteristics of protransfersomes and transfersomes as vesicular carriers”
Page 5 line 32: we have corrected the sentence of “Protransfersomes, an extremely flexible liquid lipid provesicles, provides benefits for improving the stability of transfersomes” into “Protransfersomes, extremely flexible liquid lipid provesicles, provide benefits for improving the stability of transfersomes”
Page 5 line 32: we have corrected the sentence of “protransfersomes system, which was originally” into “protransfersome, which was originally..”
Page 5 line 55: we have corrected the word of “protransfersomes” into “protransfersome”
Page 7 line 43: we have corrected the sentence of “Transfersomes acts as encapsulating agents for drug molecules” into “Transfersomes act as encapsulating carriers for various active ingredients”
Page 9 line 18,27: we have corrected the sentence of “transfersomes has been…” into “transfersome has been..”
Page 13 line 48: we have corrected the sentence of “prepared as a transfersomes” into “prepared as a transfersome”
Page 14 line11-13: we have corrected the word of “protransfersomes” into “protransfersome”
Page 14 line 48: we have corrected the word of “transfersomes” into “transfersome”
Page 15 line 11, 14, 48: we have corrected the word of “transfersomes” into “transfersome”
Page 17 line 4-7, 34-37: we have corrected the word of “transfersomes” into “transfersome”
Page 17 line 44: we have corrected the word of “protransfersomes” into “protransfersome”
Page 21 line 18: we have corrected the word of “transfersomes” into “transfersome”
Page 24 line 32: we have corrected the sentence of “Transfersomes encapsulates various drug molecules” into “Transfersome encapsulates various active ingredients”
Page 24 line 32: we have corrected the sentence of “Vesicular drug carriers consisting of “ into Transfersome is a vesicular drug carrier that consists of”

3. P1, ln 34-35: “cosmetics; for example”, use a comma instead of semi-colon
Author response and action taken:
Many thanks for the correction, we have corrected the sentence.
Page 2 line 34-35: we replaced the semicolon with comma as the following: “skin care products or cosmetics, for example, sunblocks.”

4. Stratum corneum: italicize the first time it appears and abbreviate thereafter as SC
Author response and action taken:

Many thanks for the correction, we have corrected the sentence.
Page 2 line 45-46: "…the barrier of the *Stratum Corneum* (SC) in order…."

5. P2, ln 52-53: "ingredients which can be natural products, vitamins, and proteins". This phrase is extremely reductive of what the ingredients of anti-ageing cosmetics are. Please rephrase it.
Author response and action taken:
Many thanks for the correction, we have corrected the sentence.
Page 2 line 50-53: The active ingredients that have been used as antiaging cosmetics include extracts from natural products, vitamins, and proteins.

6. P3, ln 27-28: "Thus, liposome can dissolve or carry hydrophobic, hydrophilic…". Please, be rigorous on technological terminology. Liposomes do not "dissolve". They are versatile systems but drug-carrier interaction is not as solute-solvent interaction.
Author response and action taken:
Many thanks for the correction, we have corrected the statement.
Page 2 line 527-28: "Thus, liposomes can entrap hydrophobic, hydrophilic, or amphiphilic active cosmetic ingredients inside their structures…"

7. P3, ln 44-46: "These vesicles are 105 times more deformable than conventional liposomes." If so, please insert the reference.
Author response and action taken:
Many thanks for the correction, we have added the reference.
Page 3 line 44-46: "These vesicles are 10 times more deformable than conventional liposomes [10]."
10. Hussain A, Singh S, Sharma D, Webster TJ, Shafaat K, Faruk A. Elastic liposomes as novel carriers: Recent advances in drug delivery. *International Journal of Nanomedicine*. 12, 5087–5108 (2017).

8. P3, ln 46: "However, because the stability of transfersomes remains low…". This is not true. These systems are quite stable. Please revisit the work of Cevc and co-workers, Simões and Ascenso. Transfersomes are controllably destabilized systems to be deformable. The development of protransfersomes may be justified by other reasons but not by stability issues.
Author response and action taken:
Many thanks for the comments. In this statement, we refer to the possibility of drug leakage during storage due to water diffusion stimulating transfersome-entrapped active ingredients is higher than that of protransfersome, thus causing the less stable system. In addition, transfersome vesicle has limited entrapment capacity, while protransfersome provide greater ability in encapsulating higher amount of active ingredients, as evaluated in our previous study.

Page 3 line 46: we have corrected the sentence as the following: "However, because the transfersome vesicle has limited entrapment capacity and content leakage of active ingredients still tends to occur due to water diffusion from dispersing media,.."

9. P4, ln 4-7: "…sedimentation, aggregation, fusion, leakage of trapped drugs, or hydrolysis of encapsulated drugs." The corresponding references are missing.

Author response and action taken:

Many thanks for the correction. We have added the references.

Page 4 line 4-7: " … hydrolysis of encapsulated active ingredients [12–14]"

12. Iskandarsyah, Rahmi AD, Pangesti DM. Comparison of the characteristics of transfersomes and protransfersomes containing azelaic acid. *J. Young Pharm.* 10(2), s11–s15 (2018).

13. Gupta V, Agrawal RC, Trivedi P. Reduction in cisplatin genotoxicity (micronucleus formation) in non target cells of mice by protransfersome gel formulation used for management of cutaneous squamous cell carcinoma. *Acta Pharm.* 61(1), 63–71 (2011).

14. Jain S, Sapre R, Tiwary AK, Jain NK. Proultraflexible lipid vesicles for effective transdermal delivery of levonorgestrel: development, characterization, and performance evaluation. *AAPS PharmSciTech* [Internet]. 6(3), E513-22 (2005).

10. Please, uniformize Protransfersomes or pro-transfersomes. Several typos are present: transferomal, Transfersomess, transfersom, sometimes capitalized some not, lack of spaces between words and commas, more than once "namely;"

Author response and action taken:

Many thanks for the correction. We have re checked and revised the consistency writing of transfersome, protransfersome.

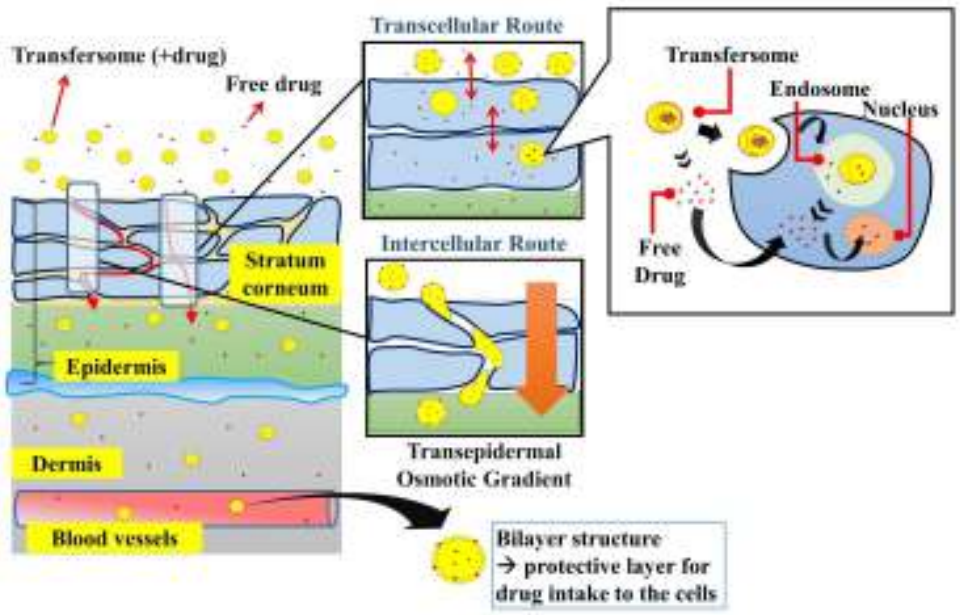
Page/line numbers: -

11. Figure 3 – What articles fundament the transcellular route of intact vesicles? If it is an assisted transport, the figure should resemble that.

Author response and action taken:

Many thanks for the comments. We have revised the the Figure 3 as the following figure.

Page/line numbers: Figure 3



12. "Transfersomes acts as encapsulating agents for drug molecules" section: there is a big confusion between therapeutic use of transfersomes and cosmetic use of transfersomes in the initial paragraphs of this section. The introductory part of this section needs to be improved.

Author response and action taken:

Many thanks for the comments. This review is intended for prospective use of ultradeformable vesicle for improving delivery of cosmetic active ingredients, not only for decorative purposes, but we also refer to active ingredients used in the formula of cosmetics that have local biological effects on the skin tissue, improving its appearance. We have changed the term of active ingredients to cosmetic active ingredient. However, the study about the use of ultradeformable vesiclefor cosmetic skin delivery is still limited. Therefore, considering the similar physicochemical properties of such active pharmaceutical ingredients such as insulin, hormones, and other as stated in this paper is aimed to give analogies of successful delivery of these substances using vesicles, making it prospective also for active cosmetic ingredients.

Page 10 line 43: we have revised the title into "Transfersomes act as encapsulating carriers for various active ingredients".

Page 10 line 51: we have added the sentence explaining the recent use on drug delivery and cosmetic as the following: **"**It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the

production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis."

Throughout the manuscript: We have also replaced the use of drugs, active pharmaceutical ingredients, cosmeceutical with active cosmetic ingredients, and cosmetic.

The abstract section
Page 1 line 10: we have revised the use of "cosmeceutical agents" with "cosmetics"
Page 1 line 17: we have revised the use of "cosmeceutical active substances" with "active cosmetic ingredients"
Page 1 line 34: we have revised the use of "active substances" with "active cosmetic ingredients"
Page 1 line 45: we have revised the use of "cosmeceutics" with "active cosmetic ingredients"
Page 2 line 37-38: we have revised the sentence of "Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of active pharmaceutical ingredients… " with "Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of local biological active cosmetic ingredients
Page 2 line 41: we have revised the use of "anti-ageing substances" with "anti-ageing ingredients"
Page 2 line 50-51: we have revised the sentence of "Anti-ageing cosmetics are formulated from various types of active pharmaceutical ingredients which can be natural products, vitamins, and proteins" with "Active anti-ageing cosmetic ingredients such as Coenzyme Q10, which demonstrates low water solubility [5,6], growth factors such as Epidermal Growth Factor (TGF), and Transforming Growth Factor-β (TGF-β) are contained in amniotic membrane stem cell metabolite products (AMSC-MPs) that have a significant molecular weight [7,8], vitamins, and other herbal and biological products [9]."
Page 3 line 11: we have revised the use of "active cosmeceutical ingredients" with "active cosmetic ingredients"
Page 3 line 23: we have revised the use of "for the drugs" with "for substances"
Page 3 line 30, 34: we have revised the use of "drugs" with "active cosmetic ingredients"
Page 3 line 48: we have deleted the word of "drugs"
Page 4 line 7: we have revised the use of "leakage of trapped drugs, or hydrolysis of encapsulated drugs" with "leakage of trapped substances, or hydrolysis of encapsulated active ingredients."

Method section
Page 4 line 26-27, we have added sentences as the following:
"The existing research into the use of transfersome in cosmetics is limited. As stated in this paper, considering the similar physicochemical properties of active pharmaceutical ingredients such as insulin and hormones is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients."

Results Section

Page 4 line 11: we have deleted the word of "drugs"
Page 4 line 29-30: we have revised the words of "the active cosmeceutical ingredient" with "active cosmetic ingredients"
Page 4 line 36,41: we have revised the words of "drug" with "active ingredient"
Page 7 line 46: we have deleted the words of "drug molecule"
Page 7 line 50: we have revised the words of "drugs" with "active ingredient"
Page 7 line 55: we have revised the words of "lipophilic drugs" with "lipophilic substances"
Page Page 10 line 57: we have deleted the words of "drug"
Page 8 line 4: we have revised the words of "drug" with "encapsulated substances"
Page 8 line 6: we have deleted the words of "drug"
Page 8 line 9: we have revised the words of "the active cosmeceutical ingredient" with "active cosmetic ingredients"
Page 8 line 9: we have revised the words of "various types of active pharmaceutical ingredients" with "various types of active ingredients"
Page 8 line 32, 33: we have revised the sentence of "The encapsulation methods of drug in transfersomes" into "The encapsulation methods of active ingredients in transfersomes"
Page 11 line 7: we have revised the sentence of "Transfersomes, loading drugs characterized by low solubility and high permeability" with "which can load active ingredients characterized by low solubility and high permeability"
Page 11 line 11-12: we have revised the words of "Active pharmaceutical ingredients belonging to the drug group include…" with "Active ingredients belonging to this group include…."
Page 11 line 23: we have deleted the words of "pharmaceutical"
Page 11 line 32: we have replaced the words of "of both drugs" with "of both active ingredients"
Page 11 line 41: we have deleted the words of "of drugs"
Page 13 line 14: we have replaced the words of "drug precipitation" with "precipitation of active ingredients"
Page 16 line 7,9,11: we have replaced the words of "the drug" with "the active ingredients"
Page 16 line 11,27,36,46: we have deleted the words of "drug" in "drug carriers" , "drug loading", "gradual drug release","drug entrapping efficiency"
Page 18 line 7,46: we have replaced the words of "drug" with "ingredients"
Page 19 line 13: we have replaced the words of "drug" with "ingredients"
Page 19 line 23: we have replaced the words of "active cosmeceutical ingredients" with "active cosmetic ingredients"
Page 19 line 29: we have deleted the words of "drug" in "free drug solutions"
Page 21 line 4,11,43: we have replaced the words of "drug" with "active ingredients"
Page 21 line 13: we have replaced the words of "drug penetration" with "skin penetration"

Discussion and conclusion section

Page 22 line 36: we have replaced the words of "active cosmeceutical ingredients" with "active cosmetic ingredients"
Page 22 line 48: we have replaced the words of "drugs" with "active ingredients"
Page 23 line 11: we have replaced the words of "cosmeceutics fields" with "cosmetics field with local biological effects"
Page 23 line 13,18,37,46: we have replaced the words of "cosmeceutical " with "cosmetic"
Page 23 line 20,55: we have replaced the words of "cosmeceutics " with "cosmetics"

Page 24 line 16,48: we have replaced the words of "cosmeceutics " with "cosmetics"
Page 24 line 20,44: we have replaced the words of "cosmeceuticals " with "cosmetics"
Page 24 line 39,41: we have replaced the words of "drugs " with "active cosmetic ingredients"
Page 25 line 9: we have replaced the words of "cosmeceuticals substances" with "cosmetic ingredient"
Page 25 line 14: we have replaced the words of "cosmeceuticals" with "cosmetic"

13. P8, ln 9-14: "The encapsulation of active cosmeceutical ingredients with large molecular weight can be based on several studies of transfersomes formulation for delivery of proteins, such as hormones, stem cells, and ribonucleic acids (RNAs)." Several studies: where are they? Several references are missing on the proper site where they should be cited.
Author response and action taken:
Many thanks for the comments. We have added the references in the statements

Page 8 line 13-14: "….hormones, stem cells, and ribonucleic acids (RNAs) [18,21,22]."
18. Guo J, Ping Q, Zhang L. Transdermal delivery of insulin in mice by using lecithin vesicles as a carrier. *Drug Delivery: Journal of Delivery and Targeting of Therapeutic Agents*. 7(2), 113–116 (2000).
21. Ibaraki H, Kanazawa T, Kurano T, Oogi C, Takashima Y, Seta Y. Anti-RelA siRNA-Encapsulated Flexible Liposome with Tight Junction-Opening Peptide as a Non-invasive Topical Therapeutic for Atopic Dermatitis. *Biological and Pharmaceutical Bulletin*. 42(7), 1216–1225 (2019).
22. Mandpe P, Prabhakar B, Shende P. Role of Liposomes-Based Stem Cell for Multimodal Cancer Therapy. *Stem Cell Reviews and Reports*. 16(1), 103–117 (2020).

14. P21, ln 25: "The lipid in the stratum corneum is negatively charged." What lipid? Lack of rigor in scientific terminology should be avoided.
Author response and action taken:
Many thanks for the correction. The lipid in this sentence refers to lipid lamellae in Stratum Corneum that consists cholesterol, free fatty acids and ceramides providing high proportion of negatively charged lipids.

Page 21 line 25: we have revised the sentences as the following: " The lipid lamellae in the SC have high proportion of negatively charged lipids [53]."

15. Why protransfersomes have no in vitro evaluation?
Author response and action taken:
Many thanks for the comment. Actually we have discussed about in vitro evaluation of protransfersome in the section of in vitro evaluation of transfersomes (page 20), which

protramsfersome transforms into transfersome in the presence of water. However, at the present, the in vitro evaluations are still limited for the characterization of protransfersome and its ability to convert to transfersome after addition of water.
Page 20 line 20: we have added protransfersomes in the section title of "*In vitro* evaluations of transfersomes and protransfersomes".

16. Results present only therapeutic applications and not cosmetic applications
Author response and action taken:
Many thanks for the comments. In this review, we only included original research article to be analyzed. There are some use of transfersome for cosmetic such as the penetration and biodistribution study of transfersome loading retinyl palmitate, which has important role in production of extracellular matrix in skin (page 17 line 43-58), the study of the use of transfersome for delivery of a combination of EGCG and hyaluronic acid as an antioxidant and antiaging (page 18 line 9-23). However, during the analysis, the study about the use of transfersome for cosmetic skin delivery is limited. Therefore, considering the similar physicochemical properties of such active pharmaceutical ingredients such as insulin, hormones, and other as stated in this paper is aimed to give analogies of successful delivery of these substances using vesicles, making it prospective also for active cosmetic ingredients.

Page 10 line 51: we have added the sentence explaining the recent use on drug delivery and cosmetic as the following: **"**It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis."

Throughout the manuscript: We have also replaced the use of drugs, active pharmaceutical ingredients, cosmeceutical with active cosmetic ingredients, and cosmetic.

17. P26, ln. 43-44: "…of edge activators, known as surfactants…", isn´t it the other way around?
Author response and action taken:

Many thanks for the comments. Edge activator plays important role as membrane-softening agent to facilitate ultradeformability of transfersome vesicle. And so far, edge activator includes glycerol, ethanol, or surfactants such as Tween, Span, Sodium Cholate, and so on. With the presence of *edge activator* (EA), the vesicles can become elastic with the result that they can enhance the penetration of active cosmetic ingredients, thus enabling them to reach deeper skin layers [8]. EAs are displaced to zones with higher curvature/stress in a lipid bilayer due to mechanical stress. Such displacement led to a minimal energetic cost for changing the shapes and volumes of transfersomes. Moreover, EAs can significantly reduce the transition temperature of lipids membranes by occupying the combined space of phospholipid molecules and disturbing the ordered arrangement of lipid bilayers [9]

Page 25 line 43-44: we have revised the sentence as the following: "the addition of surfactants as EAs.."

Page 6 line 36: we have added some explanation about edge activators as the following: "EAs are displaced by mechanical stress to zones with higher curvature/stress in a lipid bilayer. Such displacement led to the minimal energetic cost incurred in changing the shape and volume of transfersomes. Moreover, EAs can significantly reduce the transition temperature of lipid membranes by occupying the combined space of phospholipid molecules and disturbing the ordered arrangement of lipid bilayers [17]."

17. Yang C, Dai X, Yang S, *et al.* Coarse-grained molecular dynamics simulations of the effect of edge activators on the skin permeation behavior of transfersomes. *Colloids and Surfaces B: Biointerfaces* [Internet]. 183(11), 110462 (2019). Available from: https://doi.org/10.1016/j.colsurfb.2019.110462.

18. p27, ln. 16-18 "It is in consideration with cosmeceutical active agents can be locally or systemically delivered to target sites. Nonsense phrase. Please rephrase it.

Author response and action taken:

Many thanks for the correction. We have re-paraphrased the statement as the following.

Page 27 line 16-18:" The use of transfersomes and protransfersomes may facilitate penetration of active cosmetic ingredients to deep skin layers i.e. dermis layer."

REVIEWER: 3

Comments to the Author

1**. The inclusion & exclusion criteria in consort diagram needs improvement & clarity**

Author response and action taken:

Many thanks for the comments. We have stated the inclusion and exclusion criteria in the manuscript as the following (***page 7 line 41-58***): The eligibility criteria applied when selecting journal articles comprised original research, short case studies, experimental research design, and the year of publication falling within the period 1992-2020. The sites accessed for the purposes

of conducting the search included Pubmed (Scopus & Scimago) and Google Scholar which contain search keywords. Articles published in predatory journals or publishers which include review articles were excluded from the study.
Page/line numbers: -

2. During inclusion of papers, kindly ensure you are including more cosmetic related papers rather than therapeutic papers.
Author response and action taken:
Many thanks for the comment. In this review, we only included original research article to be analyzed. There are some use of transfersome for cosmetic such as the penetration and biodistribution study of transfersome loading retinyl palmitate, which has important role in production of extracellular matrix in skin (page 17 line 43-58), the study of the use of transfersome for delivery of a combination of EGCG and hyaluronic acid as an antioxidant and antiaging (page 18 line 9-23). However, during the analysis, the study about the use of transfersome for cosmetic skin delivery is limited. Therefore, considering the similar physicochemical properties of such active pharmaceutical ingredients such as insulin, hormones, and other as stated in this paper is aimed to give analogies of successful delivery of these substances using vesicles, making it prospective also for active cosmetic ingredients.

Page 10 line 51: we have added the sentence explaining the recent use on drug delivery and cosmetic as the following: **"**It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis."

3. Discussion on mechanism by which transferosomes can enhance anti-aging efficacy is missing and which type of anti-aging molecule can be used in transferosomes also missing
Author response and action taken:
Many thanks for the comment. Regarding this comment, actually we have stated the mechanism in the page as follows (page 3 line 6-16): "Therefore, the use of nanoparticulate carriers in skin delivery has the potential for anti-ageing cosmetics to improve the decreased quality of the dermis layer in aged skin. The presence of active cosmetic ingredients within a nanocarrier

system in the epidermis and dermis layers indicates that they can promote collagen and elastin repair activity which enhances skin firmness [12].” We have also added some discussion as the following.

Page 26 line 9: we have revised the statement and added some discussion as the following: “Skin ageing is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation, and disrupt the dermal extracellular matrix [101,102]. However, active cosmetic ingredients used in anti-ageing therapy such as CoQ10 and AMSC-MP, among others, suffer from skin penetration-related drawbacks including low water solubility and large molecular weight. The use of transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, i.e., the dermis, which is composed of almost 70% collagen [103].”

4. Evaluation also kindly try to include more cosmetic/ topical related papers

Author response and action taken:

Many thanks for the comment. In this review, we included original research article to be analyzed related to the use of transfersome, protransfersome for topical skin delivery. There are some use of transfersome for cosmetic such as the penetration and biodistribution study of transfersome loading retinyl palmitate, which has important role in production of extracellular matrix in skin (page 17 line 43-58), the study of the use of transfersome for delivery of a combination of EGCG and hyaluronic acid as an antioxidant and antiaging (page 18 line 9-23). However, there are limited papers discussing about the use of transfersome and protransfersome for cosmetic.

Page 10 line 51: we have added the sentence explaining the recent use on drug delivery and cosmetic as the following: **“**It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis.”

5. The entire paper needed to be re-written with improved clarity

Author response and action taken:

Many thanks for the comments. we have added some discussion in the manuscript as well as using active cosmetic ingredients instead of drug.

We reconsidered and changed the title from " Insight and future prospects of transfersomes for cosmeceutics" into "Ultradeformable vesicle: Concepts and applications relating to the delivery of skin cosmetics"
Page/line numbers:Title page

Page 4 line 26-27, we have added sentences as the following:
"The existing research into the use of transfersome in cosmetics is limited. As stated in this paper, considering the similar physicochemical properties of active pharmaceutical ingredients such as insulin and hormones is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients**."**

Page 6 line 36: we have added some explanation about edge activators as the following: "EAs are displaced by mechanical stress to zones with higher curvature/stress in a lipid bilayer. Such displacement led to the minimal energetic cost incurred in changing the shape and volume of transfersomes. Moreover, EAs can significantly reduce the transition temperature of lipid membranes by occupying the combined space of phospholipid molecules and disturbing the ordered arrangement of lipid bilayers [17]."

Page 10 line 51: we have added the sentence explaining the recent use on drug delivery and cosmetic as the following: It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis."

Page 26 line 9: we have revised the statement and added some discussion as the following:
"Skin ageing is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation, and disrupt the dermal extracellular matrix [101,102]. However, active cosmetic ingredients used in anti-ageing therapy such as CoQ10 and AMSC-MP, among others, suffer from skin penetration-related drawbacks including low water solubility and large molecular weight. The use of transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, i.e., the dermis, which is composed of almost 70% collagen [103]."

COMMENTS FROM THE EDITOR:

1. As identified by the reviewers, the structure and focus of this review could be greatly improved. Currently, the authors give some background on transfersomes, liposomes, and protransfersomes, and describe the mechanisms for skin penetration of these nanocarriers, then describe research pertaining to transfersomes. Much of the research described concerns therapeutic applications rather than cosmetic and therefore confuses the purpose of the work. Therefore, the editors suggest this article is reclassified as a Perspective rather than a Review (please make this change in ScholarOne when uploading the revised manuscript). The authors can then review all instances of transfersomes being used in the literature (therapeutic or otherwise), and then discuss what this research could mean in each case for the future of cosmetic transfersome delivery. While we welcome the speculative nature of this work, the speculation must be supported by thorough discussion of available evidence.

Author response and action taken:

Many thanks for the comments. We have revised some parts of the manuscript and added some statements and discussion about the limitation of the review for protransfersome and transfersoem in cosmetic delivery.

Page 1 line 27-28: We have added some sentences as the following: "Numerous reports exist highlighting the successful delivery of therapeutic agents such as large molecular, low water soluble, and poorly permeable active ingredients which involve penetrating the skin by means of transfersome.."

Page 1 line 34-35: We have added some sentences as the following: "However, the use of transfersome in the delivery of skin active cosmetics is limited. Considering the similar physicochemical properties of their active ingredients, transfersome should possess….."

Page 4 line 11-18: we have revised the paragraphs as the following: "Transfersome is widely reported to be employed in the topical and transdermal delivery of various active pharmaceutical ingredients. However, its applications to cosmetic delivery are limited. Moreover, the recent development of cosmetics is largely intended to improve the appearance of skin by having local biological effects on its tissues. Considering the similar physicochemical properties of the active ingredients provides direct analogies for successful skin delivery using transfersome, thereby also rendering them prospective active cosmetic ingredients. This review will demonstrate the potential use of transfersome in enhancing active ingredient penetration which promotes optimal anti-ageing activity within the cosmetic delivery system. This, in turn, increasing their effectiveness in impeding skin ageing."

Page 26 line 9: we have revised the statement and added some discussion as the following: "Skin ageing is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation, and disrupt the dermal extracellular matrix [101,102]. However, active cosmetic ingredients used in anti-ageing therapy such as CoQ10 and AMSC-MP, among others, suffer from skin penetration-related drawbacks including low water solubility and large molecular weight. The use of transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, i.e., the dermis, which is composed of almost 70% collagen [103]."

2. Few, if any, specific examples of cosmetic ingredients and their mechanisms of action are given (e.g. in the 'Result' section where the authors give an overview of ingredients). For example, insulin is presented as an example of a protein which can be delivered by transfersomes, however the cosmetic uses of insulin are not described, nor are any current technologies for the delivery of insulin for cosmetic purposes given. The editors recommend giving examples of specific cosmetic ingredients (and brief mechanisms of action) which may be delivered using transfersomes, and explain why tranfersome delivery would be an improvement compared to existing technologies.

Author response and action taken:

Many thanks for the correction. We have added some discussion about it.

Page 10 line 51: we have added a paragraph in this section as follows: "It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis. "

Page 14 line 58: we have added a paragraph in this section as follows: "The capability of transfersome to encapsulate hydrophobic molecules within the lipid bilayer would enable modification of physicochemical properties of active ingredients encapsulated in carriers which are nano-sized particles, amphiphilic self-assemble phospholipid with surfactant presence, thus affecting their dispersability, solubilization, and releases to aqueous media at the intended sites, especially dermis for antiaging therapy [92]."

Page 15 line 56: we have added a paragraph in this section as follows: "Transfersome vesicles possess the ability to modify the permeability of active ingredients due to encapsulation within the carrier which can change passive diffusion into active transport, allowing low permeable active ingredients to permeate biological membranes. The use of biomimetic phospholipid as a component of transfersome would enable vesicles to carry active ingredients via the paracellular or transcellular routes among others, or through fusion with the cell membrane. This underpins the potential of transfersome to deliver active ingredients promoting dermal repairs and rejuvenation [94]."

Page 17 line 17: we have added a paragraph in this section as follows:" According to these results, transfersome and protransfersome are able to improve the solubility and permeability of active ingredients with low water solublity and poor permeability. Their ability to entrap hydrophobic molecules within the lipid domain of the bilayer membrane as well as the amphiplic properties of the phospholipids used in transfersomes significantly improve the solubility and permeability of such compounds, rendering them useful in delivering active cosmetic ingredients."

3. In discussing specific research, please be clear in identifying oral versus topical delivery and systemic versus targeted delivery. The route of administration and the target of the active ingredient should be clarified and related back to the topic of cosmetics.
Author response and action taken:
Many thanks. We have revised it
Page/line numbers:
Page 8 line 51: We have added"via transdermal route"
Page 9 line 9: We have added "increase transdermal penetration …."
Page 9 line 18: We have added "increase transdermal penetration …."
Page 11 line 4: we have added " … can increase therapeutic effectiveness by topical administration of its transfersomal system.."
Page 12 line 18: we have added " … transdermal transfersome formulation..."
Page 12 line 25: we have added " … high penetration into skin deeper layers…"
Page 12 line 37: we have added "Diclofenac topical transfersome …"
Page 13 line 50: we have added "…improved for its transdermal bioavailability.."
Page 14 line 34: we have added "…transfersomes for transdermal delivery.."
Page 15 line 13: we have added "…for topical application..."
Page 15 line 46: we have added "…penetration abilities across skin [47]."
Page 16 line 48: we have added "…effectiveness in skin melanoma therapy [19,43,97]. The use of protransfesome and transfersome also improved cisplatin levels in plasma during transdermal application, which proves these ultradeformable vesicles successfully enhance penetration of low soluble and low permeable drug such as Cisplatin [42]."

Page 20 line 18: we have added "…after topical aplication."
Page 20 line 30: we have added "…and transdermal application of"

4. The relevance of the encapsulated ingredients to cosmetics should be discussed. For example, are there any cosmetic applications of Levonogestrel or is this simply presented as a hormone which has proved deliverable by transfersomes and therefore other, cosmetically active hormones could also be delivered this way. Equally with cisplatin, what relevance does this have to cosmetics? Please ensure that each ingredient mentioned is evaluated with respect to its relevance to the delivery of cosmetic ingredients using transfersomes.

Author response and action taken:
Many thanks for the correction. We have added a paragraph in each section of active ingredients categorization.

Page 10 line 51: we have added a paragraph in this section as follows: "It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,87,88]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [89]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,90]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis. "

Page 14 line 58: we have added a paragraph in this section as follows: "The capability of transfersome to encapsulate hydrophobic molecules within the lipid bilayer would enable modification of physicochemical properties of active ingredients encapsulated in carriers which are nano-sized particles, amphiphilic self-assemble phospholipid with surfactant presence, thus affecting their dispersability, solubilization, and releases to aqueous media at the intended sites, especially dermis for antiaging therapy [92]"

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active ingredients to permeate biological membranes. The use of biomimetic phospholipid as a component of transfersome would enable vesicles to carry active ingredients via the paracellular or transcellular routes among others, or through fusion with the cell membrane. This underpins the potential of transfersome to deliver active ingredients promoting dermal repairs and rejuvenation [94]. “

Page 17 line 17: we have added a paragraph in this section as follows: “According to these results, transfersome and protransfersome are able to improve the solubility and permeability of active ingredients with low water solublity and poor permeability. Their ability to entrap hydrophobic molecules within the lipid domain of the bilayer membrane as well as the amphiplic properties of the phospholipids used in transfersomes significantly improve the solubility and permeability of such compounds, rendering them useful in delivering active cosmetic ingredients.”

5. In the introduction, please ensure that the aims of the article are clearly stated and that the structure of the discussion is outlined so that the readers are fully informed on the purpose of the work. Additionally, please ensure the novelty and significance of the work is justified with respect to existing literature.

Author response and action taken:

Many thanks for the comments, we have added aims in the abstract and introduction

Page/line numbers:

Page 1 line 27: Numerous reports exist highlighting the successful delivery of therapeutic agents such as large molecular, low water soluble, and poorly permeable active ingredients which involve penetrating the skin by means of transfersome. Moreover, *in vitro* and *in vivo* studies have indicated that transfersome increases deposition, penetration, and efficacy of active ingredients. However, the use of transfersome in the delivery of active cosmetic ingredients is limited. Considering their similar physicochemical properties, transfersome should possess considerable potential as a delivery system for anti-aging cosmetics.

Page 4 line 11-18: we have revised the paragraphs as the following: “Transfersome is widely reported to be employed in the topical and transdermal delivery of various active pharmaceutical ingredients. However, its applications to cosmetic delivery are limited. Moreover, the recent development of cosmetics is largely intended to improve the appearance of skin by having local biological effects on its tissues. Considering the similar physicochemical properties of the active ingredients provides direct analogies for successful skin delivery using transfersome, thereby also rendering them prospective active cosmetic ingredients. This review will demonstrate the potential use of transfersome in enhancing active ingredient penetration which promotes optimal anti-ageing activity within the cosmetic delivery system. This, in turn, increasing their effectiveness in impeding skin ageing.”

6. Please review the manuscript to ensure that all statements are appropriately referenced. References that pertain to a particular sentence should be included at the end of that sentence.
Author response and action taken:
Many thanks for the comments. We have checked that all statements have been appropriately referenced
Page/line numbers:-

7. In the Publishing Agreement form, the authors state that they are government employees. For the purposes of this form, employees of a university which receives government funding are not classed as government employees. Therefore, please recomplete the Publishing Agreement form with Section A filled in, rather than Section B.
Author response and action taken:
Many thanks. We have revised the publishing agreement form by filling check in section A.

8. Please have the entire manuscript proof read by a native English speaker prior to resubmission to ensure all spelling and grammatical errors are corrected. Please note that Future Science partners with Enago to provide pre-submission editing services for our authors. Editing services include language check, copyediting and substantive editing. For more information, please visit the website here: www.enago.com/futurescience/
Author response:
many thanks. We have proofread the manuscript at Simon and The Colledge, please see the attached certificate

9**. Please make sure that all tables and boxes are clearly titled and cited in the text.**
Author response:
Many thanks, we have checked and ensured that all table and figures have been cited in the text

10. Please include reference annotations: up to 8 references should be chosen from your bibliography and highlighted as being '*' – of interest, or "" – of considerable interest, with a brief sentence explaining why in each case.**
Author response:
Reference annotations

14. Miatmoko A, Kawano K, Hattori Y, Maitani Y, Yonemochi E. Evaluation of transfersome and protransfersome for percutaneous delivery of cisplatin in hairless mice. *J Pharmaceu Pharmacol*. S(1), 1–7 (2015).*)
This reference provides information regarding enhancement of skin penetration of Cisplatin, an active ingredient which has low water solubility and poor permeability

accros biological membrane.

22. Jain S, Sapre R, Tiwary AK, Jain NK. Proultraflexible lipid vesicles for effective transdermal delivery of levonorgestrel: development, characterization, and performance evaluation. *AAPS PharmSciTech* [Internet]. 6(3), E513-22 (2005).*)
This reference provides important information about the successful use of protransfersome as well as its characterization in delivering protein substance i.e. levonorgestrel via transdermal route.

29. Guo J, Ping Q, Zhang L. Transdermal delivery of insulin in mice by using lecithin vesicles as a carrier. *Drug Deliv. J. Deliv. Target. Ther. Agents*. 7(2), 113–116 (2000). *)
This reference provides data of skin permeation of Insulin using transfersome, proving that transfersome is able to entrap hydrophilic active ingredients which have large molecular weight and deliver them acrross the skin.

31. Cevc G. Chapter 9 Material transport across permeability barriers by means of lipid vesicles. *Handb. Biol. Phys.* 1(C), 465–490 (1995).**)
This reference provides basic information regarding the use of lipid vesicle for transdermal delivery of active ingredients.

38. Avadhani KS, Manikkath J, Tiwari M, *et al.* Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. *Drug Deliv.* 24(1), 61–74 (2017).*)
This reference provides important information about the successful use of transfersome for topical delivery of poor permable active ingredients.

75. Pena-rodríguez E, Moreno MC, Blanco-fernandez B, González J, Fernández-campos F. Epidermal delivery of retinyl palmitate loaded transfersomes: Penetration and biodistribution studies. *Pharmaceutics*. 12(2) (2020).*)
This reference provides important information about the successful use of transfersome for topical delivery of low water soluble active ingredients.

88. Prakoeswa CRS, Effendy ZF, Herwanto N, Ervianty E, Rantam AF. Efficacy of topical application of a mixture of amniotic membrane stem cell metabolic products and vitamin C after microneedling treatment in patients with photoaging. *J. Pakistan Assoc. Dermatologists*. 30(3), 485–489 (2020).*)
This reference provides important information about the potential efficacy of AMSC-MP in skin cosmetics and its limitation in skin delivery, which should use microneedle to obtain good efficacy in photoaging therapy.

89. El Zaafarany GM, Awad GAS, Holayel SM, Mortada ND. Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery. *Int. J. Pharm.* 397(1–2), 164–172 (2010). **)
This reference provides important information about the surfactant type and its role in determining transferome physical properties that affect skin penetration

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Many thanks; we choose regular service and not an open access. Thank you

ABSTRACT

Skin ageing is a phenomenon resulting in reduced self-confidence, with the result that cosmetics have become a major requirement for social determinants of health. The use of active cosmetic ingredient can help prevent skin aging. Transfersome as an ultradeformable vesicle is well known to be capable of deeply penetrating the dermis. This scoping review provides an insight into transfersome and its prospective use in anti-ageing cosmetics. Numerous reports exist highlighting the successful delivery of therapeutic agents such as large molecular, low water soluble, and poorly permeable active ingredients which involve penetrating the skin by means of transfersome. Moreover, *in vitro* and *in vivo* studies have indicated that transfersome increases deposition, penetration, and efficacy of active ingredients. However, the use of transfersome in the delivery of active cosmetic ingredients is limited. Considering their similar physicochemical properties, transfersome should possess considerable potential as a delivery system for anti-aging cosmetics.

Keywords: transfersome, ultradeformable vesicle, cosmetic, social determinants of health, scoping review

Background

Skin ageing is a process of changing physical appearance that can reduce an individual's self-confidence. These skin changes are closely related to ones in the balance of the production and decomposition of collagen, elastin and glycosaminoglycans which constitute quality parameters of the dermis layer [1,2]. There are several triggers which can be internal physical factors such as Deoxyribonucleic Acid (DNA) damage due to Reactive Oxygen Species (ROS), the development of chronic diseases, and metabolic disorders connected with ageing, or external factors including exposure to sunlight and oxidant materials resulting in the skin losing elasticity and firmness and the appearance of wrinkles [1–3]. Wrinkles are a sign of ageing skin caused by collagen degradation and these visible skin folds can have an impact on quality of life and physical appearance [4].

Anti-ageing strategies that have been implemented include protection against ultraviolet (UV) rays, invasive procedures, and ski ncare products or cosmetics, for example, sunblocks. Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of local biological active cosmetic ingredients. Anti-ageing ingredients have become a popular means of improving intrinsic skin biological function. Therefore, this compound must be able to penetrate the barrier of the *Stratum Corneum* (SC) in order to reach the dermis layer and rejuvenate and repair skin wrinkles.

Active anti-ageing cosmetic ingredients such as Coenzyme Q10, which demonstrates low water solubility [5,6], growth factors such as Epidermal Growth Factor (TGF), and Transforming Growth Factor-β (TGF-β) are contained in amniotic membrane stem cell metabolite products (AMSC-MPs) that have a significant molecular

weight [7,8], vitamins, and other herbal and biological products [9]. These compounds should possess different physicochemical characteristics. However, only small molecules less than 500 Da in size and lipophilic molecules with log P values between -1 to 4 can penetrate the SC which constitutes the skin barrier [10,11]. Therefore, the use of nanoparticulate carriers in skin delivery has the potential for anti-ageing cosmetics to improve the decreased quality of the dermis layer in aged skin. The presence of active cosmetic ingredients within a nanocarrier system in the epidermis and dermis layers indicates that they can promote collagen and elastin repair activity which enhances skin firmness [12].

Certain nanocarriers such as liposome, transfersome, glycerosome, ufosome, and hybrid vesicle have been developed to improve skin drug delivery. Liposome is a lipid-based vesicular carrier consisting of an inner water phase surrounded by lipid bilayer membranes [13]. The addition of softening bilayers such as surfactants to liposome can produce transfersome [14], while the use of glycerol as the edge activator of liposomal bilayer membrane generates glycerosome [15].

Transfersomes represent the first generation of elastic liposomes which demonstrate liposome-like characteristics with the ability to deform and reform their shapes. Liposome is known to provide three different environments for substances entrapped inside them, namely; the lipid-water interface, the hydrophobic nucleus, and the aqueous interior. Thus, liposomes can entrap hydrophobic, hydrophilic, or amphiphilic active ingredients within their structures, in addition to improving their stability. With the presence of *edge activators* (EAs), the vesicles can become elastic resulting in their ability to enhance the penetration by active cosmetic ingredients, thus enabling them to reach deeper skin layers [16]. EAs are displaced by mechanical stress

to zones with higher curvature/stress in a lipid bilayer. Such displacement led to the minimal energetic cost incurred in changing the shape and volume of transfersomes. Moreover, EAs can significantly reduce the transition temperature of lipid membranes by occupying the combined space of phospholipid molecules and disturbing the ordered arrangement of lipid bilayers [17].

Transfersomes can shrink, thereby facilitating penetration of the skin via intercellular routes and pores of the SC that are much smaller than their own vesicles diameters. These vesicles are 10 times more deformable than conventional liposomes [18]. However, because the transfersome vesicle has limited entrapment capacity and content leakage of active ingredients still tends to occur due to water diffusion from dispersing media, a provesicular carrier has been developed, namely protransfersomes. Protransfersomes are lipid provesicles in the form of crystalline liquid which will turn into very flexible transfersomes vesicles *in situ* by absorbing water from the skin [19]. These characteristics enable the protransfersomes to protect the encapsulated materials as well as vesicular lipids from any unwanted chemical reactions such as hydrolysis and oxidation associated with degradation, and physical reactions such as sedimentation, aggregation, fusion, leakage of trapped substances, or hydrolysis of encapsulated active ingredients [20–22]. They extend shelf life and are capable of targeting materials encapsulated in the deeper layers of the skin [23–25].

Transfersome is widely reported to be employed in the topical and transdermal delivery of various active pharmaceutical ingredients. However, its applications to cosmetic delivery are limited. Moreover, the recent development of cosmetics is largely intended to improve the appearance of skin by having local biological effects on its tissues. Considering the similar physicochemical properties of the active ingredients

provides direct analogies for successful skin delivery using transfersome, thereby also rendering them prospective active cosmetic ingredients. This review will demonstrate the potential use of transfersome in enhancing active ingredient penetration which promotes optimal anti-ageing activity within the cosmetic delivery system. This, in turn, increasing their effectiveness in impeding skin ageing.

Methods

This review analysed the potential use of transfersome as a carrier in the delivery of anti-ageing cosmetics. The existing research into the use of transfersome in cosmetics is limited. As stated in this paper, considering the similar physicochemical properties of active pharmaceutical ingredients such as insulin and hormones is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients.

The method employed in writing this article review consisted of an electronic literature study involving the accessing of national and international journal search sites related to the keywords "ultradeformable vesicles", "transfersomes", "protransfersomes", "anti-ageing active compound", "protein for anti-ageing","topical delivery","skin delivery","deformability","skin penetration", and "topical drug classification system".

The eligibility criteria applied when selecting journal articles comprised original research, short case studies, experimental research design, and the year of publication falling within the period 1992-2020. The sites accessed for the purposes of conducting the search included Pubmed (Scopus & Scimago) and Google Scholar which contain search keywords. Articles published in predatory journals or publishers which include

review articles were excluded from the study. Identification, data correlation analysis, and paper selection were conducted on the basis of the following CONSORT diagram in Figure 1.

Result

Characteristics of protransfersomes and transfersomes as vesicular carriers for skin delivery

For topical delivery, transfersomes have many advantages relating to their high membrane elasticity and deformability. These can be achieved by combining two lipophilic or amphiphilic components, namely; phospholipids and biosurfactants at the appropriate ratio or formula to form bilayer vesicles [23]. In transfersomes, the surfactant as the EA, is in the form of a single chain surfactant capable of destabilizing the lipid bilayer and causing an increase in the fluidity and elasticity of the vesicular membrane with the result that the vesicles can change shape and pass through the pore intact by shrinking in size to one 5-10 times smaller than the original, thus increasing the penetration of the active cosmetic ingredients [26]. Protransfersomes, extremely flexible liquid lipid provesicles, provide benefits for improving the stability of transfersomes [19].

During application, the active ingredient interacts with the skin which is both attached and adheres to the SC. Due to the osmotic gradient resulting from the difference in water content of skin tissue, the active ingredient will be transported to the deeper layers of the skin by passing through the SC. Under light microscopy, the protransfersome, which is originally crystalline and lamellar-shaped, will turn into transfersome vesicles after hydration. This is due to the difference in the degree of

hydration of the surfactant and phospholipid molecules together with the change in the shape of the hydrated molecules. Because of its limited solvent content, the resulting protransfersome will form a compact palisade and vesiculation lamellae. The addition of water will further cause swelling of the bilayer and vesicless due to the interaction of the air with the surfactant head groups and tend to form a random spherical vesicles of transfersomes as presented in Figure 2 [27].

The deformability of the vesicles is influenced by the chemical structure of the surfactants, which surfactants with low hydrophilic-lipophilic balance (HLB) value generally can form smaller size vesicles. The surfactant concentration must also be proper, otherwise the vesicles will harden and be damaged [27]. Another role of surfactants is to increase the hydration properties of transfersome vesicles with the result that they tend to seek moisture in deeper skin layers after application to the skin hydrotaxis (xerophobia) [26].

Transfersomes can penetrate the skin layers by means of different mechanisms depending on their composition as presented in Figure 3, namely; (1) the vesicles maintain their intact shape (deformability) via the intercellular pathway, or (2) the vesicles fuse and mix with skin lipids (transcellular) due to destabilization of the membrane by surfactants, or (3) the vesicles go directly to deeper skin tissues via appendageal routes. Transfersomes can easily shrink to one-tenth of their original size in order to pass through the pore by means of a transdermal osmotic gradient due to differences in the water content of the skin surface, which is about 15 %, and the dermis that has high water amounted of 75 % [28]. This osmotic gradient helps the active ingredients pass through the skin passively via the hydrophilic ducts of the SC [17]. When the transfersomes are applied to skin in non-occlusive conditions, transfersomes

will dehydrate due to water evaporation, the hydrophilicity nature of which causes the vesicles to be attracted to a layer with a high water content, allowing the intact vesicles to penetrate via the intercellular spaces [29,30].

The osmotic gradient is high in the SC and decreases with skin depth to the stratum spinosum layer (0.042 mm) [31]. This osmotic gradient acts as a propulsion for most transfersomes vesicles with a total lipid mass of more than 0.1 mg/cm^2 within one hour. The occlusive means of application can cause at least 90 % of the drug to be retained in the SC due to excess hydration, with the result that only small levels of the drug can enter the bloodstream [21]. The SC is composed of corneocytes embedded in hydrophobic lipids that form a crystalline lamellar phase. The corneocytes are coated with cross-linked soft keratin [23]. The water content in the SC is not evenly distributed according to its thickness. A thin layer of water is in equilibrium with the surrounding water content, while the moisture content of a thicker layer of the epidermis is close to saturation level. Viable skin contains 70–80 % water, whereas the surface of the SC is drier than this viable dermis [24]. The electronic diffraction results of the biopsy specimens show that the water content regularly drops from 70-80 % in the stratum granulosum layer to 15-25 % in the upper layer, which means that the water is completely bound, even for fairly high water content values, in these skin layers [25].

Transfersomes act as encapsulating carriers for various active ingredients

Transfersomes as trapping carriers represent the first generation of elastic liposomes that can deliver various active ingredients with different lipophilicity and are able to encapsulate active substances with large molecular weights. Transfersome is widely reported as being used for topical and transdermal delivery of various active

pharmaceutical ingredients. However, the recent development of cosmetics is primarily intended to improve the appearance of the skin through localized biological effects on its tissues. Identifying the similar physicochemical properties of the active ingredients provides direct analogies for successful transfersome-based skin treatment, rendering them prospective active cosmetic ingredients. The active ingredients with hydrophilic properties will be encapsulated in the aqueous core, while the lipophilic substances is trapped within the lipid membrane layer. The process of encapsulating large molecules for subsequent penetration of deeper skin layers is that of forming a reservoir for slow and sustained release of the encapsulated substances, allowing for a reduction in the frequency of administration [32].

The encapsulation of active cosmetic ingredients with large molecular weight can be based on several studies of transfersome formulation for delivery of proteins, such as hormones, stem cells, and ribonucleic acids (RNAs) [29,32,33]. The reverse phase evaporation method for transfersome formulation has been used in the encapsulation of hydrophilic polypeptide molecules e.g. Insulin, by using Sodium Cholate as the EA with an entrapment efficiency of up to 81 % [34].

Table 1 shows the use of transfersomes for loading various types of active ingredients that are proven to improve stability, penetration, and effectiveness, while reducing the toxicity to enable their use as the references for ultradeformable vesicles formulation of cosmeceutical active ingredients with similar physicochemical properties. The encapsulation methods of active ingredients in transfersomes depends on the solubility and permeability as follows:

a. Protein molecules

Protein is known as an active ingredient with a large molecular weight that has been used in skin care. An appropriate delivery system is required to ensure that this active ingredient is stable and can penetrate the skin to produce therapeutic effects. A number of proteins have been formulated into transfersomes, one being insulin whose particle size can be reduced to 100 nm and which can easily penetrate deeper skin layers producing a hypoglycemic effect when compared to conventional insulin vesicles via transdermal route [29]. This is because insulin, which is composed of large molecules and demonstrates high affinity, is distributed in the skin by interstitial fluid flow through the lymphatic system in the skin dermis layer in the presence of lymphatic vessels and capillaries [34]. These anatomical characteristics can be utilized in transdermal delivery of such proteins [78].

Apart from insulin, the progestin hormone used for the purposes of oral birth control or contraception has also been formulated as transfersomes, namely; Norgestrel, which is composed of soya phosphatidylcholine and sodium cholate at a weight ratio of 90:10, and Levonogestrel containing the same elements at a weight ratio of 85:15. Transfersome has been shown to increase transdermal penetration, double contraceptive

effectiveness, enhance active ingredient stability, augment entrapment coefficiency, and facilitate greater reproducibility [22,79].

The use of Small interfering RNA (siRNA) and microRNA in the treatment of atopic dermatitis which is formulated as transfersome has been shown to increase effectiveness and reduce side effects. RNA which is enzymatically degraded and has low membrane permeability can be delivered to the deeper layers of the skin using a gene carrier with the addition of penetration enhancers containing cysteine, arginine and histidine. Such enhancers work through different mechanisms. The arginine residue forms a complex with siRNA. The histidine portion allows the complex to escape the endosome, while the cysteine constituents stabilize and release siRNA in a reducing environment. Peptide modification with stearic acid further stabilizes the complex through hydrophobic interactions. Formulated with the small unilamellar vesicles fusion method, the siRNA particle size can reach 70 nm and is protected from enzymatic degradation. The increased effectiveness of siRNA as a regulator of cytokine production, leading to a reduction in inflammatory cytokines in mice, indicated that transfersomes successfully deliver siRNA transdermally [32]. Transfersomes composed of Phospholipon® 90G and Brij® O20 combined with sponge Haliclona sp. spicula (SHS) for siRNA delivery, which acts as enhancers by making many micro channels, approximately 800 micropores per mm^2 for 48 hours at a dose of 10 mg / 1.77 cm^2 SHS through and into the SC, thereby successfully facilitate protein penetration to the deeper layers of the skin [80].

Cristiano et al. (2020) [60] have also formulated the enzyme product of Sulforaphane (1-isothiocyanate-(4R)-(methylsulfinyl)-butane) encapsulated within transfersomes consisting of Phospholipon 90G and sodium cholate at a weight ratio of

88:12, using the thin layer evaporation method for melanoma therapy. The use of transfersomes has been shown to increase penetration into the deeper layers of the skin, thereby increasing its anti-cancer activity.

The new paradigm emphasizes stem cells as an attractive biotechnology product to be formulated as anti-ageing cosmetics. The effectiveness of stem cells in regenerating damaged cells due to oxidant-induced shortening of telomere chromosomes has also been studied [81,82]. Stem cells possess the unique characteristic of being unspecialized and, as such, able to reproduce themselves repeatedly through asymmetric division [83]. Derived not only from animals, stem cells from plants are also used as cosmetics after being made into standardized stem cell extracts [84]. The characteristics of stem cells products or extracts which have large molecular weights and are unstable for transdermal preparations have prompted researchers to utilize nano-sized delivery systems, one of which is elastic liposomes which can reduce particle size <100 nm as a means of facilitating penetration into deeper skin layers [33].

It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,85,86]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [87]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,88]. The ability of transfersomes to encapsulate hydrophilic substances inside the

vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis.

b. Active substances with low solubility and high permeability

Transfersomes, which can load active ingredients characterized by low solubility and high permeability, can have an effect by means of several methods, namely; high pressure homogenation, modified coacervation phase separation and conventional thin film hydration. Active ingredients belonging to this group include Resveratrol, Quercetin, Glimepiride, Diclofenac, Ketoprofen, Rifampicin, Nifedipine, Raloxifene, and Retinyl Palmitate [23,25,28,40,63,71,74,75].

Resveratrol which has an antioxidant effect has been widely combined with various active ingredients including Psoralen which, in combination with Ultraviolet A, can stimulate melanin production and tyrosinase activity in melanocytes. The resulting transfersomes vesicles have a homogeneous particle size, are stable and demonstrate high trapping efficiency with the result that the use of transfersomes enhances the effect of the combination of both active ingredients and is able to inhibit the increase of free radicals for vitiligo therapy [37]. Arora et al. (2020) [25] prepared transfersomes using Central Composite Design and found that the transfersomes composed of cholesterol hydrochloride (DC-Chol), cholesterol (Chol) and sodium deoxycholate (SDC) can increase the depot effect on the skin. The addition of a cosmetic base cream and gel has no change effects on the physical characteristics of the vesicles. On the contrary, it can increase acceptability during use.

Quercetin, which is a phytoestrogen employed in osteoporosis therapy, has low bioavailability when taken orally. Therefore, Pandit et al. (2020) [28] formulated

Quercetin in transfersome using a fractional factorial design optimized by a complete factorial design. The results showed that quercetin loaded in transfersomes, prepared with Phosphatidylcholine and Tween 80 at a weight ratio of 2 : 1, has a homogeneous and stable particle size and can increase therapeutic effectiveness by topical administration of its transfersomal system indicated by femoral thickness, length, and density and also serum biochemical parameters such as calcium, phosphorus, alkaline phosphatase, and tartrate-resistant alkaline phosphatase.

Transfersomes are also used for delivery of Glimepiride, which is an oral anti-diabetic drug. The side effects of hypoglycaemia, as well as digestive and hepatic disorders that often occur, can be reduced by the ability to release it gradually, thereby increasing patient compliance. The Box-Benkhen design was used in the transdermal transfersome formulation which has a weight ratio of Phospholipids: Sodium Deoxycholate: Glimepiride = 200: 45: 1. Positive vesicles characteristics were obtained, thereby increasing effectiveness due to high penetration into skin deeper layers which showed a higher penetration flux than Glimepiride suspension [40]. Increased drug bioavailability which reduced both the side effects on gastrointestinal tracts and the long term therapeutic effects due to lower quantities of drugs being used during the therapy was superior to the oral administration of Glimepiride.

El Zaafarany et al. (2010) [89] studied a comparison of the characteristics of the Diclofenac topical transfersome prepared by means of two manufacturing methods, active ingredient content, phospholipid-EA ratio, and using five variations of surfactants as EAs. The preparation methods used are vortex-sonication and rotary evaporator-sonication. The manufacturing method has a significant effect, while the transfersome prepared by the rotary evaporator-sonication method produces higher trapping

efficiency than the vortex-sonication method due to perfect hydration of the vesicles. In contrast to the vortex method, visual observation indicates that lipids tend to collect and adhere to the vial walls, rendering hydration of the vesicles difficult. The vortex method is unable to disperse lipids completely, resulting in a clumpy dispersion, difficult homogenizing and susceptibility to rapid sedimentation and aggregation [89].

Adding a specific amount of the active substance to the transfersomes affects the loading capacities. Consequently, if it exceeds the optimal capacity of the vesicles, precipitation of active ingredients will occur. The phospholipids-EA ratio also greatly affects transfersomes vesicles characteristics. Optimum deformability is obtained from the phospholipids-EA ratio of 85:15. If the amount of phospholipids is excessive, vesicless will form with low deformability due to a lack of surfactant. A similar phenomenon will occur if too great a quantity of the surfactant is added due to the formation of a rigid micelle mixture [89].

The use of various types of surfactants possessing different chemical structures also results in contrasting vesicles characteristics. Comparing the effect of surfactant types with the optimal phospholipid-surfactant ratio, it was found that the vesicless containing Tween 80 had the highest deformability. This is due to the fact that Tween 80 is composed of flexible, non-bulky hydrocarbon chains. In contrast, the sodium cholate has lower deformability due to its steroid-like structure which is larger than the hydrocarbon chain of Tween 80. From the entrapment efficiency value, the largest order is the system containing Span 85> Span 80> Na cholate> Na deoxycholate> Tween 80 [89].

The anti-tuberculosis drug Rifampicin prepared as a transfersome can be improved for its transdermal bioavailability and patient compliance due to continous

drug release. A comparison of the base of the gel and the suspension confirmed the particle sizes to be similar, but the zeta potential of the gel was more negative because of the acidity of carbopol as the gelling agent. In addition, the permeation value, depot effect, and bioavailability of gel preparations were greater due to the composition of the formula containing Phospholipon 90G and Tween 80 at a weight ratio of 15: 7 between Ethanol, and D-limonene [71].

Nifedipine, an anti-hypertensive drug, constitutes a transdermal protransfersome preparation produced by coacervation phase separation method. The protransfersome consists of Phospholipid and Sodium Deoxycholate at a weight ratio of 85:15 produced a bioavailability 6.5 times greater than that of oral administration. This is supported by a high entrapment efficiency of up to 97 % and the increase in penetration ability up to three times greater than the drug suspension triggerring an increase in its anti-hypertensive effectiveness [63].

The selective estrogen receptor modulator, Raloxifene Hydrochloride, is an active therapeutical compound used in the treatment of breast cancer and osteoporosis, but has low bioavailability. Mahmood et al. (2014) [26] succeeded in increasing its bioavailability by formulating it into the transfersomes for transdermal delivery. The formula was designed with the Box-Behnken design composed of Phospholipon® 90G and Sodium Deoxycholate at a weight ratio of 300: 35, resulting in vesicles with high entrapment efficiency, good stability, and high penetration rates.

In the other study, Retinoids, which affect maturation of skin epithelial cells, can cause skin disease if the levels are low or reduced. Therefore, an external supplement in the form of Retinyl Palmitate can be used. Having low solubility, transfersome for transdermal delivery was prepared with a weight ratio of Phosphatidylcholine: Tween

80 of 18: 1. It succesfully promotes skin penetration as evidenced by the discovery of Retinyl Palmitate in various layers of the skin, with the result that the transfersomes can be used as a carrier for active ingredients with similar characteristics [75].

The capability of transfersome to encapsulate hydrophobic molecules within the lipid bilayer would enable modification of physicochemical properties of active ingredients encapsulated in carriers which are nano-sized particles, amphiphilic self-assemble phospholipid with surfactant presence, thus affecting their dispersability, solubilization, and releases to aqueous media at the intended sites, especially dermis for antiaging therapy [90].

c. Active substances with high solubility and low permeability

Within this category, there are several active substances including epigallocatechin-3-gallate (EGCG), 5-Fluorouracil, and methotrexate. The study of the use of transfersome for delivery of a combination of EGCG and hyaluronic acid as an antioxidant for topical application has been reported. The transfersome was prepared by a combination of thin layer hydration and high pressure homogenization methods. The formula optimization was performed using a Box-Behnken design which is prepared with Phosphatidylcholine: Sodium Cholate at a weight ratio of 85: 15, respectively, resulting in increased UV protection promoting its anti-oxidant and anti-aging effects [38].

The use of the transfersomes for transdermal delivery of methotrexate can increase the effect of drug deposition in the skin and can release the drug efficiently. The transfersomes prepared at a Phosphatidylcholine: Tween 80 weight ratio of 7:3 are superior to conventional liposomes in delivering drugs into the deeper layer skin [91]. In combination with Resveratrol, they can increase the anti-cancer activity of skin

melanoma and some squamous cell carcinomas such as actinic keratosis, Bowen's disease, and keratoacanthoma [44].

Methotrexate was formulated by extrusion method using Phosphatidylcholine and with two types of EAs, i.e. Tween 80 and Sodium Cholate, to compare its physicochemical characteristics and penetration abilities across skin [46]. From the study, it is clear that the resulting transfersome has a homogeneous and stable unilamellar and can increase the penetration of the methotrexate into the skin layer by up to five times. As the EA, Tween 80 was more effective at increasing vesicles deformability than Sodium Cholate [46].

Transfersome vesicles possess the ability to modify the permeability of active ingredients due to encapsulation within the carrier which can change passive diffusion into active transport, allowing low permeable active ingredients to permeate biological membranes. The use of biomimetic phospholipid as a component of transfersome would enable vesicles to carry active ingredients via the paracellular or transcellular routes among others, or through fusion with the cell membrane. This underpins the potential of transfersome to deliver active ingredients promoting dermal repairs and rejuvenation [92].

d. Active substances with low solubility and low permeability

In transdermal delivery, the active ingredients should be dissolved to maximally penetrate the skin. To overcome the problem of solubility and low permeability of active ingredients, transfersomes are used as the carriers as they have been shown to successfully deliver active ingredients to the deeper layers of the skin, including the Curcumin, Psoralen, Cisplatin, Paclitaxel, and Ketorolac [35,37,43,51,93]. The low

bioavailability of Curcumin can be increased by transfersomes prepared with purified Phosphatidylcholine (Epikuron^{TM} 200) as the phospholipids and the surfactant, i.e. Sodium Cholate, at a weight ratio of 85:15 using a thin layer hydration method followed by extrusion. Their nanovesicles characteristics, including small and homogenous particle size with high entrapment efficiency up to 93.91 % and loading amount of 7.04 %, prove useful in increasing anti-tumor activity [35].

Cisplatin, a platinum chemotherapeutic agent, has been known to produce profoundly toxic effects on healthy cells. Transfersomes composed of Soya Lecithin and Sodium Cholate at a weight ratio of 17: 3 induced a gradual release, thereby reducing the side effects. Cisplatin, either alone or together with a stabilizer such as a combination of Soya Lecithin: Pluronic: Sodium Cholate ratio at respective weight ratios of 17:1.5:1.5 or other antioxidants produced positive nanovesicles characteristics with small and homogenous particle size and high entrapment efficiency up to 97.97 %, thus increasing anti-cancer effectiveness in skin melanoma therapy [19,42,94]. The use of protransfesome and transfersome also improved cisplatin levels in plasma during transdermal application, which proves these ultradeformable vesicles successfully enhance penetration of low soluble and low permeable drug such as Cisplatin [14].

In addition, as a potent analgesic, Ketorolac can be formulated for transdermal delivery which has the advantage of gradual release, thus reducing the gastrointestinal side effects that often accompany it. To overcome the low solubility and permeability of ketorolac, Nava et al. (2011) [51] succeeded in formulating Ketorolac into the transfersome, consisting of Epikuron^{TM} 200 and Tween 80 at a respective weight ratio of 86:14. The transfersome has a particle size of approximately 127.8 nm at low polydispersity index, a relatively neutral charge with a zeta potential value of -12 mV

and high entrapment efficiency of 73.11 %. Moreover, its release is delayed in character causing it to remain in the skin for a long period, thus producing local therapeutical effects [51].

According to these results, transfersome and protransfersome are able to improve the solubility and permeability of active ingredients with low water solublity and poor permeability. Their ability to entrap hydrophobic molecules within the lipid domain of the bilayer membrane as well as the amphiplic properties of the phospholipids used in transfersomes significantly improve the solubility and permeability of such compounds, rendering them useful in delivering active cosmetic ingredients.

In vitro **evaluations of transfersomes and protransfersomes**

Several nanocarrier lipids, both conventional and elastic liposomes, have different characteristics of vesicles shapes depending on their constituent components, namely; surfactant for transfersomes and ethanol for ethosome. From microscopic observation, it is clear that all of them are spherical vesicless, but have different vesicles sizes as can be seen in the Transmission Electron Micrographs (TEM) [60].

During hydration in the presence of water, the protransfersome gel with lamellar appearance transforms into transfersome due to the hydrating fluid being absorbed by the gel system [95]. This hydrated gel forms spherical vesicular structures due to the different degrees of hydration between surfactants and phospholipids. Starting from the protransfersome with a limited amount of solvent, a mixture of lamellar liquid crystals is formed which resembles the interrelated palisade and vesiculated lamellae. The addition of excess water will cause swelling of the lipud bilayer due to the interaction of water with the surfactant hydrophilic groups above the solvent threshold concentration

with the result that the bilayer randomly forms a spherical structure which resembles a vesicles [22] and can be described as presented in Figure 2.

The increase in vesicles deformability is also evidenced by the increasing amount of active ingredients penetrating the skin which becomes the important factor in efficient skin permeation. This deformability is highly influenced by the presence of an EA in the form of a single chain surfactant with a high radius of curvature which renders the vesicles unstable and enables the double layers of vesicless to easily change shape [96]. EAs reduce the energy required to deform the vesicles with the result that transfersomes vesicles can flex to pass through tiny pores in the skin or through intercellular gaps [26]. However, this deformability can be reduced when the amount of surfactant increases [72].

The lipid lamellae in the SC have high proportion of negatively charged lipids [97]. Consequently, the ionically-charged surfactant affects the penetration of the active substances. Vesicles with cationic surfactants can increase the penetration of the active substances to a greater extent than anionic or non-ionic surfactants as revealed by the considerable fluorescent intensity of labelling agent entrapped in transfersomes. This result is due to electrostatic attraction to the SC which contains mostly negatively charged lipid lamellae. This difference in charge can strengthen the interaction between cationic transfersomes and intracellular lipids [96].

Release study of active substances from the carrier can be used to predict how the carrier can deliver active ingredients and produce therapeutic effects before being tested *in vivo* which is an expensive process. In the *in vitro* release test using Franz diffusion cell, the active substances release from transfersomes is limited by two barriers, namely; the phospholipid and the dialysis membrane. The concentration of EA has an effect on

the release of active ingredients which is directly proportional. If the concentration is low, then the release of the active substances is similarly low. This is because the lipid membrane becomes regular and does not leak easily. Meanwhile, if the concentration of EA is excessive, the vesicless will be stiffer with the result that they leak easily and are less sensitive to osmotic gradients [27]. Pena-Rodríguez et al. (2020) [75] studied the penetration of the Retinyl Palmitate by comparing transfersomes composed of Phosphatidylcholine and Tween 80 with free active ingredients. It was found that about 69 % of conventional Liposomes loaded Retinyl Palmitate could not penetrate the skin and it was only 2% reached the epidermis to be retained in the SC. Lipid vesicles can act as a reservoir system for the continuous delivery of active cosmetic ingredients. However, the vesicles of the anionic surfactant deviate from the first-order kinetics of drug release following the diffusion flow of the skin [96].

El-Alim et al. (2019) [55] compared the release rate of Diflunisal in solutions with those of liposomes, ethosomes, and transfersomes. The results showed that within two hours the amount of Diflunisal released from the solution was 84.52 %, while that from the liposomes, 68.10 %; ethosomes, 58.21 %; and transfersomes, 65.88 %. The peak level of Diflunisal release in solution is reached within three hours, whereas Diflunisal in vesicles continues for up to five hours before reaching peak levels.

In vivo evaluations of transfersomes and protransfersomes

From several studies it is known that skin penetration by drugs can be via intercellular or transcellular routes. Transfersomes can pass through these routes due to their elastic properties and the water concentration gradient in the skin layer. The nature of this tendency to attract water triggers the vesicles' ability to penetrate the deeper

layers of the skin because of their higher water content. After entering the dermis, the active substances will circulate through the blood vessels to the systemic blood circulation. Due to the higher drug penetration, effectiveness also increases.

The pharmacokinetic study of mice conducted by Jain et al. (2005) [22] indicated that, Levonorgestrel levels in blood plasma are very low for free active ingredients, which was at 0.015 ± 0.005 µg/mL, in contrast to the transfersomes-loaded levonogestrel which reaches levels of 0.139 ± 0.050 µg/mL after topical aplication. The level rises to approximately eight times higher within four hours and is maintained for up to 48 hours. Therefore, it can be proved that by using transfersomes, levonorgestrel can be gradually released over a protracted period.

A similar study was performed by Hussain et al. (2020) [71] which compared the plasma levels after oral administration of Rifampicin and transdermal application of transfersomes-loaded Rifampicin. The comparative data for C_{max} and T_{max} indicated levels of 10.5 ± 1.4 µg/mL after 2.0 hours and 6.9 ± 0.80 µg/mL after 10.6 hours respectively for oral administrations. Meanwhile, the AUC value of Rifamipicin after 24 hours for oral administration was 41.71 ± 5.2 µg/ml, while for transdermal application it was 56.23 ± 2.7 µg/ml. This suggests that the use of transfersomes for transdermal administration can increase the systemic availability of Rifampicin by reducing the dose-related side effects as well as the toxicity of the orally administered Rifampicin.

An *in vivo* test using tape stripping was used by Fernández-García et al. (2020) [69] to compare Amphotericin B levels in the SC and dermis after Amphotericin B transfersomes application to undamaged skin and by microneedle use. This study proves that Amphotericin B transfersomes can penetrate to the deeper layers of the skin, whereas using a microneedle before the application of Amphotericin B transfersomes

results in increased penetration of the active ingredient during the first hour, especially in deeper skin areas. The use of microneedles produces temporary skin micropores that aid drug delivery throughout the skin. However, these micropores close within two hours and scar tissue is formed which can reduce the surface area for the active ingredient [29]. In this study, there was no significant difference in the degree of skin penetration between transfersomes-loaded amphotericin B and Amphotericin B added to dimethyl sulfoxide (DMSO) as a skin penetration enhancer. This study proved that transfersome is capable of acting as an enhancer in itself.

Transfersomes are largely evaluated *in vivo* through the use of both human and animal subjects. In human subjects, the transfersomes can be assessed for their Transepidermal Water Loss (TEWL) value both before and after application. From the results of the tape strip, it is known that there is no significant difference in the TEWL value, therefore confirming that the transfersomes does not affect skin integrity [75]. Although transfersomes can act as a depot for epidermal absorption, the SC is desquamated with the result that the active ingredient can be lost. On the other hand, by using transfersomes, about 63 % of the Retinyl Palmitate successfully penetrated the epidermis. The fluorescent photomicrographs of transfersomes contained nile red indicating that transfersomes can deliver active ingredients penetrating the deeper layers of the skin [75]. Moreover, the fluorescence correlated with transfersomes was extensively observed in the space between the corneocytes in the epidermis [19].

Arora et al. (2020) [25] studied penetration of the antioxidant Resveratrol by transfersome carriers composed of Soya phospholipids and Sodium Cholate at a weight ratio of 85:15. At the appropriately high phospholipid content level, the lipophilic Resveratrol can be trapped within the lamellar lipids of vesicles. The use of

transfersomes succesfully increased the penetration of Resveratrol, thus improving the *ex vivo* antioxidant activity as determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. This improved effectiveness is due to an increased flux of active ingredients by disrupting the SC barrier through an amalgam effect of a combination of phospholipids and surfactants. In addition, the skin-penetrated amount of vesicles-entrapped active ingredients is increased due to the longer residence time in the skin.

In albino Wistar rat subjects, the application of transfersome-loading Timolol composed of phosphatidylcholine : Span 80 and Tween 80 at a weight ratio of 3:1 to the shaved back skin was observed for the occurrence of erythema and edema compared to conventional liposomes. Neither erythema nor edema occurred after this *in vivo* application [24].

Discussion

Based on the review, the formulation of the ultradeformable vesicles i.e. transfersomes and protransfersomes can be seen to increase the effectiveness of active ingredients due to improvements in their physicochemical characteristics and skin penetration. With the combination of phospholipids that resemble skin membranes and the addition of surfactants as EAs, the formation of vesicles can reduce the particle size enabling them to easily penetrate the intercellular gaps and skin pores. The ability to deliver active ingredients with the various characteristics of lipophilicity, solubility, permeability, and large molecular weight including proteins (RNA, hormones) also constitutes an advantage of this delivery system. Transfersomes can be applied in the cosmetics industry because the research conducted indicates that the use of a base preparation including gel and cream neither changes the skin penetration profile nor reduces the

effectiveness of the active ingredient. Rather, it can increase the length of time the drug remains in the skin and product acceptability [8, 94].

It is expected that transfersomes and protransfersomes can potentially be used in the cosmetic field with local biological effects, especially in anti-aging products. Skin ageing is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation, and disrupt the dermal extracellular matrix [99,100]. However, active cosmetic ingredients used in anti-ageing therapy such as CoQ10 and AMSC-MP, among others, suffer from skin penetration-related drawbacks including low water solubility and large molecular weight. The use of transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, i.e., the dermis, which is composed of almost 70% collagen [101]. With the increased skin penetration, the effectiveness and stability cosmetics products will be improved, providing potential use for beauty and health.

Conclusions

Transfersomes and protransfersomes demonstrate encouraging potential for use in cosmetics, especially anti-aging products. The use of phospholipids and EAs in these carriers has benefits for producing nanovesicles with desirable characteristics supporting high skin penetration, thus increasing the effectiveness of active cosmetic ingredients.

FUTURE PERSPECTIVE

Delivering cosmetic active ingredients to target sites, especially for agents affects biological functions can be ultimately supported by appropriate delivery carriers. This

review represents the underlying researches in topical or transdermal delivery of active ingredients to the development of therapeutic products for esthetic medicines and cosmetics. A positive approach of the use of ultradeformable carriers, which is called Transfersomes, and its provesicular states namely Protransfersomes, has been largely explored to improve skin penetration by utilizing the nature characters of phospholipids and EAs forming intact flexible vesicles passing through intercellular gaps. As delivery carriers, these deformable vesicles is highly potentials for transporting either hydrophobic or hydrophilic molecules with low or even large molecular weight such as protein to penetrate into deeper skin tissues, which become the main target sites of most cosmetics, especially for anti-ageing therapy. Further explorations and investigations is definitely required to comprehensively evaluate the potential use of ultradeformable vesicles in improving the efficacy of cosmeceuticals, which is currently still limited.

EXECUTIVE SUMMARY

Transfersome encapsulates various active ingredients

- Transfersome is a vesicular drug carrier that consists of bilayer membrane composed of phospholipids and biosurfactants at the appropriate ratio surrounding inner aqueous phase
- The active cosmetic ingredients with hydrophilic properties will be encapsulated in the aqueous core, while the lipophilic active ingredients is trapped within the lipid membrane layer.
- Transfersomes also provide possibility for encapsulating active cosmetic ingredients with large molecular weight

Ultradeformable liposomes as vesicular drug carriers for skin cosmetics

- Ultradeformable vesicles vesicles can change shape and pass through the pore intact by shrinking in size to one 5-10 times smaller than the original due to transepidermal osmotic gradient
- Transfersomes can penetrate the skin layers by means of different routes, namely; (1) intercellular pathway, or (2) transcellular route, and (3) via appendageal routes

Ultradeformable liposomes impore skin delivery of active cosmetic ingredients

- Lipid vesicles of transfersomes can act as a reservoir system for the continuous delivery of active cosmetic ingredients
- Transfersomes is capable of acting as an enhancer in itself
- Skin-penetrated amount of transfersomes vesicles-entrapped active ingredients is increased due to the longer residence time in the skin
- Neither erythema nor edema occurred after the *in vivo* application of transfersomes

Acknowledgement

None.

Financial Disclosures

None.

Conflict of Interest

The authors declare that no conflicts of interest for this study.

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Table 1. The utilization of transfersomes and protransfersomes as delivery carriers for various active cosmeceutical agents

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
Antioxidant	Curcumin	Low	Low (Large MW)		Transfersomes	[35,36]
	Resveratrol	Low	High	Low	Transfersomes	[25]
	Psoralen (+ Resveratrol)	Low	Low		Transfersomes	[37]
	Epigallocate-chin-3-gallate (EGCG)	High	Low	Low	Transfersomes	[38,39]
Anti-diabetic agent	Glimepiride	Low	High		Protransfersomes	[40,41]
Anti-cancer	Cisplatin	Low	Low		Protransfersomes	[14,19, 42]
	Cisplatin	Low	Low		Transfersomes & Protransfersomes	[14]
	Cisplatin + Imuquimod	Low	Low		Protransfersomes + Carbopol	[43]
	5-	High	Low		Transfersomes	[10,44]

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
	Fluorouracil (+ Resveratrol)					
	Methotrexate	High	Low		Transfersomes	[45] , [46], [47]
Analgesic	Ketoprofen	Low	High		Protransfersomes	[48], [23], [49]
	Ketorolac	Low	Low		Protransfersomes	[50]
					Transfersomes	[51], [12], [52]
	Diclofenac	Low	High		Protransfersomes	[53], [54]
	Diflunisal	Low	High		Transfersomes	[55], [56]
Hormone and protein	Levonoges-trel	Low	Low		Protransfersomes	[22,57]
	Norgestrel	Low	Low		Protransfersomes	[58]
	Insulin		Low	Low	Transfersomes	[34],

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
						[29]
	siRNA		Low	Low	Transfersomes	[59]
	Sulforaphane		Low	Low	Transfersomes	[60]
	Phytoestro-gen Quercetin	Low	High	Low	Transfersomes	[28], [61]
Anti-hipertentive agent	Timolol	Low	Low		Protransfersomes	[27], [24], [62]
	Nifedipine	Low	High		Protransfersomes	[63], [64]
	Minoxidil + Caffein	Low	Low		Transfersomes	[65], [66],[67]
Anti-infection	Azaleic Acid	Low	Low		Transfersomes & Protransfersomes	[20]
	Amphotericin B	Low	Low		Transfersomes	[68], [69], [70]
	Rifampicin	Low	High		Transfersomes	[71], [72]
Selective	Raloxifene	Low	High		Transfersomes	[73],

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
estrogen receptor modulator (SERM)	hydrochlo-ride					[74]
Vitamins	Retinoyl Palmitate	Low	High	Low	Transfersomes	[75]
	Tochopherol	Low	High	Low	Transfersomes	[76]
Herbal products	Emu oil from *Dromaius novaehollan-diae*	Low	Low		Transfersomes	[77]

Figure Legends

Figure 1 The consort diagram of article screening and selection

Figure 2 The physical formation of transfersomes from protransfersome gel

Figure 3 The presence of transdermal osmotic gradient leads to skin penetration of transfersomes via transcellular and intercellular routes into deeper skin layers

Reparaphrase: Under the mechanical stress due to trans-epidermal osmotic gradient force, EAs will be transferred to areas of higher curvature or pressure in the lipid bilayer. This process causes changes in the shape and volume of the transfersome vesicle with minimal energy requirements. In addition, the addition of EA can reduce the ordered degree of the arrangement of phospholipid molecules within spaces in the lipid bilayers significantly reducing the transition temperature of the bilayer membrane of transfersome [17].

Page 12 line 9: "The current use of siRNA can be an alternative method for anti-aging therapy which it is known that siRNA can regulate the expression of certain genes that are closely related to skin aging [81]. This is expected to increase the ability of skin cells to repair themselves, where in aging skin, the skin layer tends to become thin and easily damaged due to decreased skin matrix production and lipid synthesis, lower antioxidant capacity, and hyperpigmentation [82]. However, there are several obstacles related to skin penetration of this oligonucleotide, including the large molecular size so that it is difficult to enter the skin layer, as well as its negative charge so that difficult to be internalized by the cells [83,84]. The use of nanocarriers sucah as transfersome to encapsulate and modify natural properties of this siRNA could be improving biological efficacy and stability for cosmetic delivery."

Page 14 line 27: "In cosmetics, Resveratrol has been shown to increase the proliferation of fibroblasts, thus increasing the production of collagen matrix having good potential to be used in anti-aging therapy [92]. In addition, its high antioxidant capacity has an important role in preventing oxidative damage to cells in skin tissues due to UV exposures and slowing down the photo-aging process [92,93]. Although it has good permeability for topical delivery [93], however, resveratrol has low water solubility and great issues in its stability [94,95]."

Page 14 line 55:" Quercetin, a polyphenol compound, has been reported having anti-fibrotic effect that is able to reduce skin's car formation and accelerate wound healing [96]. The use of Quercetin has also been reported effectively protect human skin tissues from photoaging through inhibition of matrix metalloproteinase-1 expression preventing collagen degradation [97,98].

However, the use of Quercetin is largely limited by its low water solubility with partition coefficient value of 1.82 [98,99]"

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Page 19 line 30 : Ketorolac-like active cosmetic ingredient, such as Ascorbic Acid [121],,.."

Page 19 line 59 : "Numerous reports showed that Curcumin can be the potential agents for reversing aging. Its high antioxidant capacity protecting from bad effects of free radicals, as well as anti-inflammatory effects could stimulate production of TGF-β, thus stimulating fibroblast and inducing extracellular matrix production and angiogenesis, that play big role in skin health and repairs [124–127]. Curcumin has been known to have low water solubility and poor permeability for oral and topical delivery [128]."

Page 20 line 19 : "It has been known that Cisplatin, a platinum chemotherapeutic agent, has a very low skin penetration, which the main route is through skin appendages [129]. Moreover, Cisplatin also has limited solubility in water, thus it is often requires solubilizing agents as well as absorption enhancers to improve it [130]."

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ABSTRACT

Skin ageing is a phenomenon resulting in reduced self-confidence, thus becoming a major requirement for social determinants of health. The use of active cosmetic ingredient can help prevent skin aging. Transfersome is well known to be capable of deeply penetrating the dermis. This scoping review provides an insight into transfersome and its prospective use in anti-ageing cosmetics. Numerous reports exist highlighting the successful skin delivery of therapeutic agents such as large molecular, low water soluble, and poorly permeable active ingredients by means of transfersome. Moreover, *in vitro* and *in vivo* studies have indicated that transfersome increases deposition, penetration, and efficacy of active ingredients. However, the use of transfersome in the delivery of active cosmetic ingredients is limited. Considering their similar physicochemical properties, transfersome should possess considerable potential as a delivery system for anti-aging cosmetics.

Keywords: transfersome, ultradeformable vesicle, cosmetic, social determinants of health, scoping review

Background

Skin ageing is a process of changing physical appearance that can reduce an individual's self-confidence. These skin changes are closely related to ones in the balance of the production and decomposition of collagen, elastin and glycosaminoglycans which constitute quality parameters of the dermis layer [1,2]. There are several triggers which can be internal physical factors such as Deoxyribonucleic Acid (DNA) damage due to Reactive Oxygen Species (ROS), the development of chronic diseases, and metabolic disorders connected with ageing, or external factors including exposure to sunlight and oxidant materials resulting in the skin losing elasticity and firmness and the appearance of wrinkles [1–3]. Wrinkles are a sign of ageing skin caused by collagen degradation and these visible skin folds can have an impact on quality of life and physical appearance [4].

Anti-ageing strategies that have been implemented include protection against ultraviolet (UV) rays, invasive procedures, and ski ncare products or cosmetics, for example, sunblocks. Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of local biological active cosmetic ingredients. Anti-ageing ingredients have become a popular means of improving intrinsic skin biological function. Therefore, this compound must be able to penetrate the barrier of the *Stratum Corneum* (SC) in order to reach the dermis layer and rejuvenate and repair skin wrinkles.

Active anti-ageing cosmetic ingredients such as Coenzyme Q10, which demonstrates low water solubility [5,6], growth factors such as Epidermal Growth Factor (TGF), and Transforming Growth Factor-β (TGF-β) are contained in amniotic membrane stem cell metabolite products (AMSC-MPs) that have a significant molecular

weight [7,8], vitamins, and other herbal and biological products [9]. These compounds should possess different physicochemical characteristics. However, only small molecules less than 500 Da in size and lipophilic molecules with log P values between -1 to 4 can penetrate the SC which constitutes the skin barrier [10,11]. Therefore, the use of nanoparticulate carriers in skin delivery has the potential for anti-ageing cosmetics to improve the decreased quality of the dermis layer in aged skin. The presence of active cosmetic ingredients within a nanocarrier system in the epidermis and dermis layers indicates that they can promote collagen and elastin repair activity which enhances skin firmness [12].

Certain nanocarriers such as liposome, transfersome, glycerosome, ufosome, and hybrid vesicle have been developed to improve skin drug delivery. Liposome is a lipid-based vesicular carrier consisting of an inner water phase surrounded by lipid bilayer membranes [13]. The addition of softening bilayers such as surfactants to liposome can produce transfersome [14], while the use of glycerol as the edge activator of liposomal bilayer membrane generates glycerosome [15].

Transfersomes represent the first generation of elastic liposomes which demonstrate liposome-like characteristics with the ability to deform and reform their shapes. Liposome is known to provide three different environments for substances entrapped inside them, namely; the lipid-water interface, the hydrophobic nucleus, and the aqueous interior. Thus, liposomes can entrap hydrophobic, hydrophilic, or amphiphilic active ingredients within their structures, in addition to improving their stability. With the presence of *edge activators* (EAs), the vesicles can become elastic resulting in their ability to enhance the penetration by active cosmetic ingredients, thus enabling them to reach deeper skin layers [16]. Under the mechanical stress resulting

from trans-epidermal osmotic gradient force, EAs will be transferred to areas of higher curvature or pressure in the lipid bilayer. This process causes changes in the shape and volume of transfersome vesicles with minimal energy requirements. Moreover, the addition of EA can disrupt the ordered arrangement of phospholipid molecules within spaces in the lipid bilayers, significantly reducing the transition temperature of the transfersome bilayer membrane [17].

Transfersomes can shrink, thereby facilitating penetration of the skin via intercellular routes and pores of the SC that are much smaller than their own vesicles diameters. These vesicles are 10 times more deformable than conventional liposomes [18]. However, because the transfersome vesicle has limited entrapment capacity and content leakage of active ingredients still tends to occur due to water diffusion from dispersing media, a provesicular carrier has been developed, namely protransfersomes. Protransfersomes are lipid provesicles in the form of crystalline liquid which will turn into very flexible transfersomes vesicles *in situ* by absorbing water from the skin [19]. These characteristics enable the protransfersomes to protect the encapsulated materials as well as vesicular lipids from any unwanted chemical reactions such as hydrolysis and oxidation associated with degradation, and physical reactions such as sedimentation, aggregation, fusion, leakage of trapped substances, or hydrolysis of encapsulated active ingredients [20–22]. They extend shelf life and are capable of targeting materials encapsulated in the deeper layers of the skin [23–25].

Transfersome is widely reported to be employed in the topical and transdermal delivery of various active pharmaceutical ingredients. However, its applications to cosmetic delivery are limited. Moreover, the recent development of cosmetics is largely intended to improve the appearance of skin by having local biological effects on its

tissues. Considering the similar physicochemical properties of the active ingredients provides direct analogies for successful skin delivery using transfersome, thereby also rendering them prospective active cosmetic ingredients. This review will demonstrate the potential use of transfersome in enhancing active ingredient penetration which promotes optimal anti-ageing activity within the cosmetic delivery system. This, in turn, increasing their effectiveness in impeding skin ageing.

Methods

This review analysed the potential use of transfersome as a carrier in the delivery of anti-ageing cosmetics. The existing research into the use of transfersome in cosmetics is limited. As stated in this paper, considering the similar physicochemical properties of active pharmaceutical ingredients such as insulin and hormones is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients**.**

The method employed in writing this article review consisted of an electronic literature study involving the accessing of national and international journal search sites related to the keywords "ultradeformable vesicles", "transfersomes", "protransfersomes", "anti-ageing active compound", "protein for anti-ageing","topical delivery","skin delivery","deformability","skin penetration", and "topical drug classification system".

The eligibility criteria applied when selecting journal articles comprised original research, short case studies, experimental research design, and the year of publication falling within the period 1992-2020. The sites accessed for the purposes of conducting the search included Pubmed (Scopus & Scimago) and Google Scholar which contain

search keywords. Articles published in predatory journals or publishers which include review articles were excluded from the study. Identification, data correlation analysis, and paper selection were conducted on the basis of the following CONSORT diagram in Figure 1.

Result

Characteristics of protransfersomes and transfersomes as vesicular carriers for skin delivery

For topical delivery, transfersomes have many advantages relating to their high membrane elasticity and deformability. These can be achieved by combining two lipophilic or amphiphilic components, namely; phospholipids and biosurfactants at the appropriate ratio or formula to form bilayer vesicles [23]. In transfersomes, the surfactant as the EA, is in the form of a single chain surfactant capable of destabilizing the lipid bilayer and causing an increase in the fluidity and elasticity of the vesicular membrane with the result that the vesicles can change shape and pass through the pore intact by shrinking in size to one 5-10 times smaller than the original, thus increasing the penetration of the active cosmetic ingredients [26]. Protransfersomes, extremely flexible liquid lipid provesicles, provide benefits for improving the stability of transfersomes [19].

During application, the active ingredient interacts with the skin which is both attached and adheres to the SC. Due to the osmotic gradient resulting from the difference in water content of skin tissue, the active ingredient will be transported to the deeper layers of the skin by passing through the SC. Under light microscopy, the protransfersome, which is originally crystalline and lamellar-shaped, will turn into

transfersome vesicles after hydration. This is due to the difference in the degree of hydration of the surfactant and phospholipid molecules together with the change in the shape of the hydrated molecules. Because of its limited solvent content, the resulting protransfersome will form a compact palisade and vesiculation lamellae. The addition of water will further cause swelling of the bilayer and vesicless due to the interaction of the air with the surfactant head groups and tend to form a random spherical vesicles of transfersomes as presented in Figure 2 [27].

The deformability of the vesicles is influenced by the chemical structure of the surfactants, which surfactants with low hydrophilic-lipophilic balance (HLB) value generally can form smaller size vesicles. The surfactant concentration must also be proper, otherwise the vesicles will harden and be damaged [27]. Another role of surfactants is to increase the hydration properties of transfersome vesicles with the result that they tend to seek moisture in deeper skin layers after application to the skin hydrotaxis (xerophobia) [26].

Transfersomes can penetrate the skin layers by means of different mechanisms depending on their composition as presented in Figure 3, namely; (1) the vesicles maintain their intact shape (deformability) via the intercellular pathway, or (2) the vesicles fuse and mix with skin lipids (transcellular) due to destabilization of the membrane by surfactants, or (3) the vesicles go directly to deeper skin tissues via appendageal routes. Transfersomes can easily shrink to one-tenth of their original size in order to pass through the pore by means of a transdermal osmotic gradient due to differences in the water content of the skin surface, which is about 15 %, and the dermis that has high water amounted of 75 % [28]. This osmotic gradient helps the active ingredients pass through the skin passively via the hydrophilic ducts of the SC [17].

When the transfersomes are applied to skin in non-occlusive conditions, transfersomes will dehydrate due to water evaporation, the hydrophilicity nature of which causes the vesicles to be attracted to a layer with a high water content, allowing the intact vesicles to penetrate via the intercellular spaces [29,30].

The osmotic gradient is high in the SC and decreases with skin depth to the stratum spinosum layer (0.042 mm) [31]. This osmotic gradient acts as a propulsion for most transfersomes vesicles with a total lipid mass of more than 0.1 mg/cm^2 within one hour. The occlusive means of application can cause at least 90 % of the drug to be retained in the SC due to excess hydration, with the result that only small levels of the drug can enter the bloodstream [21]. The SC is composed of corneocytes embedded in hydrophobic lipids that form a crystalline lamellar phase. The corneocytes are coated with cross-linked soft keratin [23]. The water content in the SC is not evenly distributed according to its thickness. A thin layer of water is in equilibrium with the surrounding water content, while the moisture content of a thicker layer of the epidermis is close to saturation level. Viable skin contains 70–80 % water, whereas the surface of the SC is drier than this viable dermis [24]. The electronic diffraction results of the biopsy specimens show that the water content regularly drops from 70-80 % in the stratum granulosum layer to 15-25 % in the upper layer, which means that the water is completely bound, even for fairly high water content values, in these skin layers [25].

Transfersomes act as encapsulating carriers for various active ingredients

Transfersomes as trapping carriers represent the first generation of elastic liposomes that can deliver various active ingredients with different lipophilicity and are able to encapsulate active substances with large molecular weights. Transfersome is

widely reported as being used for topical and transdermal delivery of various active pharmaceutical ingredients. However, the recent development of cosmetics is primarily intended to improve the appearance of the skin through localized biological effects on its tissues. Identifying the similar physicochemical properties of the active ingredients provides direct analogies for successful transfersome-based skin treatment, rendering them prospective active cosmetic ingredients. The active ingredients with hydrophilic properties will be encapsulated in the aqueous core, while the lipophilic substances are trapped within the lipid membrane layer. The process of encapsulating large molecules for subsequent penetration of deeper skin layers is that of forming a reservoir for slow and sustained release of the encapsulated substances, allowing for a reduction in the frequency of administration [32].

The encapsulation of active cosmetic ingredients with large molecular weight can be based on several studies of transfersome formulation for delivery of proteins, such as growth hormones contained in amniotic membrane stem cell-metabolite products (AMSC-MP), stem cells, and ribonucleic acids (RNAs) [7,29,32–34]. The reverse phase evaporation method for transfersome formulation has been used in the encapsulation of hydrophilic polypeptide molecules e.g. Insulin, by using Sodium Cholate as the EA with an entrapment efficiency of up to 81 % [35].

Table 1 shows the use of transfersomes for loading various types of active ingredients that are proven to improve stability, penetration, and effectiveness, while reducing the toxicity to enable their use as the references for ultradeformable vesicles formulation of cosmetic active ingredients with similar physicochemical properties. The encapsulation methods of active ingredients in transfersomes depend on the solubility and permeability as follows:

a. Protein molecules

Protein is known as an active ingredient with a large molecular weight that has been used in skin care. An appropriate delivery system is required to ensure that this active ingredient is stable and can penetrate the skin to produce therapeutic effects. A number of proteins have been formulated into transfersomes, one being insulin whose particle size can be reduced to 100 nm and which can easily penetrate deeper skin layers producing a hypoglycemic effect when compared to conventional insulin vesicles via transdermal route [29]. This is because insulin, which is composed of large molecules and demonstrates high affinity, is distributed in the skin by interstitial fluid flow through the lymphatic system in the skin dermis layer in the presence of lymphatic vessels and capillaries [35]. These anatomical characteristics can be utilized in transdermal delivery of such proteins [79].

Apart from insulin, the progestin hormone used for the purposes of oral birth control or contraception has also been formulated as transfersomes, namely; Norgestrel, which is composed of soya phosphatidylcholine and sodium cholate at a weight ratio of 90:10, and Levonogestrel containing the same elements at a weight ratio of 85:15. Transfersome has been shown to increase transdermal penetration, double contraceptive effectiveness, enhance active ingredient stability, augment entrapment efficiency, and facilitate greater reproducibility [22,80].

The current use of siRNA can represent an alternative anti-aging therapy since it is known to be capable of regulating the expression of certain genes that are intimately involved in the skin aging process [81]. This is expected to enhance the ability of skin cells to repair themselves since during aging the skin layer tends to become thin and easily damaged due to reduced skin matrix production and lipid synthesis, lower

antioxidant capacity, and hyperpigmentation [82]. However, several obstacles exist to skin penetration by this oligonucleotide including its large molecular size that renders passing through the skin layer difficult, in addition to its negative charge that hinders internalization by the cells [83,84]. The use of nanocarriers such as transfersomes in encapsulating and modifying natural properties of this siRNA could enhance the biological efficacy and stability of cosmetic delivery.

The use of small interfering RNA (siRNA) and microRNA in the treatment of atopic dermatitis which is formulated as transfersome has been shown to increase effectiveness and reduce side effects. RNA which is enzymatically degraded and has low membrane permeability can be delivered to the deeper layers of the skin using a gene carrier with the addition of penetration enhancers containing cysteine, arginine and histidine. Such enhancers work through different mechanisms. The arginine residue forms a complex with siRNA. The histidine portion allows the complex to escape the endosome, while the cysteine constituents stabilize and release siRNA in a reducing environment. Peptide modification with stearic acid further stabilizes the complex through hydrophobic interactions. Formulated with the small unilamellar vesicles fusion method, the siRNA particle size can reach 70 nm and is protected from enzymatic degradation. The increased effectiveness of siRNA as a regulator of cytokine production, leading to a reduction in inflammatory cytokines in mice, indicated that transfersomes successfully deliver siRNA transdermally [32]. Transfersomes composed of Phospholipon® 90G and Brij® O20 combined with sponge Haliclona sp. spicula (SHS) for siRNA delivery, which acts as enhancers by making many micro channels, approximately 800 micropores per mm^2 for 48 hours at a dose of 10 mg / 1.77 cm^2 SHS

through and into the SC, thereby successfully facilitate protein penetration to the deeper layers of the skin [85].

Cristiano et al. (2020) [61] have also formulated the enzyme product of Sulforaphane (1-isothiocyanate-(4R)-(methylsulfinyl)-butane) encapsulated within transfersomes consisting of Phospholipon 90G and sodium cholate at a weight ratio of 88:12, using the thin layer evaporation method for melanoma therapy. The use of transfersomes has been shown to increase penetration into the deeper layers of the skin, thereby increasing its anti-cancer activity.

The new paradigm emphasizes stem cells as an attractive biotechnology product to be formulated as anti-ageing cosmetics. The effectiveness of stem cells in regenerating damaged cells due to oxidant-induced shortening of telomere chromosomes has also been studied [86,87]. Stem cells possess the unique characteristic of being unspecialized and, as such, able to reproduce themselves repeatedly through asymmetric division [88]. Derived not only from animals, stem cells from plants are also used as cosmetics after being made into standardized stem cell extracts [89]. The characteristics of stem cells products or extracts which have large molecular weights and are unstable for transdermal preparations have prompted researchers to utilize nano-sized delivery systems, one of which is elastic liposomes which can reduce particle size <100 nm as a means of facilitating penetration into deeper skin layers [33].

It has been reported that AMSC-MPs contain numerous cytokines and growth factors including Epidermal Growth Factors (EGF), Transforming Growth Factors (TGF)-β, basic Fibroblast Growth Factor (bFGF), and Keratinocyte Growth Factor [7,84,90]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing epidermal keratinocyte proliferation and dermal

fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [91]. Recently, microneedle and laser-assisted drug delivery has been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8] which hinders their penetration of the deep skin layers [7,34]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis.

b. Active substances with low solubility and high permeability

Transfersomes, which can load active ingredients characterized by low solubility and high permeability, can have an effect by means of several methods, namely; high pressure homogenation, modified coacervation phase separation and conventional thin film hydration. Active ingredients belonging to this group include Resveratrol, Quercetin, Glimepiride, Diclofenac, Ketoprofen, Rifampicin, Nifedipine, Raloxifene, and Retinyl Palmitate [23,25,28,41,64,72,75,76].

In cosmetics, Resveratrol has been shown to promote the proliferation of fibroblasts, in turn increasing the production of collagen matrix, which renders it a potential anti-aging therapy [92]. Moreover, its high antioxidant capacity plays an important role in preventing oxidative damage to skin tissue cells due to exposure to UV and retarding the photo-aging process [92,93]. Despite demonstrating high levels of permeability in topical delivery [93], resveratrol has low water solubility and significant issues exist with regard to its stability [94,95]. Resveratrol has been combined with various active ingredients including Psoralen which, in combination with Ultraviolet A, can stimulate melanin production and tyrosinase activity in melanocytes. The resulting

transfersomes vesicles have a homogeneous particle size, are stable and demonstrate high trapping efficiency with the result that the use of transfersomes enhances the effect of the combination of both active ingredients and is able to inhibit the increase of free radicals for vitiligo therapy [38]. Arora et al. (2020) [25] prepared transfersomes using Central Composite Design and found that the transfersomes composed of cholesterol hydrochloride (DC-Chol), cholesterol (Chol) and sodium deoxycholate (SDC) can increase the depot effect on the skin. The addition of a cosmetic base cream and gel has no change effects on the physical characteristics of the vesicles. On the contrary, it can increase acceptability during use.

Quercetin, a polyphenol compound, has been reported as having an anti-fibrotic effect capable of reducing scar formation and accelerating wound healing [96]. The use of Quercetin has also been reported as effective in protecting human skin tissues from photoaging through inhibition of matrix metalloproteinase-1 expression which prevents collagen degradation [97,98]. However, the use of Quercetin is highly restricted by its low water solubility with a partition coefficient value of 1.82 [98,99]. In a previous report, Quercetin as a a phytoestrogen employed in osteoporosis therapy, has low bioavailability when taken orally. Therefore, Pandit et al. (2020) [28] formulated Quercetin in transfersome using a fractional factorial design optimized by a complete factorial design. The results showed that quercetin loaded in transfersomes, prepared with Phosphatidylcholine and Tween 80 at a weight ratio of 2 : 1, has a homogeneous and stable particle size and can increase therapeutic effectiveness by topical administration of its transfersomal system indicated by femoral thickness, length, and density and also serum biochemical parameters such as calcium, phosphorus, alkaline

phosphatase, and tartrate-resistant alkaline phosphatase. Thus, the use of transfersome successfully improved topical delivery of Quercetin.

In another study, Retinoids, which affect the maturation of skin epithelial cells, can cause skin disease where levels are low or reduced. Therefore, an external supplement in the form of Retinyl Palmitate can be applied. In the presence of enzymes in the skin, Retinyl palmitate will be converted to retinol and oxidized to form tretinoin which induces thickening of the epidermal layer and collagen production [100]. Having low solubility, for the purposes of dermal delivery, transfersome was prepared with a weight ratio of Phosphatidylcholine: Tween 80 of 18: 1. It successfully promotes skin penetration as evidenced by the discovery of Retinyl Palmitate in various layers of the skin, with the result that the transfersomes can be used as carriers for active ingredients with similar characteristics [76].

Interestingly, the topical use of 3% diclofenac sodium with hyaluronic acid repairs, to a great extent, signs of skin damage due to chronic UV exposure including irregular pigmentation and coarseness. This is probably due to its promotion of cyclooxygenase inhibition which reduces melanin transfer to the epidermal keratinocytes [101,102]. Diclofenac itself has come to be regarded as a low water soluble substance with good permeability [103]. El Zaafarany et al. (2010) [104] studied a comparison of the characteristics of the Diclofenac topical transfersome prepared by means of two manufacturing methods, active ingredient content, phospholipid-EA ratio, and using five variations of surfactants as EAs. The preparation methods used are vortex-sonication and rotary evaporator-sonication. The manufacturing method has a significant effect, while the transfersome prepared by the rotary evaporator-sonication method produces higher trapping efficiency than the vortex-sonication method due to

perfect hydration of the vesicles. In contrast to the vortex method, visual observation indicates that lipids tend to collect and adhere to the vial walls, rendering hydration of the vesicles difficult. The vortex method is unable to disperse lipids completely, resulting in a clumpy dispersion, difficult homogenizing and susceptibility to rapid sedimentation and aggregation [104].

Adding a specific amount of the active substance to the transfersomes affects the loading capacities. Consequently, if it exceeds the optimal capacity of the vesicles, precipitation of active ingredients will occur. The phospholipids-EA ratio also greatly affects transfersomes vesicles characteristics. Optimum deformability is obtained from the phospholipids-EA ratio of 85:15. If the amount of phospholipids is excessive, vesicless will form with low deformability due to a lack of surfactant. A similar phenomenon will occur if too great a quantity of the surfactant is added due to the formation of a rigid micelle mixture [104].

The use of various types of surfactants possessing different chemical structures also results in contrasting vesicles characteristics. Comparing the effect of surfactant types with the optimal phospholipid-surfactant ratio, it was found that the vesicless containing Tween 80 had the highest deformability. This is due to the fact that Tween 80 is composed of flexible, non-bulky hydrocarbon chains. In contrast, the sodium cholate has lower deformability due to its steroid-like structure which is larger than the hydrocarbon chain of Tween 80. From the entrapment efficiency value, the largest order is the system containing Span 85> Span 80> Na cholate> Na deoxycholate> Tween 80 [104]. The use of Tween 80 in transfersome loading diclofenac sodium effectively improves deformability of the vesicles thus increasing skin delivery in non-occlusive topical application.

Nifedipine, an anti-hypertensive drug, has been reported to effectively repair wrinkles as well as promote skin elasticity and hydration as a 0.5% topical preparation [105]. It blocks muscular contraction and relaxes facial muscular fibers, thus reducing the depth of wrinkles [106]. On the other hand, Nifedipine demonstrates very low solubility in water with a high partition coefficient which limits its use in dermal delivery [107]. Nifedipine constitutes a transdermal protransfersome preparation produced by coacervation phase separation method. The protransfersome consists of Phospholipid and Sodium Deoxycholate at a weight ratio of 85:15 produced a bioavailability 6.5 times greater than that of oral administration. This is supported by a high entrapment efficiency of up to 97 % and the increase in penetration ability up to three times greater than the drug suspension triggerring an increase in its anti-hypertensive effectiveness [64].

Raloxifene Hydrochloride, is an active therapeutical compound used in the treatment of breast cancer and osteoporosis, but it has low bioavailability. It has been claimed in recent reports that Raloxifene is able to improve both collagen synthesis by fibroblasts in human skin tissue and skin elasticity due to its effects on selective estrogen receptor modulators [108,109]. Mahmood et al. (2014) [26] succeeded in increasing its bioavailability by formulating it into the transfersomes for transdermal delivery. The formula was designed with the Box-Behnken design composed of Phospholipon® 90G and Sodium Deoxycholate at a weight ratio of 300: 35, resulting in vesicles with high entrapment efficiency, good stability, and high penetration rates.

Transfersomes are also used for delivery of Glimepiride, which is an oral anti-diabetic drug. The side effects of hypoglycaemia, as well as digestive and hepatic disorders that often occur, can be reduced by the ability to release it gradually, thereby

increasing patient compliance. The Box-Benkhen design was used in the transdermal transfersome formulation which has a weight ratio of Phospholipids: Sodium Deoxycholate: Glimepiride = 200: 45: 1. Positive vesicles characteristics were obtained, thereby increasing effectiveness due to high penetration into skin deeper layers which showed a higher penetration flux than Glimepiride suspension [41]. Increased drug bioavailability which reduced both the side effects on gastrointestinal tracts and the long term therapeutic effects due to lower quantities of drugs being used during the therapy was superior to the oral administration of Glimepiride.

The anti-tuberculosis drug Rifampicin prepared as a transfersome can be improved for its transdermal bioavailability and patient compliance due to continous drug release. A comparison of the base of the gel and the suspension confirmed the particle sizes to be similar, but the zeta potential of the gel was more negative because of the acidity of carbopol as the gelling agent. In addition, the permeation value, depot effect, and bioavailability of gel preparations were greater due to the composition of the formula containing Phospholipon 90G and Tween 80 at a weight ratio of 15: 7 between Ethanol, and D-limonene [72].

The capability of transfersome to encapsulate hydrophobic molecules with physicochemical properties similar to Glimepiride and Rifampicin, such as Coenzyme Q10 [110], α-Tocopherol [111], Idebenone [112], α-Lipoic Acid [112,113], Ferulic Acid [114], and Tretinoin [115], within the lipid bilayer would enable modification of physicochemical properties of active ingredients encapsulated in carriers which are nano-sized particles, amphiphilic self-assemble phospholipid with surfactant presence, thus affecting their dispersability, solubilization, and releases to aqueous media at the intended sites, especially dermis for antiaging therapy [116].

c. Active substances with high solubility and low permeability

Within this category, there are several active substances including epigallocatechin-3-gallate (EGCG), 5-Fluorouracil, and methotrexate. The study of the use of transfersome for delivery of a combination of EGCG and hyaluronic acid as an antioxidant for topical application has been reported. The transfersome was prepared by a combination of thin layer hydration and high pressure homogenization methods. The formula optimization was performed using a Box-Behnken design which is prepared with Phosphatidylcholine: Sodium Cholate at a weight ratio of 85: 15, respectively, resulting in increased UV protection promoting its anti-oxidant and anti-aging effects [39].

It has been previously reported that 5-Fluorouracil can be used to manage actinic keratosis and is able to induce collagen synthesis during matrix remodeling and wound healing through a 5% topical administration of 5-Fluorouracil reversing photoaging [117]. The use of transfersome dispersed in a carbopol-based gel has been observed to successfully enhance penetration through hypertrophic scar tissue to the dermal layer, even penetrating deeper skin layers without physical changes or allergic reaction [118]. Another report suggested that using Tween 80 as the edge activator in transfersome-loaded carbopol gel significantly improves skin deposition and penetration of 5-Fluorouracil [119].

In addition, as a potent analgesic, Ketorolac can be formulated for transdermal delivery which has the advantage of gradual release, thus reducing the gastrointestinal side effects that often accompany it. To overcome the low permeability of ketorolac [120], Nava et al. (2011) [52] succeeded in formulating Ketorolac into the transfersome, consisting of Epikuron^{TM} 200 and Tween 80 at a respective weight ratio of 86:14. The

transfersome has a particle size of approximately 127.8 nm at low polydispersity index, a relatively neutral charge with a zeta potential value of -12 mV and high entrapment efficiency of 73.11 %. Moreover, its release is delayed in character causing it to remain in the skin for a long period, thus producing local therapeutical effects [52].

Transfersome vesicles possess the ability to modify the permeability of active ingredients due to encapsulation within the carrier which can change passive diffusion into active transport, allowing low permeable Ketorolac-like active cosmetic ingredients such as Ascorbic Acid [121], to permeate biological membranes. The use of biomimetic phospholipid as a component of transfersome would enable vesicles to carry active ingredients via the paracellular or transcellular routes among others, or through fusion with the cell membrane. This underpins the potential of transfersome to deliver active ingredients promoting dermal repairs and rejuvenation [122].

d. Active substances with low solubility and low permeability

In transdermal delivery, the active ingredients should be dissolved to maximally penetrate the skin. To overcome the problem of solubility and low permeability of active ingredients, transfersomes are used as the carriers as they have been shown to successfully deliver active ingredients to the deeper layers of the skin, including the Curcumin, Psoralen, Cisplatin, Paclitaxel, and Ketorolac [36,38,44,52,123]. Numerous reports have demonstrated that Curcumin can be a potential agent for reversing aging. Its high antioxidant capacity offers protection against the negative effects of free radicals, as well as anti-inflammatory effects which potentially stimulate the production of TGF-β and fibroblasts, while also inducing extracellular matrix production and angiogenesis that both play a significant role in repairing skin and maintaining its health

[124–127]. Curcumin has been seen to demonstrate low water solubility and poor permeability for oral and topical delivery [128]. The low bioavailability of Curcumin can be increased by transfersomes prepared with purified Phosphatidylcholine (Epikuron^{TM} 200) as the phospholipids and the surfactant, i.e. Sodium Cholate, at a weight ratio of 85:15 using a thin layer hydration method followed by extrusion. Their nanovesicles characteristics, including small and homogenous particle size with high entrapment efficiency up to 93.91 % and loading amount of 7.04 % with an improved skin permeability, prove useful in increasing anti-tumor activity [36].

Cisplatin has been known to act as a platinum chemotherapeutic agent with extremely low skin penetration through the main route of skin appendages [129]. Moreover, Cisplatin also demonstrates limited solubility in water and, consequently, often requires solubilizing agents as well as absorption enhancers to improve its effects [130]. Transfersomes composed of Soya Lecithin and Sodium Cholate at a weight ratio of 17: 3 produced a gradual release, thereby reducing the side effects of Cisplatin on healthy cells. Cisplatin, either alone or together with a stabilizer such as a combination of Soya Lecithin: Pluronic: Sodium Cholate ratio at respective weight ratios of 17:1.5:1.5 or other antioxidants produced positive nanovesicles characteristics with small and homogenous particle size and high entrapment efficiency up to 97.97 %, thus increasing anti-cancer effectiveness in skin melanoma therapy [19,43,131]. The use of protransfesome and transfersome also improved Cisplatin levels in plasma during transdermal application, which proves these ultradeformable vesicles successfully enhance penetration of low soluble and low permeable active ingredients such as Cisplatin [14].

The use of the transfersomes for transdermal delivery of methotrexate can increase the effect of drug deposition in the skin and can release the drug efficiently. The transfersomes prepared at a Phosphatidylcholine: Tween 80 weight ratio of 7:3 are superior to conventional liposomes in delivering drugs into the deeper layer skin [132]. In combination with Resveratrol, they can increase the anti-cancer activity of skin melanoma and some squamous cell carcinomas such as actinic keratosis, Bowen's disease, and keratoacanthoma [45].

Methotrexate was formulated by extrusion method using Phosphatidylcholine and with two types of EAs, i.e. Tween 80 and Sodium Cholate, to compare its physicochemical characteristics and penetration abilities across skin [47]. From the study, it is clear that the resulting transfersome has a homogeneous and stable unilamellar and can increase the penetration of the methotrexate into the skin layer by up to five times. As the EA, Tween 80 was more effective at increasing vesicles deformability than Sodium Cholate [47].

According to these results, transfersome and protransfersome are able to improve the solubility and permeability of active cosmetic ingredients with low water solubility and poor permeability, such as Kinetin [133,134], and Superoxide Dismutase, which has also large molecular weight of 30 kDa [135]. Their ability to entrap hydrophobic molecules within the lipid domain of the bilayer membrane as well as the amphiplic properties of the phospholipids used in transfersomes significantly improve the solubility and permeability of such compounds, rendering them useful in delivering active cosmetic ingredients.

In vitro evaluations of transfersomes and protransfersomes

Several nanocarrier lipids, both conventional and elastic liposomes, have different characteristics of vesicles shapes depending on their constituent components, namely; surfactant for transfersomes and ethanol for ethosome. From microscopic observation, it is clear that all of them are spherical vesicless, but have different vesicles sizes as can be seen in the Transmission Electron Micrographs (TEM) [61].

During hydration in the presence of water, the protransfersome gel with lamellar appearance transforms into transfersome due to the hydrating fluid being absorbed by the gel system [136]. This hydrated gel forms spherical vesicular structures due to the different degrees of hydration between surfactants and phospholipids. Starting from the protransfersome with a limited amount of solvent, a mixture of lamellar liquid crystals is formed which resembles the interrelated palisade and vesiculated lamellae. The addition of excess water will cause swelling of the lipud bilayer due to the interaction of water with the surfactant hydrophilic groups above the solvent threshold concentration with the result that the bilayer randomly forms a spherical structure which resembles a vesicles [22] and can be described as presented in Figure 2.

The increase in vesicles deformability is also evidenced by the increasing amount of active ingredients penetrating the skin which becomes the important factor in efficient skin permeation. This deformability is highly influenced by the presence of an EA in the form of a single chain surfactant with a high radius of curvature which renders the vesicles unstable and enables the double layers of vesicless to easily change shape [137]. EAs reduce the energy required to deform the vesicles with the result that transfersomes vesicles can flex to pass through tiny pores in the skin or through intercellular gaps [26]. However, this deformability can be reduced when the amount of surfactant increases [73].

The lipid lamellae in the SC have high proportion of negatively charged lipids [138]. Consequently, the ionically-charged surfactant affects the penetration of the active substances. Vesicles with cationic surfactants can increase the penetration of the active substances to a greater extent than anionic or non-ionic surfactants as revealed by the considerable fluorescent intensity of labelling agent entrapped in transfersomes. This result is due to electrostatic attraction to the SC which contains mostly negatively charged lipid lamellae. This difference in charge can strengthen the interaction between cationic transfersomes and intracellular lipids [137].

Release study of active substances from the carrier can be used to predict how the carrier can deliver active ingredients and produce therapeutic effects before being tested *in vivo* which is an expensive process. In the *in vitro* release test using Franz diffusion cell, the active substances release from transfersomes is limited by two barriers, namely; the phospholipid and the dialysis membrane. The concentration of EA has an effect on the release of active ingredients which is directly proportional. If the concentration is low, then the release of the active substances is similarly low. This is because the lipid membrane becomes regular and does not leak easily. Meanwhile, if the concentration of EA is excessive, the vesicless will be stiffer with the result that they leak easily and are less sensitive to osmotic gradients [27]. Pena-Rodríguez et al. (2020) [76] studied the penetration of the Retinyl Palmitate by comparing transfersomes composed of Phosphatidylcholine and Tween 80 with free active ingredients. It was found that about 69 % of conventional Liposomes loaded Retinyl Palmitate could not penetrate the skin and it was only 2% reached the epidermis to be retained in the SC. Lipid vesicles can act as a reservoir system for the continuous delivery of active cosmetic ingredients.

However, the vesicles of the anionic surfactant deviate from the first-order kinetics of drug release following the diffusion flow of the skin [137].

El-Alim et al. (2019) [56] compared the release rate of Diflunisal in solutions with those of liposomes, ethosomes, and transfersomes. The results showed that within two hours the amount of Diflunisal released from the solution was 84.52 %, while that from the liposomes, 68.10 %; ethosomes, 58.21 %; and transfersomes, 65.88 %. The peak level of Diflunisal release in solution is reached within three hours, whereas Diflunisal in vesicles continues for up to five hours before reaching peak levels.

In vivo evaluations of transfersomes and protransfersomes

From several studies it is known that skin penetration by drugs can be via intercellular or transcellular routes. Transfersomes can pass through these routes due to their elastic properties and the water concentration gradient in the skin layer. The nature of this tendency to attract water triggers the vesicles' ability to penetrate the deeper layers of the skin because of their higher water content. After entering the dermis, the active substances will circulate through the blood vessels to the systemic blood circulation. Due to the higher drug penetration, effectiveness also increases.

The pharmacokinetic study of mice conducted by Jain et al. (2005) [22] indicated that, Levonorgestrel levels in blood plasma are very low for free active ingredients, which was at 0.015 ± 0.005 μg/mL, in contrast to the transfersomes-loaded levonogestrel which reaches levels of 0.139 ± 0.050 μg/mL after topical aplication. The level rises to approximately eight times higher within four hours and is maintained for up to 48 hours. Therefore, it can be proved that by using transfersomes, levonorgestrel can be gradually released over a protracted period.

A similar study was performed by Hussain et al. (2020) [72] which compared the plasma levels after oral administration of Rifampicin and transdermal application of transfersomes-loaded Rifampicin. The comparative data for C_{max} and T_{max} indicated levels of 10.5 ± 1.4 µg/mL after 2.0 hours and 6.9 ± 0.80 µg/mL after 10.6 hours respectively for oral administrations. Meanwhile, the AUC value of Rifamipicin after 24 hours for oral administration was 41.71 ± 5.2 µg/ml, while for transdermal application it was 56.23 ± 2.7 µg/ml. This suggests that the use of transfersomes for transdermal administration can increase the systemic availability of Rifampicin by reducing the dose-related side effects as well as the toxicity of the orally administered Rifampicin.

An *in vivo* test using tape stripping was used by Fernández-García et al. (2020) [70] to compare Amphotericin B levels in the SC and dermis after Amphotericin B transfersomes application to undamaged skin and by microneedle use. This study proves that Amphotericin B transfersomes can penetrate to the deeper layers of the skin, whereas using a microneedle before the application of Amphotericin B transfersomes results in increased penetration of the active ingredient during the first hour, especially in deeper skin areas. The use of microneedles produces temporary skin micropores that aid drug delivery throughout the skin. However, these micropores close within two hours and scar tissue is formed which can reduce the surface area for the active ingredient [29]. In this study, there was no significant difference in the degree of skin penetration between transfersomes-loaded amphotericin B and Amphotericin B added to dimethyl sulfoxide (DMSO) as a skin penetration enhancer. This study proved that transfersome is capable of acting as an enhancer in itself.

Transfersomes are largely evaluated *in vivo* through the use of both human and animal subjects. In human subjects, the transfersomes can be assessed for their

Transepidermal Water Loss (TEWL) value both before and after application. From the results of the tape strip, it is known that there is no significant difference in the TEWL value, therefore confirming that the transfersomes does not affect skin integrity [76]. Although transfersomes can act as a depot for epidermal absorption, the SC is desquamated with the result that the active ingredient can be lost. On the other hand, by using transfersomes, about 63 % of the Retinyl Palmitate successfully penetrated the epidermis. The fluorescent photomicrographs of transfersomes contained nile red indicating that transfersomes can deliver active ingredients penetrating the deeper layers of the skin [76]. Moreover, the fluorescence correlated with transfersomes was extensively observed in the space between the corneocytes in the epidermis [19].

Arora et al. (2020) [25] studied penetration of the antioxidant Resveratrol by transfersome carriers composed of Soya phospholipids and Sodium Cholate at a weight ratio of 85:15. At the appropriately high phospholipid content level, the lipophilic Resveratrol can be trapped within the lamellar lipids of vesicles. The use of transfersomes succesfully increased the penetration of Resveratrol, thus improving the *ex vivo* antioxidant activity as determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. This improved effectiveness is due to an increased flux of active ingredients by disrupting the SC barrier through an amalgam effect of a combination of phospholipids and surfactants. In addition, the skin-penetrated amount of vesicles-entrapped active ingredients is increased due to the longer residence time in the skin.

In albino Wistar rat subjects, the application of transfersome-loading Timolol composed of phosphatidylcholine : Span 80 and Tween 80 at a weight ratio of 3:1 to the shaved back skin was observed for the occurrence of erythema and edema compared to

conventional liposomes. Neither erythema nor edema occurred after this *in vivo* application [24].

Discussion

Based on the review, the formulation of the ultradeformable vesicles i.e. transfersomes and protransfersomes can be seen to increase the effectiveness of active ingredients due to improvements in their physicochemical characteristics and skin penetration. With the combination of phospholipids that resemble skin membranes and the addition of surfactants as EAs, the formation of vesicles can reduce the particle size enabling them to easily penetrate the intercellular gaps and skin pores. The ability to deliver active ingredients with the various characteristics of lipophilicity, solubility, permeability, and large molecular weight including proteins (RNA, hormones) also constitutes an advantage of this delivery system. Transfersomes can be applied in the cosmetics industry because the research conducted indicates that the use of a base preparation including gel and cream neither changes the skin penetration profile nor reduces the effectiveness of the active ingredient. Rather, it can increase the length of time the drug remains in the skin and product acceptability [8, 94].

It is expected that transfersomes and protransfersomes can potentially be used in the cosmetic field with local biological effects, especially in anti-aging products. Skin ageing is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation, and disrupt the dermal extracellular matrix [140,141]. However, active cosmetic ingredients used in anti-ageing therapy such as CoQ10 and AMSC-MP, among others, suffer from skin penetration-related drawbacks including low water solubility and large molecular weight. The use of

transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, i.e., the dermis, which is composed of almost 70% collagen [142]. With the increased skin penetration, the effectiveness and stability cosmetics products will be improved, providing potential use for beauty and health.

Conclusions

Transfersomes and protransfersomes demonstrate encouraging potential for use in cosmetics, especially anti-aging products. The use of phospholipids and EAs in these carriers has benefits for producing nanovesicles with desirable characteristics supporting high skin penetration, thus increasing the effectiveness of active cosmetic ingredients.

FUTURE PERSPECTIVE

Delivering cosmetic active ingredients to target sites, especially for agents affects biological functions can be ultimately supported by appropriate delivery carriers. This review represents the underlying researches in topical or transdermal delivery of active ingredients to the development of therapeutic products for esthetic medicines and cosmetics. A positive approach of the use of ultradeformable carriers, which is called Transfersomes, and its provesicular states namely Protransfersomes, has been largely explored to improve skin penetration by utilizing the nature characters of phospholipids and EAs forming intact flexible vesicles passing through intercellular gaps. As delivery carriers, these deformable vesicles is highly potentials for transporting either hydrophobic or hydrophilic molecules with low or even large molecular weight such as protein to penetrate into deeper skin tissues, which become the main target sites of most

cosmetics, especially for anti-ageing therapy. Further explorations and investigations is definitely required to comprehensively evaluate the potential use of ultradeformable vesicles in improving the efficacy of cosmeceuticals, which is currently still limited.

EXECUTIVE SUMMARY

Transfersome encapsulates various active ingredients

- Transfersome is a vesicular drug carrier that consists of bilayer membrane composed of phospholipids and biosurfactants at the appropriate ratio surrounding inner aqueous phase
- The active cosmetic ingredients with hydrophilic properties will be encapsulated in the aqueous core, while the lipophilic active ingredients is trapped within the lipid membrane layer.
- Transfersomes also provide possibility for encapsulating active cosmetic ingredients with large molecular weight

Ultradeformable liposomes as vesicular drug carriers for skin cosmetics

- Ultradeformable vesicles vesicles can change shape and pass through the pore intact by shrinking in size to one 5-10 times smaller than the original due to transepidermal osmotic gradient
- Transfersomes can penetrate the skin layers by means of different routes, namely; (1) intercellular pathway, or (2) transcellular route, and (3) via appendageal routes

Ultradeformable liposomes impore skin delivery of active cosmetic ingredients

- Lipid vesicles of transfersomes can act as a reservoir system for the continuous delivery of active cosmetic ingredients

- Transfersomes is capable of acting as an enhancer in itself
- Skin-penetrated amount of transfersomes vesicles-entrapped active ingredients is increased due to the longer residence time in the skin
- Neither erythema nor edema occurred after the *in vivo* application of transfersomes

Acknowledgement

None.

Financial Disclosures

None.

Conflict of Interest

The authors declare that no conflicts of interest for this study.

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Table 1. The utilization of transfersomes and protransfersomes as delivery carriers for various active cosmeceutical agents

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
Antioxidant	Curcumin	Low	Low (Large MW)		Transfersomes	[36,37]
	Resveratrol	Low	High	Low	Transfersomes	[25]
	Psoralen (+ Resveratrol)	Low	Low		Transfersomes	[38]
	Epigallocate-chin-3-gallate (EGCG)	High	Low	Low	Transfersomes	[39,40]
Anti-diabetic agent	Glimepiride	Low	High		Protransfersomes	[41,42]
Anti-cancer	Cisplatin	Low	Low		Protransfersomes	[14,19, 43]
	Cisplatin	Low	Low		Transfersomes & Protransfersomes	[14]
	Cisplatin + Imuquimod	Low	Low		Protransfersomes + Carbopol	[44]

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
	5-Fluorouracil (+ Resveratrol)	High	Low		Transfersomes	[10,45]
	Methotrexate	Low	Low		Transfersomes	[46], [47], [48]
Analgesic	Ketoprofen	Low	High		Protransfersomes	[49], [23], [50]
	Ketorolac	High	Low		Protransfersomes	[51]
					Transfersomes	[52], [12], [53]
	Diclofenac	Low	High		Protransfersomes	[54], [55]
	Diflunisal	Low	High		Transfersomes	[56], [57]
Hormone and protein	Levonoges-trel	Low	Low		Protransfersomes	[22,58]
	Norgestrel	Low	Low		Protransfersomes	[59]

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
	Insulin		Low	Low	Transfersomes	[35], [29]
	siRNA		Low	Low	Transfersomes	[60]
	Sulforaphane		Low	Low	Transfersomes	[61]
	Phytoestro-gen Quercetin	Low	High	Low	Transfersomes	[28], [62]
Anti-hipertentive agent	Timolol	Low	Low		Protransfersomes	[27], [24], [63]
	Nifedipine	Low	High		Protransfersomes	[64], [65]
	Minoxidil + Caffein	Low	Low		Transfersomes	[66], [67],[68]
Anti-infection	Azaleic Acid	Low	Low		Transfersomes & Protransfersomes	[20]
	Amphotericin B	Low	Low		Transfersomes	[69], [70], [71]
	Rifampicin	Low	High		Transfersomes	[72], [73]

Therapeutic Class	Drug or Therapeutic Agents	Properties			Type of Drug Carriers	Refe-rences
		Water solubility	Permeability	Stability		
Selective estrogen receptor modulator (SERM)	Raloxifene hydrochlo-ride	Low	High		Transfersomes	[74], [75]
Vitamins	Retinoyl Palmitate	Low	High	Low	Transfersomes	[76]
	Tochopherol	Low	High	Low	Transfersomes	[77]
Herbal products	Emu oil from *Dromaius novaehollan-diae*	Low	Low		Transfersomes	[78]

Figure Legends

Figure 1 The consort diagram of article screening and selection

Figure 2 The physical formation of transfersomes from protransfersome gel

Figure 3 The presence of transdermal osmotic gradient leads to skin penetration of transfersomes via transcellular and intercellular routes into deeper skin layers