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Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Molecular Pharmaceutics - Manuscript ID mp-2022-00936n

1 message

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06-Nov-2022

Journal: Molecular Pharmaceutics
Manuscript ID: mp-2022-00936n
Title: "Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: A Proof of Concept Study"
Authors: Syafika, Nur; Azis, Sumayya Binti Abd; Enggi, Cindy Kristina; Qonita, Hanin Azka; Mahmud, Tiara Resky Anugrah; Abizart, Ahmad; Asri, Rangga; Permana, Andi Dian
Manuscript Status: Submitted

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Thank you for submitting your manuscript to Molecular Pharmaceutics.

Sincerely,

Molecular Pharmaceutics

BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Revision Requested for Manuscript ID mp-2022-00936n

2 messages

Molecular Pharmaceutics <onbehalfof@manuscriptcentral.com> Thu, Dec 15, 2022 at 12:37 AM
Reply-To: suryanarayanan-office@mp.acs.org
To: andi.dian.permana@farmasi.unhas.ac.id

14-Dec-2022

Journal: Molecular Pharmaceutics
Manuscript ID: mp-2022-00936n
Title: "Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: A Proof of Concept Study"
Author(s): Syafika, Nur; Azis, Sumayya Binti Abd; Enggi, Cindy Kristina; Qonita, Hanin Azka; Mahmud, Tiara Resky Anugrah; Abizart, Ahmad; Asri, Rangga; Permana, Andi Dian

Dear Dr. Permana:

We have received the reviews of your manuscript and they are enclosed with this letter. The reviewers have raised points that require consideration and revision of the manuscript before it is suitable for publication. However, with thorough response and revision, the manuscript may be acceptable for publication in Molecular Pharmaceutics. We would like to receive your revision as soon as possible, and no later than 11-Jan-2023.

This revision should address the reviewers' comments and include a point-by-point response to the concerns. On revision, please provide 2 copies of the final manuscript file:

a) The final revised manuscript file that does not contain any highlighting or editing marks. This file should be uploaded as the primary manuscript document file.

b) A marked copy of the revised manuscript that shows changes made on revision clearly highlighted. This file should be uploaded SEPARATELY FROM THE FINAL MANUSCRIPT FILE as Supporting Information for Review Only.

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We look forward to receiving your revised manuscript for publication in Molecular Pharmaceutics.

Sincerely,

Raj Suryanarayanan
Associate Editor
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Reviewer(s)' Comments to Author:

Reviewer: 2

Recommendation: Publish after minor revisions noted.

Comments:
This is a nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited. The corresponding author has come originally from a major group in the area. So good to see him developing his own independent ideas here now he has his own group.

The authors should consider translation of this technology. Will regulatory bodies demand sterility of microneedle patches? What manufacturing and distribution challenges will this present? What about storage stability? How would this device be reproducibly inserted by patients or their carer? Would an applicator be used or would the microneedles be inserted by hand? If inserted by hand, how would the patient or carer know for sure they had pressed the microneedles in to the skin with sufficient force? How would they obtain feedback? How long would the microneedles need to be left in the skin for effective delivery? Would this be practical, given the rapid delivery of medicines when taken orally? What about disposal? How would this be done safely and securely in resource-poor settings?

These microneedles deposit polymer in skin. PVA and PVP are biocompatible, but not biodegradable. Would they accumulate in skin or the draining lymph node local to the site of application? How would it be excreted? These are

important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of patches required for a commercialised product?

The scientific English could be improved in places. Please look for grammatical errors throughout

Additional Questions:
Does this manuscript contain fundamental results of sufficient novelty and significance to justify publication in Molecular Pharmaceutics?: Yes

Reviewer: 3

Recommendation: Publish after minor revisions noted.

Comments:
The manuscript talks about some nice work done in the transdermal delivery of actives by using physical enhancement techniques like microneedles for diabetes. The real life application of these microneedles would be low due to various reasons. In vivo and clinical testing to understand the pharmacokinetic and pharmacodynamic parameters is a must. However, the study is a thorough proof of concept study and will contribute to the existing literature in the field. I recommend the manuscript for publication after making minor revisions.
1. I strongly recommend authors to add more details in the methods section of the manuscript. Some more information about the type of instrument used for each characterization will help in better understanding and aid in reproducibility. No instrument details have been mentioned in any methods subsection.
2. What was the desired target concentration of metformin in all studies? How were the calculations done to determine the transdermal delivery? Was the percentage bioavailability considered in calculations?
3. How was the pH of solid microneedles measured? Which glass electrode probe was used?
4. There are too many abbreviations in the manuscript. It becomes very confusing to follow because of multiple abbreviations.
The purpose of abbreviations is met only if it reduces the overall word count. Abbreviating metformin as MTF does not serve any purpose. I recommend authors to either reduce the number of abbreviations or provide a through list of abbreviations at the start of the manuscript. Also, provide all full forms for all abbreviations used in the figures. Figures should be stand alone.
5. Details about procurement of rat skin should be mentioned.
6. Determining skin barrier integrity (Trans epidermal water loss, or skin electrical resistance) is crucial before conducting ex vivo/ in vitro skin permeation studies. A skin sample with no barrier integrity will lead to misleading results. Authors should explain this point.
7. In real-life application, what will be the duration of application of the MN? How do the authors justify no occurrences of hypoglycemia or hyperglycemia?

Additional Questions:
Does this manuscript contain fundamental results of sufficient novelty and significance to justify publication in Molecular Pharmaceutics?: Yes

Reviewer: 4

Recommendation: Publish after minor revisions noted.

Comments:
The manuscript has been revised appropriately.

Please see minor revisions below:
1. In the manuscript, please rephrase sentences beginning with a number written as a figure. E.g., Line 90, 196, etc. You could spell out the number if you wish.
2. Line 173: "It was then reacted for 1". Please add missing unit for 1.
3. Line 197: Remove extra bracket
4. Please make sure the method descriptions are written in past tense. Present tense has been used in some instances.
5. Line 202: “Analysis using DSC was carried out by weighing the sample as much as 3-5 mg and placed it on an aluminum pan and sealed it.”
Please rephrase to 'Analysis using DSC was carried out by weighing as much as 3-5 mg by placing it on an aluminum pan and sealing it.'
6. Are there SEM images to further define the shape of microparticles?
7. Please add the necessary statistical significance to graphs.

Additional Questions:

Does this manuscript contain fundamental results of sufficient novelty and significance to justify publication in Molecular Pharmaceutics?: Yes

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Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id> Thu, Dec 15, 2022 at 5:47 AM
To: Nur Syafika <nursyafika2341@gmail.com>, Maya Sumayya <sumayyamaya253@gmail.com>

[Quoted text hidden]

BUKTI SUBMIT HASIL REVIEW

Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Molecular Pharmaceutics - Manuscript ID mp-2022-00936n.R1

1 message

Molecular Pharmaceutics <onbehalfof@manuscriptcentral.com> Mon, Jan 9, 2023 at 11:49 AM
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08-Jan-2023

Journal: Molecular Pharmaceutics
Manuscript ID: mp-2022-00936n.R1
Title: "Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: A Proof of Concept Study"
Authors: Syafika, Nur; Azis, Sumayya Binti Abd; Enggi, Cindy Kristina; Qonita, Hanin Azka; Mahmud, Tiara Resky Anugrah; Abizart, Ahmad; Asri, Rangga; Permana, Andi Dian
Manuscript Status: Submitted

Dear Dr. Permana:

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Journal: Molecular Pharmaceutics

Manuscript ID: mp-2022-00839h

Title: "Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: An Ex Vivo Proof of Concept Study"

Author(s): Syafika, Nur; Azis, Sumayya Binti Abd; Enggi, Cindy Kristina; Qonita, Hanin Azka; Asri, Rangga Meidianto; Permana, Andi Dian

Response to Reviewers:

We are very thankful to the expert Reviewers who have taken the time to read and review our manuscript, as well as provide very helpful feedback to improve our manuscript. We have made some changes to the manuscript as a result of the feedback that has been given. We believe that this manuscript is now substantially more improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

Reviewer: 2

Recommendation: Publish after minor revisions noted.

Comments:

This is a nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited. The corresponding author has come originally from a major group in the area. So good to see him developing his own independent ideas here now he has his own group.

Response:

We are very grateful to the Reviewer who have taken the time to read and provide very helpful feedback. We are glad to know that the Reviewer considered this study to be well planned, carried out with care, and that the data and conclusions we provide are appropriate. We have made several important changes to this manuscript as a result of the feedback provided. We

believe that this manuscript is now substantially more improved.

The authors should consider translation of this technology. Will regulatory bodies demand sterility of microneedle patches?

Response:

We thank the Reviewer for the suggestion. As a result, we have added more information in the revised manuscript, as follows:

One of the critical points in the development of DMN system is the translation of this technology. It has been suggested that a cooperative collaboration between industry and academia regarding the line scale-up production and manufacturing of DMNs, particularly in pharmacopoeial standards and good manufacturing practice (GMP) guidelines.[1] Additionally, the sterilization issue of products that will be inserted into the skin, such as DMN, is undoubtedly something that needs to be considered. The holes formed from the administration of MNs can be a place for microbes to enter. However, this possibility returns to how deep and extensive the hole is. The gap created by MNs is much smaller than that produces by an injection needle (needs sterility), hence recovery from the skin would be faster and the possibility of penetration of microbes into the skin also much less. It was shown that even after repeated use on the skin, it did not interfere with the skin barrier function. In addition, the application of polymer-based MN formulas such as DMN and hydrogel-forming MN was found not to stimulate the humoral immune system.[2] Furthermore, it has been reported that the administration of MNs did not cause any microorganism penetration across the skin. Accordingly, it could be estimated that the risk of infection should not be caused by the administration of MN. However, further study is required to investigate the sterility of this combination approach.

What manufacturing and distribution challenges will this present?

Response:

We thank the Reviewer for the suggestion. As a result, we have included these in the revised manuscript as follows:

Unfortunately, until now, there is still no official GMP standard for scale-up production from MN. Although some previously marketed MN products, in reality, the products being marketed were

not MN but were described as very short hypodermic needles.[3] Therefore, the absence of an official GMP standard is one of the significant obstacles, both in scaled-up manufacturing and distribution of MN products.

What about storage stability?

Response:

We thank the Reviewer for the suggestion and the very useful insight. We have included this in the revised manuscript, as follows:

Evaluation of the water vapor transmission rate was carried out to assess the integrity of DMN against high humidity in the storage. As per the result of WVTR assay, it was found that a low WVTR indicates the ability of the DMN to maintain its integrity against high humidity and exhibits the long-term stability of the DMN.

How would this device be reproducibly inserted by patients or their carer? Would an applicator be used or would the microneedles be inserted by hand? If inserted by hand, how would the patient or carer know for sure they had pressed the microneedles in to the skin with sufficient force? How would they obtain feedback?

Response:

We thank the Reviewers for the comment, we have included this in the revised manuscript, as follows:

In applying the microneedle, an applicator can ensure that the pressure applied to the microneedle is appropriate so that the needle can be inserted entirely into the stratum corneum. An example of a commonly used applicator is a spring-operated applicator specially designed for MN insertion.[4]

In addition, a previous study has provided an innovation in applying microneedle to the stratum corneum by using a pressure-indicating sensor film that will change color when the applied force reaches 30N, and the color will be more concentrated when given a greater force, which Based on previous research, the minimum pressure required to insert a microneedle array of 1 cm2 into the stratum corneum is 32 Ncm^{-2}.[2]

How long would the microneedles need to be left in the skin for effective delivery? Would this be practical, given the rapid delivery of medicines when taken orally?

Response:

We thank the Reviewer for the suggestion. As a result, we have included these in the revised manuscript, as follows:

For effective delivery, needles containing drug microparticles must completely dissolve in the skin. Based on the dissolution time test, the time required for DMN to dissolve entirely in the skin is 15 minutes. Therefore, it could be recommended to apply DMN to the skin for 15 minutes, and then it can be removed from the skin.

DMN can penetrate the skin barrier, *stratum corneum*. When applied, the DMN polymer would form a number of pores as an aqueous channel that the drug would reach the deeper layers of the skin, without causing pain. Furthermore, the DMN polymer would directly enter the epidermis or upper dermis layer and go directly to the systemic circulation through a diffusion mechanism and show a therapeutic response when it reaches the site of action.[5,6] In some studies, delivery via DMN is more practical because it could potentially delivery the drugs directly to the systemic circulation via dermis circulation more quickly than delivery via oral. This could increase bioavailability, provide sustained drug release, reduce unfavorable side effects, and improve pharmacological response.[5,7]

Importantly, our *in vivo* studies exhibited that the administration of our approach could maintain the glucose level in the normal level with longer time compared to oral administration indicate that MTF by DMN could delivered better than taken orally.

What about disposal? How would this be done safely and securely in resource-poor settings?

Response:

We thank the Reviewer for the suggestion and the very useful insight. We have included this in the revised manuscript, as follows:

DMN consists of biocompatible and biodegradable polymers that can dissolve in the skin and leave no harmful sharp biohazards.[6] Therefore, DMN that has been applied and removed from

the skin can be disposed of without special treatment. Even in resource-poor settings, this DMN disposal can be done safely and securely.

These microneedles deposit polymer in skin. PVA and PVP are biocompatible, but not biodegradable. Would they accumulate in skin or the draining lymph node local to the site of application? How would it be excreted?

Response:

We thank the Reviewers for the comment, we have included this in the revised manuscript, as follows:

In chronic condition like T2DM, it should be noted that the treatment would be done for long period. Therefore, the deposition of polymers in the skin should be considered. Several studies show that PVP and PVA are biocompatible materials.[8–10] Some studies focusing on the application of DMNs containing insulin have shown that the polymers were deposited in the skin.[11,12] However, the deposition of the polymer following long term and repeated application has not been studied. It has been suggested that to avoid the high deposition of the polymer in the skin following the application of DMNs, biodegradable polymers with low molecular weight could be used.[11] In this study, we used PVP and PVA with low molecular weight such as PVP (K30) and PVA (10 kDa).

These are important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of patches required for a commercialised product?

Response:

We thank the Reviewer for the suggestion and the very useful insight. We have included this in the revised manuscript, as follows:

With respect to the scaled-up manufacturing of this system, in this study, we used the micro molding method by utilizing centrifugation to remove bubbles and fill microholes perfectly. This method has proven to produce microneedles with perfect physical shape. However, it is undeniable that this fabrication method has limitations for scaled-up manufacturing. Therefore,

it is necessary to reconsider the fabrication method for scaled-up manufacturing. One promising method is the fabrication method in the form of a double-penetration female mold (DPFM) combined with the positive pressure microperfusion technique (PPPT), showing that through this system, microneedles can be produced in large quantities, with optimal physical form.

The scientific English could be improved in places. Please look for grammatical errors throughout

Response:

We thank the Reviewer for the suggestion. We have made a great effort to improve the English, we believe that the manuscript more improved now.

Reviewer: 3

Recommendation: Publish after minor revisions noted.

Comments:

The manuscript talks about some nice work done in the transdermal delivery of actives by using physical enhancement techniques like microneedles for diabetes. The real life application of these microneedles would be low due to various reasons. In vivo and clinical testing to understand the pharmacokinetic and pharmacodynamic parameters is a must. However, the study is a thorough proof of concept study and will contribute to the existing literature in the field. I recommend the manuscript for publication after making minor revisions.

We are very grateful to the Reviewer who have taken the time to read and provide very helpful feedback. We are glad to know that the Reviewer considered this study will contribute to the existing literature in the field. We have revised our manuscript based on the recommendation and suggestion that given by Reviewer. We believe that this manuscript is now substantially more improved and it is worth to reconsider.

1. I strongly recommend authors to add more details in the methods section of the manuscript. Some more information about the type of instrument used for each characterization will help in

better understanding and aid in reproducibility. No instrument details have been mentioned in any methods subsection.

Response:

We thank the Reviewer for the suggestion. We have added mor information about the instrument in the revised manuscript.

2. What was the desired target concentration of metformin in all studies? How were the calculations done to determine the transdermal delivery? Was the percentage bioavailability considered in calculations?

Response:

We thank the Reviewer for the suggestion. As a result, we have added more information in the revised manuscript, as follows:

MTF in MP-MTF-GR formulation was 100 mg. To the best of our knowledge, this is the first study developing glucose responsive material for the delivery of metformin. As a proof of concept study, in this study, we did not targeted the concentration of MTF in the formula according to the concentration that can have pharmacological effect yet. The concentration of MTF in the MP-MTF-GR formula is the concentration of MTF that can be optimally entrapped in microparticle. Our in vivo study showed that our approach successfully controlled the release of metformin in the diabetic rat models. However, further study to determine the pharmacokinetic parameters is required.

3. How was the pH of solid microneedles measured? Which glass electrode probe was used?

Response:

We thank the Reviewer for the suggestion. We have added more information in the revised manuscript, as follows:

To measure the pH value, pH meter (Horiba Scientific, Kyoto, Japan) was used. A composite glass electrode was held close to the surface of the microneedle to be measured.

4. There are too many abbreviations in the manuscript. It becomes very confusing to follow

because of multiple abbreviations.

The purpose of abbreviations is met only if it reduces the overall word count. Abbreviating metformin as MTF does not serve any purpose. I recommend authors to either reduce the number of abbreviations or provide a through list of abbreviations at the start of the manuscript. Also, provide all full forms for all abbreviations used in the figures. Figures should be stand alone.

Response:

We thank the Reviewer for the suggestion. As a result, we have provided a list of abbreviations in the revised manuscript and completed the forms of abbreviations in the figure.

5. Details about procurement of rat skin should be mentioned.

Response:

We thank the Reviewer for the suggestion. Therefore, we have added more information in the revised manuscript, as follows:

Rat skins were obtained from sacrificed rats. All animal studies were approved by the Ethics Committee of the Faculty of Medicine, Hasanuddin University (protocol number: UH22070351). Rat skin is carefully shaved using a spatula and hair removal cream. Skin was excised as full thickness then soaked and cleaned with saline solution to remove the fat on the skin.

6. Determining skin barrier integrity (Trans epidermal water loss, or skin electrical resistance) is crucial before conducting ex vivo/ in vitro skin permeation studies. A skin sample with no barrier integrity will lead to misleading results. Authors should explain this point.

Response:

We thank the Reviewer for the suggestion. In the in vivo study, we evaluated the skin moisture of the rats. Water content of the skin is considered as one of the parameters of the skin barrier integrity.[13] Accordingly, in our study, we measured the skin moisture of the rats before and after the experiment. The results showed that the moisture content of the rats before the experiment were 71.43 ± 2.13%, 73.12 ± 3.53%, 74.29 ± 2.98%, 72.44 ± 2.78%, 74.81 ± 3.81% for healthy group, control group, oral group, DMN group with glucose responsive material, DMN group

without glucose responsive material, and DMN group with pure metformin, respectively, indicating the normal skin condition of the rats. After the experiments, the respective groups showed the skin moisture values of 72.65 ± 3.42%, 74.18 ± 2.76 %, 72.09 ± 3.12%, 71.98 ± 3.41% and 73.87 ± 4.32%. Analyzed statistically, there were no significant differences ($p > 0.05$) in the skin moisture values in all rats. Therefore, the skin integrity could be maintained during the experiment.

7. In real-life application, what will be the duration of application of the MN?

Response:

We thank the Reviewers for the comment, we have included this in the revised manuscript, as follows:

DMN application duration can be determined from DMN dissolving time, it is obtained that for effective delivery, needles containing drug microparticles must completely dissolve in the skin. Based on the dissolution time test, the time required for DMN to dissolve entirely in the skin is 15 minutes. Therefore, for full drug delivery, it is recommended to apply DMN to the skin for 15 minutes, and then it can be removed from the skin.

8. How do the authors justify no occurrences of hypoglycemia or hyperglycemia?

Response:

It shows that the combination of MP-MTF-GR with DMN was able to produce better glucose control. In addition to the prolonged MTF effect of the MP form, the presence of a GR ingredient, namely PBA, also produces a more controlled effect. PBA causes MTF to be more controlled according to glucose, and the high BGL triggers the release of the MTF. The results of the glycemic control effect obtained were found to be longer. Administration of DMN2 decreased BGL after 3 hours and maintained normal BGL for 8 hours with a minimum BGL of 101.31 ± 11.28 mg/dL. In addition, when the PBA-glucose complex reaches the equilibrium stage, PBA will maintain its neutral form and will no longer bind to glucose. The PBA reaction results in metformin no longer being released when glucose levels are normal, and preventing hypoglycemia's effects.[14]

Reviewer: 4

Recommendation: Publish after minor revisions noted.

Comments:

The manuscript has been revised appropriately.

We are very grateful to the Reviewer who have taken the time to read and provide very helpful feedback. We have revised our manuscript based on the recommendation and suggestion that given by Reviewer. We believe that this manuscript is now substantially more improved and it is worth to reconsider.

Please see minor revisions below:

1. In the manuscript, please rephrase sentences beginning with a number written as a figure. E.g., Line 90, 196, etc. You could spell out the number if you wish.

Response:

We thank the Reviewer for pointing this out, we have corrected the sentence in our revised manuscript.

2. Line 173: "It was then reacted for 1". Please add missing unit for 1.

Response:

We thank the Reviewer for pointing this out, in our revised manuscript, we have corrected the sentence.

3. Line 197: Remove extra bracket.

Response:

We thank the Reviewer for pointing this out, we have corrected and removed the extra bracket in the revised manuscript.

4. Please make sure the method descriptions are written in past tense. Present tense has been used in some instances.

Response:

We thank the Reviewer for pointing this out, we have corrected the sentence in our revised manuscript.

5. Line 202: “Analysis using DSC was carried out by weighing the sample as much as 3-5 mg and placed it on an aluminum pan and sealed it.”
Please rephrase to 'Analysis using DSC was carried out by weighing as much as 3-5 mg by placing it on an aluminum pan and sealing it.'

Response:

We thank the Reviewer for pointing this out, in our revised manuscript, we have corrected the sentence.

6. Are there SEM images to further define the shape of microparticles?

Response:

We thank the Reviewer for the suggestion. As a result, we have added SEM images of the microparticles in the revised manuscript.

7. Please add the necessary statistical significance to graphs.

Response:

We thank the Reviewer for the suggestion. We have added the necessary statistical significance to graphs in the revised manuscript.

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Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: A Proof of Concept Study

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder that is one of the most common health problems in the world, primarily type 2 DM (T2DM). Metformin (MTF), as the first line of DMT2, is effective in lowering glucose levels, but its oral administration causes problems, including gastrointestinal side effects, low bioavailability, and the risk of hypoglycemia. In this study, we formulated MTF into microparticles incorporating a glucose-response polymer (MP-MTF-GR), which could potentially increase the bioavailability, and extend and control the release of MTF according to glucose levels. This system was delivered by dissolving microneedle (MP-MTF-GR-DMN), applied through the skin, thereby preventing gastrointestinal side effects of orally administered MTF. MP-MTF-GR was formulated using various concentrations of gelatin as a polymer combined with phenylboronic acid (PBA) as a glucose-response material. MP-MTF-GR was encapsulated in DMN using polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA) as DMN polymer. The physico-chemical evaluation of MP-MTF-GR showed that MTF could be completely entrapped in MP with the percentage of MTF trapped increasing with increasing gelatin concentration without changing the chemical structure of MTF and producing stable MP. In addition, the results of the physico-chemical evaluation of MP-MTF-GR-DMN showed that DMN had adequate mechanical strength properties and penetration ability and was stable to environmental changes. The results of the *in vitro* release and *ex vivo* permeation study on media with various concentrations of glucose showed that the release and permeation of MTF from the formula increased with increasing glucose levels in the media. The MP-MTF-GR-DMN formula successfully delivered MTF through the skin by 11.30 ± 0.29 µg, 23.31 ± 1.64 µg, 36.12 ± 3.77 µg, and 53.09 ± 3.01 µg from PBS, PBS + glucose 1%, PBS + glucose 2%, and PBS + glucose 4%, respectively at 24 h, which indicates glucose-response permeation and release behavior. The

formula developed was also proven to be non-toxic based on hemolysis tests. Importantly, *in vivo* study on the diabetic rats model showed that this combination approach could provide a better glucose reduction compared to other routes, reducing the blood glucose level at normal level after 3 h and maintain this level for 8 h. This MP-MTF-GR-DMN is a promising alternative to MTF delivery to overcome MTF problems and increase the effectiveness of T2DM therapy.

KEYWORDS: Metformin, glucose-response, microparticle, dissolving microneedle, transdermal delivery

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels. The case of DM is increasing in recent years. Data from the International Diabetes Foundation (IDF) shows that in 2021 there were 536.6 million people with DM worldwide, and it was estimated to be 643 million and 783 million in 2030 and 2045, respectively. More than 90% of these cases are type 2 diabetes mellitus (T2DM).[1]

The first line treatment of T2DM is metformin (MTF), a biguanide antihyperglycemic agent. More than 120 million people with DMT2 are used MTF as their treatment. MTF is currently available in the form of tablets, modified tablets, capsules, suspensions, and solutions, which are administered orally.[2] The safety and efficacy of MTF in lowering blood glucose levels have been proven.[2,3] The previous meta-analysis showed that MTF could reduce HbA1c (glycated hemoglobin) levels by about 1.5 – 2.0% and FBG (fasting blood sugar) by about 50 – 70 mg/dL.[2] MTF can reduce blood glucose levels by inhibiting hepatic gluconeogenesis, reducing glucose absorption in the intestine, and increasing glucose uptake in tissues.[3–5] MTF can reduce adenosine triphosphate (ATP) and increase adenosine monophosphate (AMP) by reducing mitochondrial complex I activity. Hence, MTF causes the activation of adenosine monophosphate-activated protein kinase (AMPK), an enzyme that plays an important role in regulating energy metabolism. Activated AMPK will inhibit gluconeogenesis in the liver and increase fatty acid oxidation, thereby lowering blood glucose levels.[4,6]

In addition, MTF also works to reduce glucose levels through its activity in the intestines. Unfortunately, this site action of MTF is also the site that was resulting adverse effects. MTF can increase glucose uptake and glucose metabolism through anaerobic metabolism in the intestine. This mechanism produces lactic acid in enterocytes, which is then transported to the liver, which

causes lactic acidosis.[5,7,8] 1-3 per 30,000 MTF users are found to have lactic acidosis yearly.[4,6] MTF can change the microbiome in the gut, which causes an increase in GLP-1, thereby increasing the regulation of glucose levels.[4,5,7,8] However, changes in the microbiome also lead to increased production of lactic acid and decreased absorption of bile acids. This mechanism causes diarrhea and other gastrointestinal side effects, including nausea, vomiting, constipation, stomach pain and cramps, and flatulence.[5,7,8] Some studies reported that 20-30% of patients treated with MTF experienced gastrointestinal effects that led to intolerance and caused 5% of them could not to continue treatment or need dose adjustment.[2,4]

Although the number of cases of hypoglycemia with MTF is smaller than with the use of sulfonylureas and insulin, there are still cases of hypoglycemia. A previous study showed 112 cases of hypoglycemia from 4,072 cases of MTF overdose.[9,10] This risk of hypoglycemia can occur because of the long-term and undisciplined administration of MTF. The other limitation of MTF in the treatment of T2DM is the varying absorption profile. MTF has a bioavailability of about 50-60%, which can change according to food intake. Its bioavailability will increase in the fasting state and decrease when the intake of high-fat foods. This causes the amount of MTF absorbed to be different and affects the resulting therapeutic effect as well.[5,8]

MTF delivery via the transdermal route can be an alternative to overcome these problems. Previously, transdermal delivery of MTF has been developed through a hydrogel-forming microneedle (MN) system.[5] This system can be an alternative to transdermal delivery. Its permeation was significantly increased compared to pure MTF. MN as transdermal delivery has many advantages but still has some limitations. Drug released from MN results in uncontrolled release behavior and do not demand physiological changes in the body. As a result, the toxicity due to overdose or ineffective treatment due to lack of dose is difficult to avoid.[11] Therefore, the

release of drugs from the MN system on demand is needed to balance the therapeutic effects and side effects. Several methods have been developed to provide on demand drug release in MN by utilizing magnetic fields, ultrasound, and light.[12–14] However, these methods were quite complicated and not safe for long-term use.

On demand drug release can be done by using particular materials that have bioresponsive properties that can respond to physiological changes. The on demand drug release system using bioresponsive material has been applied in the treatment of DM using glucose-responsive (GR) materials. In the presence of GR material, drug release would occur in response to increased blood glucose levels. As a result, drug release would increase with increasing blood glucose levels and decrease when blood glucose levels are normal.[15] This system can prevent and reduce the risk of hypoglycemia from MTF. There are three types of GR materials that have been investigated for drug delivery for DM therapy, including phenylboronic acid (PBA) and its derivatives, con-canavalin A (Con A), and glucose oxidase (GOx). Con A and GOx are proteins that can be immunogenic, can be inactivated, less stable, and high cost. On the other hand, PBA is non-immunogenic, more stable *in vivo*, and low cost.[13,15,16] This study developed MTF delivery with GR system using PBA attached to gelatin polymer. Gelatin is a natural polymer that has been widely used for drug delivery. Its structure consisting of amino and carboxyl groups gives it the advantage of undergoing structural modification with compounds from other materials.[17]

MTF will be encapsulated in PBA-attach gelatin. To prolong the release and permeation time, MTF will be formulated into microparticles (MP). Many previous studies have shown that MP can provide an extended release.[18,19] MTF and PBA microparticles as GR material (MP-MTF-GR) will be delivered transdermally through the dissolvable MN (DMN) system. DMN can penetrate the skin barrier, *stratum corneum*. When applied, the DMN polymer would form a

number of pores as an aqueous channel that the drug would reach the deeper layers of the skin, without causing pain. Furthermore, the DMN polymer would directly enter the epidermis or upper dermis layer and go directly to the systemic circulation through a diffusion mechanism and show a therapeutic response when it reaches the site of action.[20,21] In some studies, delivery via DMN is more practical because it could potentially delivery the drugs directly to the systemic circulation via dermis circulation more quickly than delivery via oral. This could increase bioavailability, provide sustained drug release, reduce unfavorable side effects, and improve pharmacological response.[20,22] DMN consists of biocompatible and biodegradable polymers that can dissolve in the skin and leave no harmful sharp biohazards.[21] Therefore, DMN that has been applied and removed from the skin can be disposed of without special treatment. Even in resource-poor settings, this DMN disposal can be done safely. The polymers commonly used in DMN are water-soluble polymers, including polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), hyaluronic acid, maltose, dextran, albumin, and chondroitin sulfate.[23] Previous studies have shown that combining PVA and PVP as polymers in DMN provides adequate mechanical strength and penetration ability.[21]

In this study, for the first time, we developed the delivery system of MTF in MP form incorporated in PBA-attach gelatin which was delivered transdermally through the DMN system with a combination of PVP and PVP polymers as a potential enhancer of T2DM therapy. The MP was characterized to select the optimum formulation which was further formulated into DMN. Finally, MTF loaded DMNs was evaluated for their release behavior in the condition mimicking hyperglycemic condition in *ex vivo* studies. The outcomes of this study serve as a proof of concept for new delivery system to selectively deliver MTF *via* transdermal route to overcome the limitation of oral administration of MTF.

2. Experimental Section

2.1. Materials. Metformin (MTF) of analytical grade was purchased from Tokyo Chemical Industry Co., LTD, Tokyo, Japan. Phenylborornic acid (PBA), gelatine, acetic acid, glutaraldehyde, 1-etil-3(3-dimethylaminapropyl) carbodiimide hydrochloride (EDC.HCl), N-hydroxysuccinimide (NHS), and dimethyl sulfoxide (DMSO), poly(vinyl pyrrolidone) PVP (K30) and poly(vinyl alcohol) PVA (10 kDa) were purchased from Sigma-Aldrich (Singapore). D-glucose was obtained from Merck (Germany).

2.2. Preparation of Microparticle MTF-GR (MP-MTF-GR). The MP-MTF-GR was prepared using a similar method from the previous study.[17] Five formulations of MP-MTF-GR were prepared as shown in Table 1. Initially, predetermined amount of gelatin and 0.2 mL of acetic acid was added into 2 mL of water. To obtain a cloudy mixture, 5 mL of ethanol was added gradually. Then, as a cross-linking agent, 25 µL of glutaraldehyde (25%) was added to the mixture. The mixture was then centrifuged at 7000 rpm for 15 minutes. The supernatant was taken out and 3 mL of water was added to the precipitate (MP1). Subsequently, 12 mg of 3-CPBA was dissolved in 1 mL of DMSO. It was then reacted for 1 with 16.6 mg of EDC.HCl and 10 mg of NHS. The mixture was added to 3 mL of MP1 and stirred overnight at room temperature. To remove un-reacted 3-CPBA, it was centrifuged at 7000 rpm for 1 minute (MP2). Moreover, 100 mg of MTF was dissolved in MP2 and stirred overnight. To remove the un-loaded MTF, the mixture was centrifuged at 9000 rpm for 15 min. Finally, MP-MTF-GR was obtained. Morphology of MP-MTF-GR was observed using a light microscope.

Table 1. Composition of MP-MTF-GR

Composition	(mg)				
	MP1	MP2	MP3	MP4	MP5
Gelatin	100	150	200	250	300
MTF	100	100	100	100	100

2.3. Determination of Entrapment Efficiency (EE) and Drug Loading (DL). Measurement of entrapment efficiency and drug loading was measured by centrifuging the formula for 15 minutes at 7000 rpm to separate the unencapsulated MTF. After that, the supernatant was taken, then the absorbance was measured using a UV-Vis spectrometer. The absorbance findings were then used to calculate the concentration of unencapsulated MTF, then EE and DL were calculated using Eq. 1 and Eq. 2 below.[24]

$$\%EE = \frac{(\text{weight of initial MTF-weight of unencapsulated MTF})}{\text{weight of initial mtf}} \times 100 \qquad \text{Eq. 1}$$

$$\%DL = \frac{\text{amount of entrapped drug in microparticle}}{\text{total weight of microparticle}} \times 100 \qquad \text{Eq. 2}$$

2.4. Determination of Particle Size and Polydispersity Index (PDI). The particle size was determined by observing the formula with a light microscope with a magnification of 100 x then the particle size and PDI were measured using an Optilab Raster Image®.[25]

2.5. Determination of Zeta Potential. A total of 0.3 mg/mL of the formula was suspended in deionized water, and then the zeta potential was measured using Mastersizer 2000 size analyzer (Malvern Instruments, Malvern, UK).[13]

2.6. Physiochemical Characterization of MP-MTF-GR using Fourier Transform Infrared Spectroscopy (FTIR). 2 mg of each sample was mixed with potassium bromide and

compressed to form a pellet. IR spectra were recorded using an FTIR spectrometer ((Accutrac FT/IR-4100™ Series, Perkin Elmer, USA), spectra were collected in the wavelength range of 4000-400 cm^{-1}.[13]

2.7. Physical Form Characterization of MP-MTF-GR using Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). Physical characterization was carried out using DSC and XRD instruments. Analysis using DSC (DSC 2920, TA Instruments, Surrey, UK) was carried out by weighing the sample as much as 3-5 mg and placed it on an aluminum pan and sealed it. The sample is then heated at a constant increase of 2^{o}C/min from 0 to 300^{o}C under a dry atmosphere of nitrogen.

Characterization using XRD was done by collecting XRD patterns using the instrument Rigaku Corporation, Kent, England with Cu Kα radiation. Then, the diffraction patterns were collected over the 2theta range of 0-50^{o}.[13]

2.8. *In vitro* Drug Release Study. The *in vitro* drug release study was carried out to observe the release of MTF from the MP-MTF-GR, MP-MTF, and pure MTF. This study was performed using the dialysis method. PBS and glucose-containing PBS (1%, 2%, and 4%) were used as the release medium. An amount of formulation (equivalent to 20 mg/mL of MTF) was inserted into the dialysis membrane. Then, it was placed in 100 mL of release medium at 37°C. The release study was performed at 100 rpm. At predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24 h), an aliquot of 1 mL was taken and replaced by the same amount of fresh release medium. To determine the amount of drug released, samples were then analyzed using a UV-Vis spectrophotometer at wavelength 234.2 nm. All measurement was done in triplicate. Furthermore, the data obtained were fitted to five different mathematic models using DD Solver® software,

namely zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell to determine the release kinetics of MTF.[26] The mathematic model was described in Eq. 3 – 7 as follows:

Zero order: $C_t = C_0 + k_0 t$ Eq. 3

First order: $lnC_t = lnC_0 + k_1 t$ Eq. 4

Higuchi: $C_t = k_H \sqrt{t}$ Eq. 5

Korsmeyer-Peppas: $C_t = k_{KP} t^n$ Eq. 6

Hixson-Crowell: $\sqrt[3]{C_t} = \sqrt[3]{C_0} k_{HC} t$ Eq. 7

Where C_t represents the percentage of MTF released at time t, C_0 represents the initial concentration of MTF in the medium, t represents the time, n represents the exponent of diffusion release, k_0, k_1 k_{KP}, k_H, and k_{HC} represent the release coefficient for zero order, first order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell, respectively.

To test the adaptability of GR-MP-MTF release depending on glucose levels in the media, samples were first incubated in a PBS medium containing 100 mg/dL glucose for 30 min. Afterward, the sample was removed and incubated in a medium containing glucose 400 mg/dL for 30 minutes.[27] This cycle was then repeated several times until 360 minutes. MTF released was then analyzed using a UV-Vis spectrophotometer at wavelength 234.2 nm

2.9. Formulation of DMN containing MP-MTF-GR (MP-MTF-GR-DMN). MP-MTF-GR-DMN was prepared using a silicone mould (needle density 20 x 20, pyramidal needles, 700 m height). The aqueous blend of polymers containing various PVA and PVP concentrations was mixed with 25% w/w MP-MTF-GR. The concentration was present in Table 2. Initially, 0.3 – 0.5 g mixture was poured into the silicone mold, then centrifuged at 3500 rpm for 30 minutes to fill the mould. DMN was then dried at room temperature for 24 hours and further dried at 37°C for 24

hours. The dried DMN was removed from mould, and the morphology of the DMN was observed under a light microscope

Table 2. Composition of MP-MTF-GR-DMN

Formulation	Composition (%w/w)			
	MP-MTF-GR	PVP	PVA	Water
DMN1	25	25	10	40
DMN2	25	25	15	35
DMN3	25	25	20	30
DMN4	25	20	15	40
DMN5	25	15	15	45

2.10. Evaluation of Mechanical Strength and Penetration Properties of MP-MTF-GR-DMN. The mechanical strength of DMN was evaluated using the TA.TX2 Texture Analyzer in compression mode as previous study.[21] DMN was given a force of 32 N/array for 30 seconds. DMN was observed using a light microscope, the height before and after the test was determined using Image Raster® software. The percentage reduction in needle height was calculated by Eq. 12, where h_B is the height before testing and h_A is the height after testing.

$$\%\text{Height needle reduction} = \frac{h_B - h_A}{h_B} \times 100\% \qquad \text{Eq. 12}$$

Penetration properties of DMN were evaluated using Parafilm®M as a validated artificial skin model.[28] DMN was applied to eight layers of Parafilm®M and given a force of 32 N/array for 30 seconds. The number of holes formed in each layer was calculated and the deepest layer of the hole was determined.

2.11. Calculation of Theoretical Drug Content in Needles and Determination of Drug Recovery. Theoretical drug content was determined by calculating the densities of all formulas

first. To calculate the density of the formula, the blank polymer mixture (without MP-MTF-GR) was prepared on a flat block and then dried. The dried blocks were weighed and the dimensions were measured to determine the volume. The volume of the block is calculated by Eq. 8.

$$\text{Volume} = \text{width} \times \text{length} \times \text{height} \qquad \text{Eq. 8}$$

The density of formulas was determined by Eq. 9.

$$\text{Density} = \frac{\text{mass}}{\text{volume}} \qquad \text{Eq. 9}$$

Needle volume was determined using the dimensions of the needle, then the mass of needle was determined using the volume of needles and density of formulas. Mass of MP in needle was determined by calculating the percentage of dry MP in needle mass. Finally, MTF content theoretically was calculated by Eq. 10.

$$\text{MTF content in needle} = \text{drug loading} \times \text{mass of MP in needle} \qquad \text{Eq. 10}$$

Drug content was determined by dissolving DMN in 5 mL of distilled water. 10 mL methanol was added to this mixture, then the mixture was sonicated for 30 minutes. Then this mixture was centrifuged at 5000 rpm in 10 minute and supernatant was collected. Absorbance of MTF in was determined using UV-Vis spectrophotometer at a wavelength of 234.2 nm and concentration was determined (concentration found). Drug content recovery was determined used Eq. 11.

$$\text{Drug content recovery (\%)} = \frac{\text{concentration found}}{\text{theoretical drug content}} \times 100\% \qquad \text{Eq. 11}$$

2.12. Evaluation of Surface pH. In this study, 20 mg of microneedles were stored in a beaker containing 50 ml of double-distilled water, and then the microneedles were allowed to swell for 15 min at room temperature. To measure the pH value, bring the composite glass electrode close to the surface of the microneedle to be measured. The pH was then measured after an equilibration time of 1 minute.[29]

2.13. Evaluation of Water Vapor Transmission Rate (WVTR). WVTR was assessed using a glass vial containing 1 g of anhydrous calcium chloride. DMN has sealed a glass vial with the help of adhesive tape. This vial was then placed in a desiccator containing a saturated solution of potassium chloride (%RH 85%). The vial was weighed at the predetermined time. WVTR is calculated by Eq. 14

$$\text{WVTR} = \frac{\text{(final mass-initial mass of vial) x thickness of DMN}}{\text{surface area}} \qquad \text{Eq. 13}$$

2.14. Evaluation of Moisture Absorption Ability (MAA). MAA testing was carried out using the method from the previous study.[30] MAA testing was conducted to determine the ability of DMN to absorb moisture at different %RH. DMN was placed in different desiccators. The desiccator contained magnesium chloride (33% RH), sodium nitrite (69% RH), and potassium sulfate (97% RH). MN weights were weighed every 48 hours for 14 days. %MAA was calculated by Eq. 13.

$$\%\text{MAA} = \frac{\text{final mass-initial mass}}{\text{initial mass}} \text{ x } 100\% \qquad \text{Eq. 14}$$

2.15. Dissolution Study. A dissolution time test was carried out using rat skin to determine the time required for DMN to dissolve in the skin completely. DMN was applied to the rat skin, and to ensure penetration, manual pressure was applied. Above the DMN was given a 5 g stainless-steel cylindrical (11 cm) to ensure the DMN does not move from its place. At the predetermined time, DMN was removed and DMN morphology was observed under a microscope. The dissolution test was carried out until the DMN was completely dissolved.

2.16. *Ex vivo* Permeation Study. Ex vivo permeation study was carried out to obtain MTF permeation profiles of DMN containing MP-MTF-GR, MP-MTF, and pure MTF on media PBS pH 7.4 and glucose containing PBS (1%, 2%, and 4%). This study was performed using Franz diffusion cells. Rat skin was used as a permeation membrane and placed between the donor

compartment and receptor compartment in Franz cells with the *stratum corneum* facing upwards. DMN was applied in the center of the skin by using manual pressure for 30 seconds. A 5.0 g cylinder was placed over the DMN to ensure the DMN stayed in its place. In the receptor compartment, PBS pH 7.4 media was placed as a normal body fluids model and PBS+glucose 1%, PBS+glucose 2%, and PBS+glucose 4% as body fluids model with increased glucose levels. The medium was stirred at 100 rpm and the temperature was maintained at 37 ± 1°C. The donor compartment was placed above the receptor compartment and was sealed using Parafilm®M to reduce evaporation. 0.5 mL of media was sampled through the sampling arm and replaced by the same amount of fresh media at predetermined time intervals (0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h). The absorbance of sampled media was determined using a UV-Vis spectrophotometer at a wavelength of 234.2 nm. The concentration obtained is plotted against the sampling time.

2.17. *In Vivo Study in Diabetic Rats.* The animal research protocol has been approved by the Ethics Committee of Medical Faculty, Hasanuddin University. In this study, 30 male Wistar rats were used, weighing 220.36 ± 9.62 g.

Wistar rats were induced with type 2 diabetes mellitus using streptozotocin (STZ). Induction of type 2 diabetes mellitus was carried out in the diabetes group (n = 25) using low dose two injections of STZ (100 mg/kg) in 0.1M citrate buffer (pH 4.5) by intraperitoneal injection. Fasting blood glucose (FBG) levels were measured after eight days of STZ injection using a glucose meter (Nesco® Multicheck). Rats with FBG levels of more than 400 mg/dl were considered diabetic and used in further analysis.

Rats were grouped into six groups (n=5), namely healthy group, control group, oral group, DMN-F4-PBA group, DMN-F4 group, and DMN-MTF group. Groups of healthy rats were prepared by providing a standard feed without diabetes induction. The control group was prepared

by inducing type 2 diabetes mellitus using STZ and maintaining blood glucose levels. The oral group was prepared by giving metformin tablets with a dose of 100 mg/kg body weight, to rat induced by type 2 diabetes mellitus. All rat in the DMN-F4-PBA, DMN-F4, and DMN-MTF groups were treated transdermally with DMN-F4-PBA (DMN with GR compound), DMN-F4 (DMN without GR compound), and DMN-MTF (DMN without MP) as much as six patches which was equivalent to the dose of 75.58 µg/patch (453.48 µg in total). Rat blood was collected from veins in the tail at intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12 hours to detect blood glucose levels (BGL) using a glucose meter (Nesco® Multicheck).

2.18. Hemolysis Test. The hemolysis test was carried out by first collecting erythrocytes from rats by centrifuging whole blood for 20 minutes at 2000 rpm. The collected erythrocytes were then washed using PBS with three washing cycles. After that, the erythrocytes were resuspended in PBS to obtain a concentration of 10% v/v. After that, the suspension was added to the sample with a concentration of 500 g/mL, 50 g/mL, and 5 g/mL.

Next, the mixture was incubated at 37oC for 1 hour, followed by centrifugation at 7000 rpm for 10 minutes. Finally, the absorbance of the supernatant from the centrifugation was measured using a UV-Visible spectrometer at 540 nm to estimate the number of free erythrocytes. PBS and distilled water were used as positive and negative controls, respectively.[29]

The percentage of hemolysis is calculated using Eq. 15 as follows:

$$\text{Hemolysis (\%)} = \frac{\text{absorbance (test sample)-absorbance (negative control)}}{\text{absorbance (positive control)-absorbance (negative control)}} \times 100\% \qquad \text{Eq. 15}$$

2.18. Statistical Analysis. All data are shown as mean ± standard deviation (SD) of the mean. All values were obtained using Microsoft Excel® (Microsoft Corporation, Redmond, USA). GraphPad Prism® version 5.03 (GraphPad Software, San Diego, California, USA) was used for statistical analysis. One-way ANNOVA analysis was used for the analysis of multiple groups,

while the t-Test was used to analyze two groups. In all cases, $p < 0.05$ was considered as a significant difference.

3. Result and Discussion

3.1. Preparation of Microparticle MTF-GR (MP-MTF-GR). In this study, MTF was formulated into glucose-sensitive microparticle. Microparticle delivery systems offer various advantages, including adequate protection and prolonged effects of drugs.[19] Gelatine and PBA as polymer and glucose-sensitive agents, respectively. The microparticle fabrication was carried out by cross-linking gelatine with glutaraldehyde through the nucleophilic addition-type reaction between the aldehyde groups with free non-protonated ε-amino groups of lysine or hydroxylysine.[31] Furthermore, PBA was loaded into the microparticle to obtain glucose-sensitive release of MTF to avoid its adverse effect. Morphology of MP-MTF-GR was observed using microscope and represented in Fig. 1(A). All formulations were evaluated to obtain the optimum formulation.

3.2. Determination of Entrapment Efficiency (EE) and Drug Loading (DL). Entrapment efficiency is an important parameter that informs the percentage of drugs successfully entrapped into the microparticles.[32] In the MP-MTF-GR system, the MTF was entrapped in polymer matrix due to cross-linking between gelatin and glutaraldehyde. The value of EE obtained was shown in Fig. 1(B). It could be observed that higher polymer concentration would result in higher EE value due to prevention of drug diffusion across continuous phases.[33] Analyzed statistically, EE value for MP1, MP2, and MP3 was significantly different ($p > 0.05$). Furthermore, it was found that the EE value of MP4 (69.53±6.46%) was significantly higher than MP3 ($p < 0.05$) but exhibited non-significant different with EE value of MP5 ($p > 0.05$). Therefore, in order

to minimize the use of excipient in the formulation, MP4 was chosen as the optimum formulation and was further investigated.

Drug loading measurement is one of the key parameters. It was performed to determine the amount of total entrapped drug compared to the total microparticle weight. The DL value obtained is shown in Fig. 1(C). Subsequently, the DL value for MP1, MP2, MP3, MP4, and MP5 was 12.09±1.15%, 13.43±1.24%, 18.21±1.38%, 20.93±1.63%, and 18.02±1.28%, respectively. All formulations showed high DL value, which is > 10%.[34]

3.3. Determination of Particle Size and Polydispersity Index (PDI). The result of particle size analysis was shown in Fig. 1(D). Subsequently, particle size was found to be 4.04±0.43 µm, 6.19±0.71 µm, 7.03±0.43 µm, 8.19±0.66 µm, and 12.83±1.04 µm for MP1, MP2, MP3, MP4, and MP5, respectively. The particle size of all formulations was significantly different (p > 0.05). It could be observed that there is an increase in particle size with higher polymer concentration, probably due to the tendency of polymer to coalesce.

In order to determine the heterogeneity of the microparticles based on the size, PDI measurement was carried out. The result obtained was shown in Fig. 1(E). PDI value range from 0 - 1. A low PDI value indicates a perfectly uniform sample with respect to particle size, while a high PDI value indicates highly polydisperse particles. PDI value < 0.2 is considered acceptable for polymer-based system.[25] Therefore, it can be concluded that all formulations showed desired PDI value, which is <0.2. This finding implies that the formulation is monodisperse.

3.4. Determination of Zeta Potential. The zeta potential is one of the parameters that can be used to ensure the stability of microparticles developed from a physical point of view.[13] The results of the zeta potential measurements of all formulas are shown in Fig. 1(F). It is found that all formulas have high zeta potential values, which are above -30 mV. This value indicates a stable

suspension particle shape and is not susceptible to rapid coagulation or flocculation.[19] The PBA matrix linked to the gelatin polymer in incorporating MTF has many hydroxyl groups from PBA as well as carbonyl groups from gelatin which gives a negative charge on the surface of the microparticles. This explains the negative charge obtained in the zeta potential measurement results from all formulas.[17]

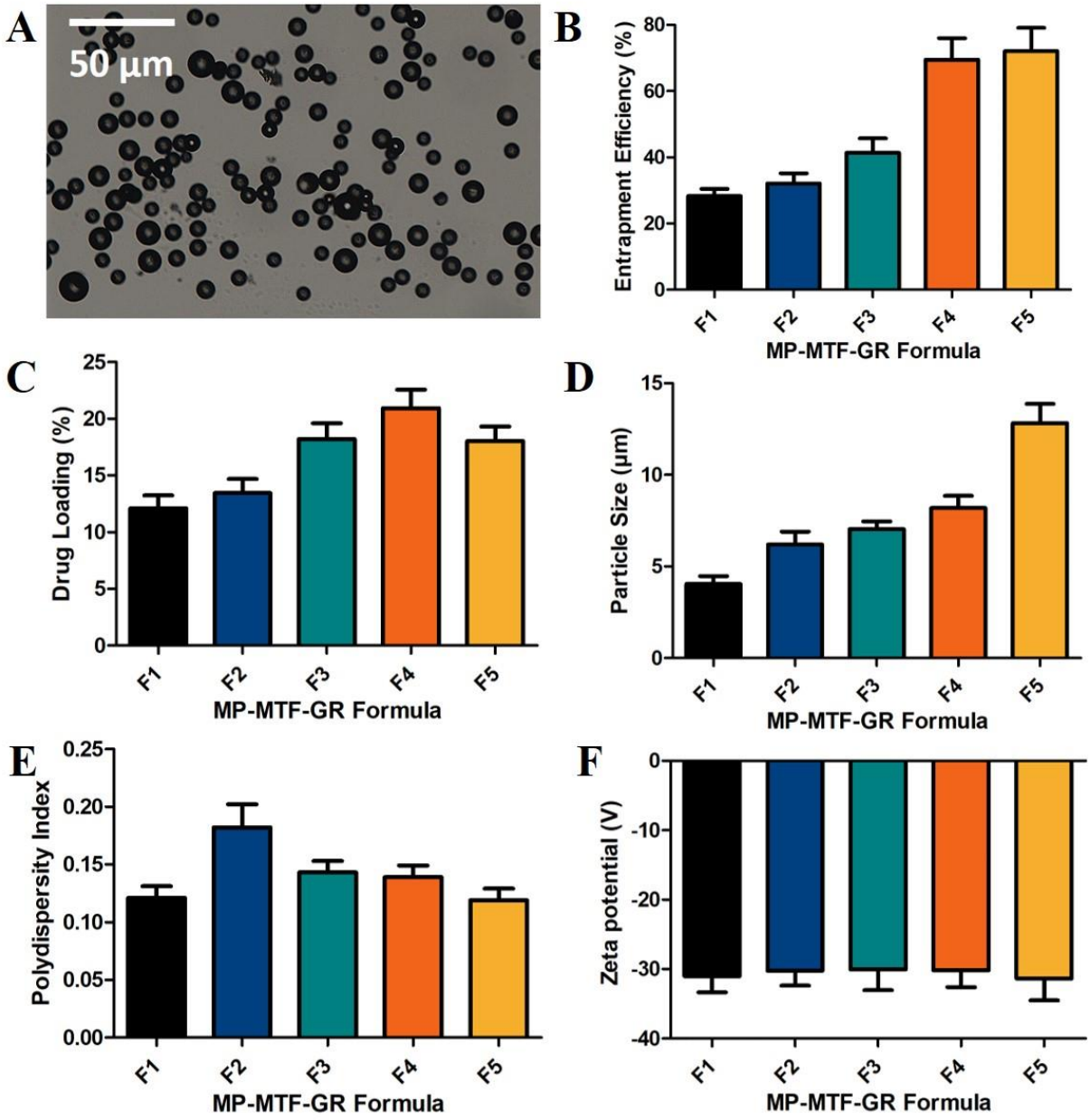

Fig. 1. (A) Microscopic image, (B) entrapment efficiency, (C) drug loading, (D) particle size, (E) polydispersity index, and (F) zeta potential of MP-MTF-GR (means ± SD, n = 3).

3.5. Physiochemical Characterization of MP-MTF-GR using Fourier Transform Infrared (FTIR) Spectroscopy. The presence of MTF in the developed microparticle system can be determined by characterization using FTIR spectroscopy. The results of the characterization of pure MTF using FTIR can be seen in Fig. 2(A), showing peaks at 3369 cm-1 and 3295 cm-1 indicating the presence of NH-stretching, which is a typical group possessed by the MTF structure.[35] In addition, CN-stretching was seen at 1619 cm-1 and 1568 cm-1, CH-stretching was seen at 3181, and CH-deformed at 929 cm-1. It can be seen that the IR MP spectrum containing MTF shows the same peaks as in the pure MTF IR spectrum. This indicates that the MTF in the MP formula does not undergo structural changes and indicates the presence of MTF trapped in the MP system. In addition, the formula containing PBA (F4) showed a peak at 1639 cm-1, which indicated the C=O amide stretching vibration of PBA, and a peak at 1319 cm-1 due to bending vibrations from benzene and stretching vibrations from boric acid, which indicates that the PBA was successfully attached to the MP.

3.6. Physical Form Characterization of MP-MTF-GR using Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). The presence and physical form of MTF in microparticles can be determined by characterization using DSC and XRD spectroscopy. The results of physical characterization using DSC are shown in Fig. 2(B). The endothermic relaxation of pure MTF was seen at a sharp peak formed at 234°C indicates pure MTF in crystalline form, this shows similarities to the previous research literature.[36] This sharp peak then disappeared and amorphous endothermic relaxation was formed in the MP DSC data, both with PBA (F4) and MP without PBA (F4 without PBA). These findings indicate that the MTF formulated as MP is

perfectly encapsulated in the polymer matrix. These findings were supported by the XRD characterization results shown in Fig. 2 (C). X-ray diffraction pattern of pure MTF shows a sharp peak at 2θ of 10 – 50° which indicates the crystalline form of MTF. Similar to the DSC data findings, the F4 formula without PBA produces an x-ray diffraction pattern with wide and sloping peaks. This shows the amorphous diffraction pattern in formula F4 without PBA. In addition, the semi-crystalline structure of formula F4 is characterized by X-ray diffraction patterns with wide peaks. However, at 2θ=19°, sharp peaks were seen, probably coming from the crystalline form of PBA linked by gelatin.[37]

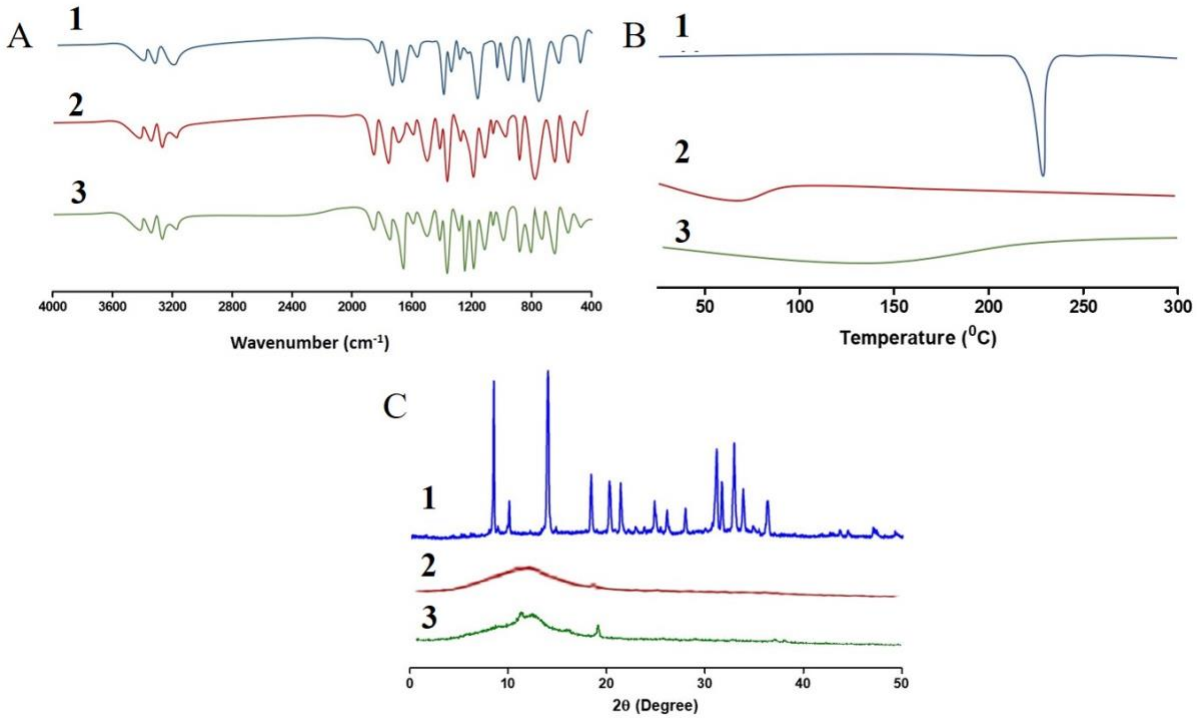

Fig. 2. (A) FTIR spectra, (B) DSC thermogram, and (C) X-ray diffractogram of (1) pure MTF, (2) MP-MTF, and (3) MP-MTF-GR.

3.7. *In vitro* Drug Release Study. The in vitro release behavior of pure MTF, MP-MTF (F4 without PBA), and MP-MTF-GR (F4) in four different release mediums was illustrated in Fig. 3. The result clearly showed that after 2 h, pure MTF was completely released in all mediums.

Specifically, MFT release from pure MTF was 98.12±9.11%, 99.01±8.31%, 97.05±9.21%, and 98.0±9.19% in PBS, glucose-containing PBS (1%), glucose-containing PBS (2%), and glucose-containing PBS (4%), respectively. Analysis statistically showed that no significant difference was found in the release of MTF from pure MTF in all mediums ($p > 0.05$). This result implies that the release of MTF from pure MTF is not affected by the presence of glucose.

While for MP-MTF, after 24 h the release of MTF in PBS, glucose-containing PBS (1%), glucose-containing PBS (2%), and glucose-containing PBS (4%) was 97.01±8.13%, 96.93±8.83%, 98.09±9.3%, and 99.19±9.16%, respectively. Despite the difference, the MTF release profile from F4 without PBA is not significantly different ($p > 0.05$). Similar to the released profile from pure MTF, formulation of MTF in form of MP-MTF exhibits release behavior that does not depend on the absence/presence of glucose. Compared to release profile from pure MTF, it was observed that formulation of MTF into microparticles could prolonged the release of MTF up to 24 h and was following previous studies.[18,19] This was due to the cross-linking of gelatin in microparticle formulation.[38] These findings indicated that formulation of MTF into microparticles was crucial to obtaining prolonged drug release.

Conversely, the release profile of MTF from MP-MTF-GR was found to decrease significantly in PBS compared to pure MTF and MP-MTF ($p < 0.05$). After 24 h, only 4.98±3.98 % of MTF was released. As the concentration of glucose in the medium increased, a higher amount of MTF was released from MP-MTF-GR. Subsequently, 29.34±2.64%, 99.09±8.92%, and 99.18±8.93% of MTF were released after 24 h in glucose-containing PBS (1%), glucose-containing PBS (2%), and glucose-containing PBS (4%), respectively. This indicates successful glucose-responsive drug delivery of MTF. The glucose responsive effect was obtained by the addition of PBA into the formulation as glucose sensitive agent. PBA exhibit reversible reaction

with *cis*-diol compound, found in glucose structure, through boronate formation. There are two forms of PBA in an aqueous solution, the hydrophobic neutral trigonal-planar and the hydrophilic negatively charged tetrahedral boronate. There is an equilibrium between these two forms. Both forms bind specifically to 1,2- or 1,3-diols found in glucose to form a cyclic boronic ester. This binding lead to increased hydrophilicity of PBA-containing materials, thus inducing swelling and dissembling of drug carrier, resulting in glucose-triggered drug release.[39]

To determine the release kinetics of MTF from GR-MP-MTF, the *in vitro* release data were fitted to several mathematic models. The results showed that MTF exhibit Korsmeyer-Peppas kinetics following test in PBS, glucose-containing PBS (1%), and glucose-containing PBS (2%) with the correlation coefficient of 0.9993, 0.9980, and 0.9403, respectively. While in glucose-containing PBS (4%), MTF showed Hixson-Crowell kinetics with a correlation coefficient of 0.9358. The Korsmeyer-Peppas kinetics describe drug release from polymeric systems. Moreover, the Hixson-Crowell kinetics describes release from systems with a change in surface area and diameter of particles.[40]

Fig. 3E. showed a pulsating profile of MTF release from the MP-MTF-GR formula in each incubation medium. It was found that less MTF was released when the formula was incubated in a medium containing glucose 100 mg/dL, and a high drug release was achieved when the formula was incubated in a medium containing glucose 400 mg/dL. Significant different ($p < 0.05$) was obtained in the MP-MTF formula without the GR compound. A constant and unchanged release profile was obtained during the change of the incubation medium. This shows that the release of MTF from the MP-MTF-GR formula could adapt according to changes in blood glucose levels in the media.

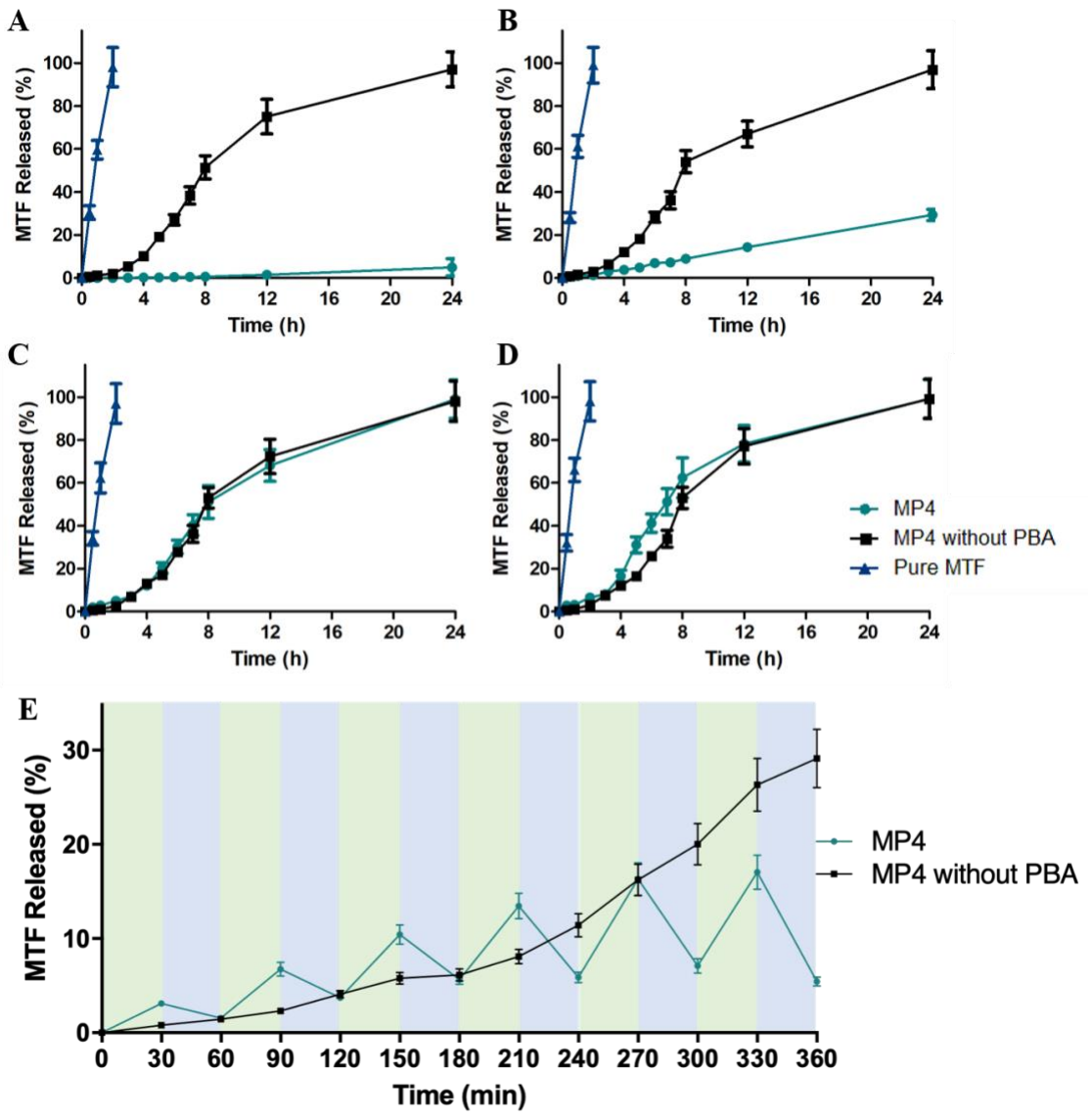

Fig. 3. *In vitro* drug release of MP4, MP4 without PBA, and pure MTF in (A) PBS media, (B) PBS + glucose 1% media, (C) PBS + glucose 2% media, and (D) PBS + glucose 4% media. (E) Pulsatile release profile of MP4 and MP4 without PBA in glucose concentration 100 mg/dL (blue) and 400 mg/dL (green) media (means ± SD, n = 3).

3.8. Formulation of DMN containing MP-MTF-GR (MP-MTF-GR-DMN). In this study, an aqueous blend of PVA and PVP was used in the DMN formulation with various concentrations, as shown in Table 2. Previous studies have shown that the combination of these

two polymers results in better mechanical strength and penetration ability than the single-use.[21,23] PVP is a polymer with good hardness, but when used alone it will provide inadequate mechanical strength for DMN due to its hygroscopicity.[23,41] The addition of PVA will fix the shortcomings of PVP. The combination of PVA and PVP causes the formation of hydrogen bonds between the C=O group of PVP and the –OH group of PVA to provide adequate mechanical strength.[26] All formulas added MP-MTF-PBA with the same concentration, 25% w/w. Fig. 4(A) shows the morphology of all DMN containing MP-MTF-PBA obtained using SEM. All formulas result in a homogeneous polymer blend with the DMN that formed a sharp needle tip.

3.9. Evaluation of Mechanical Strength and Penetration Properties of MP-MTF-GR-DMN. The mechanical strength of the DMN was evaluated to assess the ability of the DMN to withstand compression. The resistance of the DMN to compression is important because the DMN must be applied by manual compression to ensure that the DMN penetrates the *stratum corneum*. Furthermore, the mechanical strength was evaluated by providing a force of 32 N/array equivalents to manual compression strength. Morphology of MP-MTF-GR-DMN after the evaluation was obtained using microscope and shown in Fig. 4(B). The percentage reduction in needle height after compression indicates the mechanical strength of DMN.[26] The results of this evaluation are shown in Fig. 4(C). The percentage reduction in needle height were 24.02 ± 1.63%, 5.14 ± 2.66%, 5.55 ± 2.67%, 11.32 ± 1.17%, and 27.92 ± 1.30% for DMN1, DMN2, DMN3, DMN4, and DMN5, respectively. Interestingly, the percentage of needle height reduction in DMN2 and DMN3was <10% and significantly different ($p < 0.05$) compared to all formulas, which resulted an adequate mechanical strength.

Penetration ability was evaluated by using 8 layers of Parafilm®M. These parafilm layers have been developed as an artificial skin model and validated in previous studies.[28] The results of

514 the evaluation of the penetration ability (Fig. 4(D)) showed that DMN1, DMN4, and DMN5
515 penetrated only until the third layer of parafilm that equivalent to 378 µm of the total needle height
516 of 700 µm or 54% of the total needle height. On the other hand, DMN2 and DMN3 could penetrate
517 deeper up to the fourth layer of parafilm that equivalent to a thickness of 504 m, indicating that
518 72% of the needles could penetrate. Furthermore, there was no significant difference ($p > 0.05$)
519 between the number of holes formed in the fourth layer compared to DMN2 and DMN3. However,
520 the number of holes formed by DMN2 is more than DMN3, indicating better penetration ability.
521 Based on the result, DMN2 was considered for further tests.

522 **3.10. Calculation of Theoretical Drug Content in Needles and Determination of Drug**
523 **Recovery.** Before determining the drug content in the needle, it is necessary to determine the
524 density of the DMN formula. The DMN density was found to be 1.05 ± 0.01 mg/mm3, 1.06 ± 0.01
525 mg/mm3, 1.06 ± 0.06 mg/mm3, 1.07 ± 0.02 mg/mm3, and 1.07 ± 0.01 mg/mm3 for DMN1,
526 DMN2, DMN3, DMN4, and DMN5. Theoretically, the weights of MTF in needles were found to
527 be 81.44 g, 75.58 g, 71.43 g, 82.64 g, and 91.08 g for DMN1, DMN2, DMN3, DMN4, and DMN5.
528 Furthermore, the MTF content in the formula was determined by a UV-Vis spectrophotometer and
529 compared with the theoretical weights. Drug recovery for all formulas was in Fig. 4(E). Drug
530 recovery obtained is in the range of drug recovery requirements by ICH, 95 – 105% [42]. This
531 indicates that the formulation of MTF in the form of microparticles with additional glucose-
532 response reformulated in DMN did not affect the concentration of MTF.

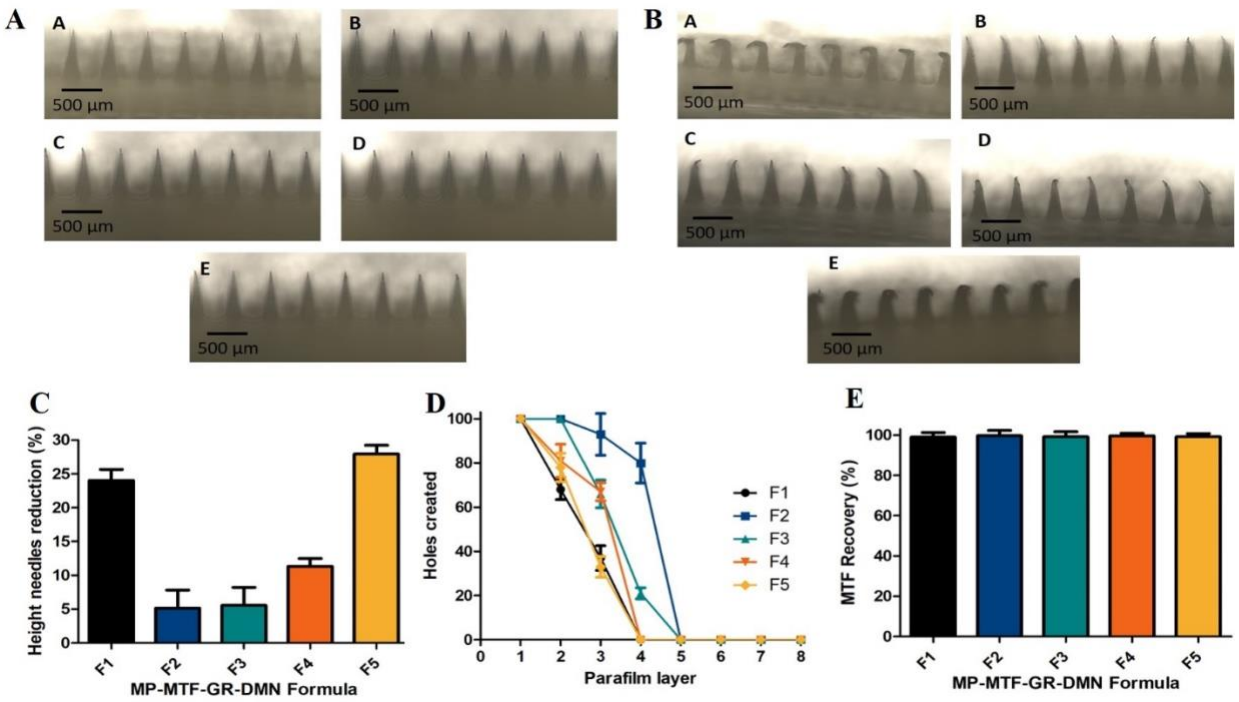

Fig. 4. Microscopic image of MP-MTF-GR-DMN (A) before evaluation and (B) after evaluation of DMN1 (A), DMN2 (B), DMN3 (C), DMN4 (D), and DMN5 (E). (C) mechanical strength, (D) penetration properties, and MTF recovery of MP-MTF-GR-DMN (means ± SD, n = 3).

3.11. Evaluation of Surface pH. One of the important things to consider in the formulation of intradermal dosage forms is the surface pH. This is important to note because human skin can have reactions that can create discomfort when in contact with materials with a pH that is incompatible with the pH that can be tolerated by human skin. The results of the surface pH evaluation of the MP-MTF-GR-DMN formula can be seen in Fig. 5(A). From the evaluation results, the MP-MTF-GR-DMN formulas DMN1, DMN2, DMN3, DMN4, and DMN5 had surface pH values of 5.72 ± 0.41, 5.82 ± 0.34, 5.78 ± 0.32, 5.69 ± 0.41, and 5.75 ± 0.33, respectively. The surface pH value of each formula was not significantly different ($p > 0.05$) and showed a safe pH value for the skin, which is 4.1 - 5.8.[43] This indicates that the MP-MTF-GR-DMN formula is safe to apply to human skin without causing irritation or other things that can cause discomfort.

3.12. Evaluation of Water Vapor Transmission Rate (WVTR). Evaluation of the water vapor transmission rate was carried out to assess the integrity of DMN against high humidity in the storage. The water vapor transmission rates of all formulas are shown in Fig. 5(B). WVTR increases with increasing polymer concentration. This is because WVTR increases with increasing polymer hydrophilicity.[44] However, this increase was not statistically significant ($p > 0.05$). After 7 days, the WVTR of all formulas obtained was lower than that of the previous study.[45] A low WVTR indicates the ability of the DMN to maintain its integrity against high humidity and exhibits the long-term stability of the DMN.

It is important to remember that WVTR does not affect DMN dissolution when applied to the skin. The selection of biodegradable polymers and the presence of interstitial fluid and skin elasticity can cause damage to the integrity of DMN and quickly dissolve after insertion.[46]

3.13. Evaluation of Moisture Absorption Ability (MAA). Moisture absorption ability is one of the crucial tests of DMN. The ability to absorb moisture from the outside during the storage period can affect the mechanical strength and penetration ability of DMN. In this study, we used 3 levels of humidity. The results can be seen in Fig. 5(C). After 14 days, the moisture absorption of all formulas at all humidity was found to be below 10%. In all formulas, moisture absorption increases with increasing humidity. The moisture absorption ability of each formula was also different. The moisture absorption ability increases as the concentration of polymer in the formula increases. The highest moisture absorption ability was obtained at DMN3, followed by DMN2, DMN1, DMN4, and DMN5. The increase in moisture absorption ability according to the polymer concentration was due to the hygroscopicity of the polymers used in both PVP and PVA. PVP and PVA are hygroscopic polymers because their structure contains hydrophilic groups, namely

carbonyl and hydroxyl groups.[30,41] The presence of carbonyl and hydroxyl groups can bind with water in the environment to form hydrogen bonds.

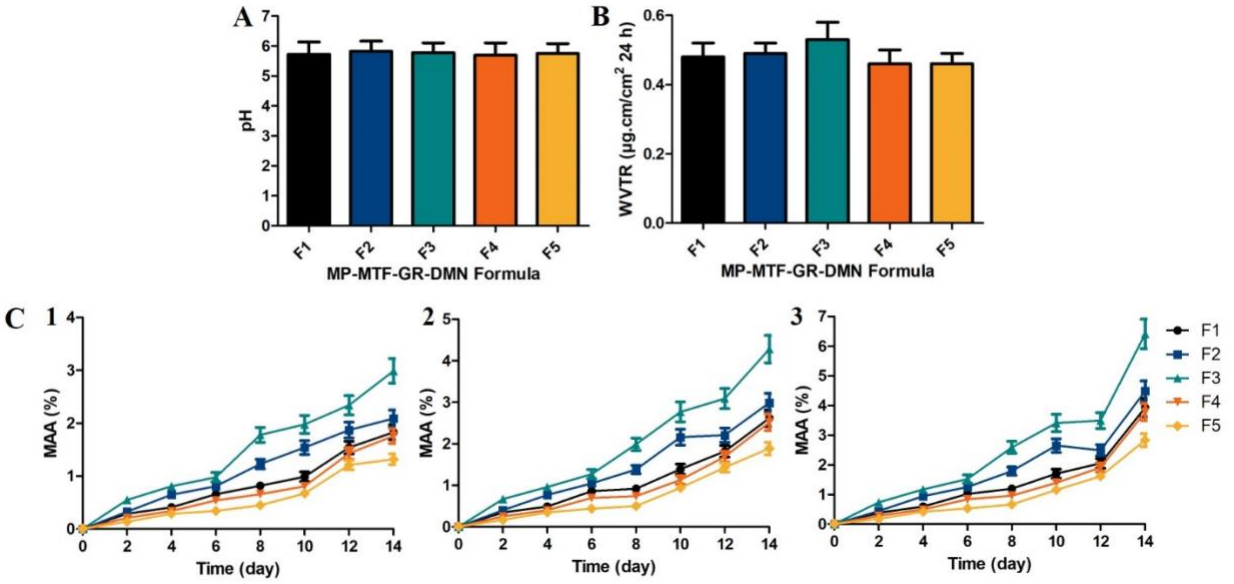

Fig. 5. Evaluation of (A) pH surface and (B) WVTR of MP-MTF-GR-DMN. (C) MAA of MP-MTF-GR-DMN in (1) RH 33%, (2) RH 69%, and (3) RH 97% (means ± SD, *n* = 3).

3.14. Dissolution Study. Dissolution time is an important test of DMN. According to its characteristics, DMN must be soluble when applied to the skin. The dissolving time test results are in Fig. 6. DMN2 was found to dissolve completely within 15 minutes, with a decrease in needle height after 3 minutes of observation. For effective delivery, needles containing drug microparticles must completely dissolve in the skin. Based on the dissolution time test, the time required for DMN to dissolve entirely in the skin is 15 minutes. Therefore, for full drug delivery, it is recommended to apply DMN to the skin for 15 minutes, and then it can be removed from the skin.

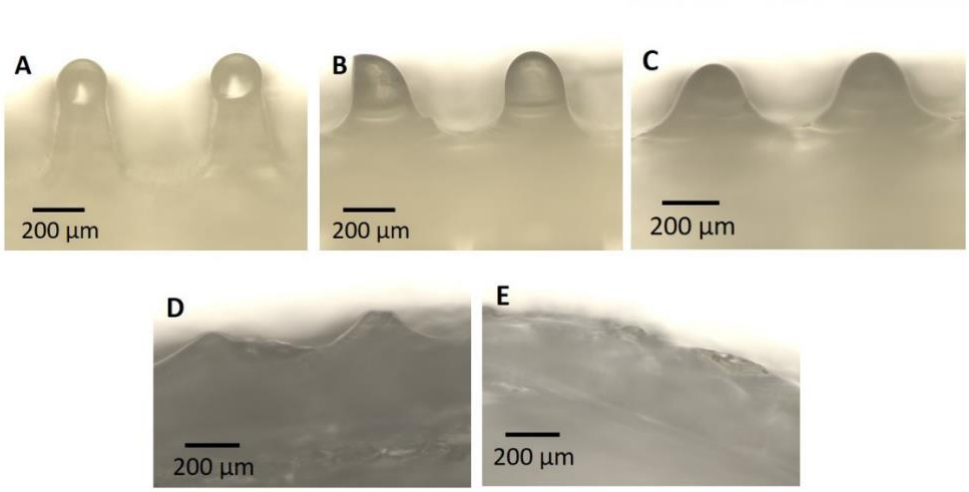

Fig. 6. Microscopic image of the dissolution of DMN2 at (A) 3 minutes, (B) 6 minutes, (C) 9 minutes, (D) 12 minutes, and (E) 15 minutes.

3.15. *Ex vivo* Permeation Study. *Ex vivo* permeation study was performed to determine the ability of DMN to permeate through rats' skin. The DMN permeation profile of MP-MTF-GR (DMN2), MP-MTF (DMN2 without PBA), and pure MTF in 4 media is shown in Fig. 7. After 24 hours, the permeated MTF from pure MTF-DMN was 65.70 ± 2.63 µg, 63.81 ± 2.41 µg, 64.79 ± 2.79 µg, and 66.98 ± 1.16 µg for PBS, PBS + glucose 1%, PBS + glucose 2%, and PBS + glucose 4%, respectively. Furthermore, there was no significant difference ($p > 0.05$) in the permeated MTF in all media which indicated that the presence of glucose had no effect on the pure MTF permeation from DMN.

Interestingly, similar results were obtained for the permeated MTF from MP-MTF-DMN. After 24 hours, the permeated MTF was 11.24 ± 0.55 µg, 11.48 ± 0.61 µg, 10.88 ± 1.47 µg, and 11.70 ± 0.67 µg from PBS, PBS + glucose 1%, PBS + glucose 2%, and PBS + glucose 4% media, respectively. There was no significant difference ($p > 0.05$) in the permeated MTF in all media, indicating that the presence of glucose also had no effect on MP-MTF permeation from DMN. However, the permeated MTF from MP-MTF-DMN was significantly lower ($p < 0.05$) than the

permeated MTF from pure MTF-DMN in all media. When applied, DMN will form small pores as channels for the drug or drug microparticles to pass through.[26] DMN loaded drugs can deliver drugs directly, meanwhile for drug microparticles it is necessary to pass through the polymer matrix first after being released from DMN.[23] This causes the microparticles in the DMN to permeate less than the pure drug in the same time duration and provides longer MTF availability.

On the other hand, the MTF permeation from MP-MTF-GR-DMN obtained 11.30 ± 0.29 µg, 23.31 ± 1.64 µg, 36.12 ± 3.77 µg, and 53.09 ± 3.01 µg from PBS, PBS + glucose 1%, PBS + glucose 2%, and PBS + glucose 4%, respectively. There was a significant increase ($p < 0.05$) of the permeated MTF with increasing glucose levels in the media. Accordingly, these results indicate that PBA as a glucose-response material is not only successful in regulating the *in vitro* release of MTF, but also able to regulate *ex vivo* permeation of MTF based on the increase in glucose levels.

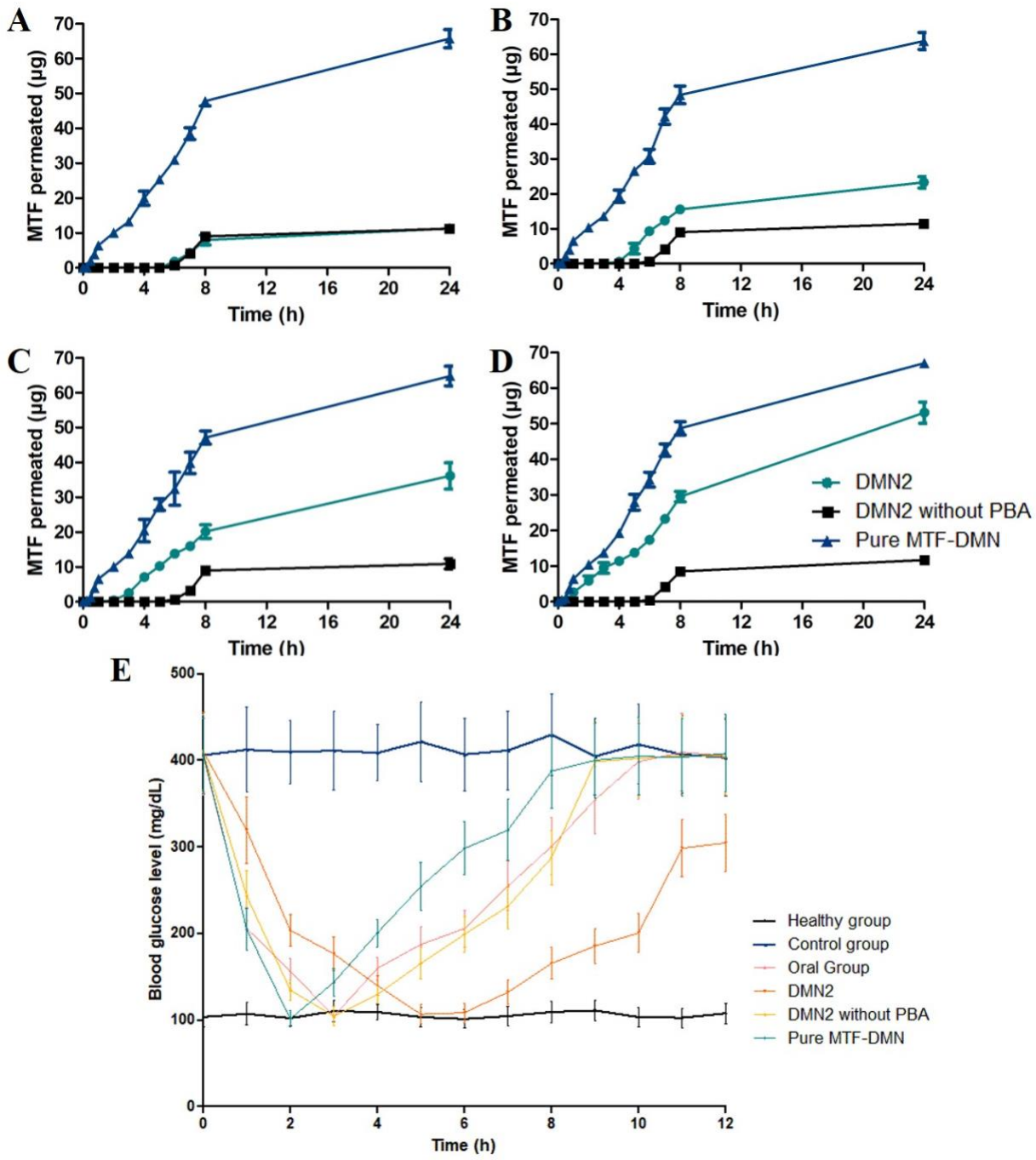

Fig. 7. *Ex vivo* permeation of DMN2 (MP-MTF-GR-DMN), DMN2 without PBA (MP-MTF-DMN), and pure MTF-DMN in (A) PBS media, (B) PBS + glucose 1% media, (C) PBS + glucose 2% media, and (D) PBS + glucose 4% media. (means ± SD, n = 3). (E) Blood glucose

levels of diabetic rat model following the administration of DMN, DMN without PBA, and pure MTF-DMN and MTF orally (means ± SD, n = 5).

It is necessary to bear in mind that the dissolution time of DMN in the skin and the time required for metformin to be permeated entirely into the systemic tract were different. DMN may be dissolved in 15 minutes, but the encapsulation system of metformin in the form of microparticles can prolong the permeation time of metformin into the systemic tract. In addition, the glucose-responsive material in the form of PBA linked to the polymer constituent of the microparticles provides a permeation effect of metformin which is accelerated as the glucose level in the media increases.[18]

In addition to considering the number of MTF that successfully permeated, flux was also an important parameter in determining the profile and permeation behavior of MTF. Flux is defined as the number of drug molecules from the preparation that is able to permeate through a barrier in a certain time.[47,48] The results of flux calculations from formulas DMN2, DMN2 without PBA, and pure MTF in various media are listed in Table 3. Based on the data, the highest flux was obtained in pure MTF and there was no significant difference ($p > 0.05$) in pure MTF flux in each media. Meanwhile, the lowest flux was obtained in DMN2 without PBA ($p > 0.05$) and there was no significantly different between the media. DMN2 shows a decrease in flux than pure MTF, which shows a controlled release profile.[49] The flux of DMN2 increased with increasing glucose levels in the medium, indicating that glucose levels affected the rate and MTF permeation profile of DMN2. This also proves that the presence of PBA in formula DMN2 could produce glucose-sensitive permeation behavior.

Table 3. Flux permeation of DMN2, DMN2 without PBA, and Pure MTF in PBA, PBS + Glucose 1%, PBS + Glucose 2%, and PBS + Glucose 4% Media (mean ± SD, n = 3)

Media	Flux at 24 h (μg/cm^2/h)		
	DMN2	DMN2 without PBA	Pure MTF-DMN
PBS	0.871 ± 0.04	0.874 ± 0.06	7.534 ± 0.12
PBS + Glucose 1%	2.122 ± 0.11	0.881 ± 0.07	7.614 ± 0.37
PBS + Glucose 2%	3.301 ± 0.23	0.831 ± 0.08	7.630 ± 0.37
PBS + Glucose 4%	4.974 ± 0.17	0.868 ± 0.06	7.869 ± 0.15

3.16. *In Vivo Study in Diabetic Rats.* To determine the effect of glucose control resulting from the formula, *in vivo* study on diabetic rats was carried out (Figure 7E). *In vivo* study was carried out on five groups of animals, namely the healthy group (negative control), the diabetic group without treatment (positive control), the diabetic group that was given MTF orally, the diabetic group that was given DMN2, the diabetic group that was given DMN2 without PBA, and the diabetic group was given pure MTF-DMN. MTF oral group was given MTF at dose 100 mg/kg BW meanwhile all of DMN groups were given 6 patches with the total MTF load 453.48 μg. Diabetic induction was done by giving STZ injection. STZ injection causes glucose levels to exceed 400 mg/dL. In the positive diabetes group, a BGL of more than 400 mg/dL persisted until the end of the test. Meanwhile, in the healthy group, BGL was at normal levels (<200 mg/dL) and continued until the end of the test.

MTF oral group was given metformin 100 mg/kg BW showed a decrease in blood glucose levels after 2 hours of administration with a minimum BGL at 104.31 ± 11.47 mg/dL achieved after 3 hours. Metformin given orally could maintain glucose levels <200 mg/dL for up to 4 hours. Meanwhile, the diabetic group given MTF-DMN showed a decrease in BGL that was achieved after 2 hours and could be maintained for up to 3 hours. The minimum BGL in the MTF-DMN group was 101.34 ± 9.12 mg/dL, achieved after 2 hours. The diabetic group given DMN without

PBA also showed a decrease in normal glucose levels after 2 hours with a minimum BGL of 104.34 ± 11.48 mg/dL. However, this formulation could produce a more prolonged glucose control effect (5 hours) than oral metformin and pure MTF-DMN, indicating that the microparticle form could increase the prolonged MTF release. Hence, it could provide a longer glycemic effect.

The combination of MP-MTF-GR with DMN was able to produce better glucose control. In addition to the prolonged MTF effect of the MP form, the presence of a GR ingredient, namely PBA, also produces a more controlled effect. PBA causes MTF to be more controlled according to glucose. The high BGL triggers the release of the MTF. The results of the glycemic control effect obtained were found to be longer. Administration of DMN2 produced a decrease in BGL after 3 hours and maintained normal BGL for 8 hours with a minimum BGL of 101.31 ± 11.28 mg/dL.

The result showed that the GR-MP-MTF-DMN formula can deliver metformin sufficiently to exert a glycemic control effect even with a reduced dose. Administration via DMN makes it possible to reduce the dose given because the DMN system could deliver drug particles efficiently to the systemic channel without losing the drug during the delivery.[20] However, these results need to be supported by pharmacokinetic data to determine the dose of loaded metformin in the formula and the size of the patch applied to the patient.

3.17. Hemolysis Test. The hemolysis test is an early-stage test conducted to determine the toxicity of newly developed pharmaceutical preparations. Hemolysis tests were carried out on several samples to ensure that each material used was not potentially toxic to erythrocytes.[29]

Here, we performed hemolysis tests on the active substance MTF, the compound that composes the MP, the compound that is glucose responsive, and the compound that composes the DMN (Fig. 8.). The test result showed that water as positive control causes hemolysis, which is

indicated by the color of the test results that changes to red which indicates that the erythrocyte sample is lysis and mixed with water. Meanwhile, PBS solution as negative control did not cause hemolysis which was indicated by the color of the solution that did not change. The same results were also obtained in all test samples. In addition, the calculation of the percentage of hemolysis in all samples obtained a value of less than 5%. It was found that all samples were not potentially toxic to erythrocytes.[50]

Finally, in this research, a combination of GR-MP-MTF compounds was obtained, which is delivered through the optimal DMN system, which can deliver MTF efficiently and control its release with constituent compounds that are not toxic to the body's erythrocytes.

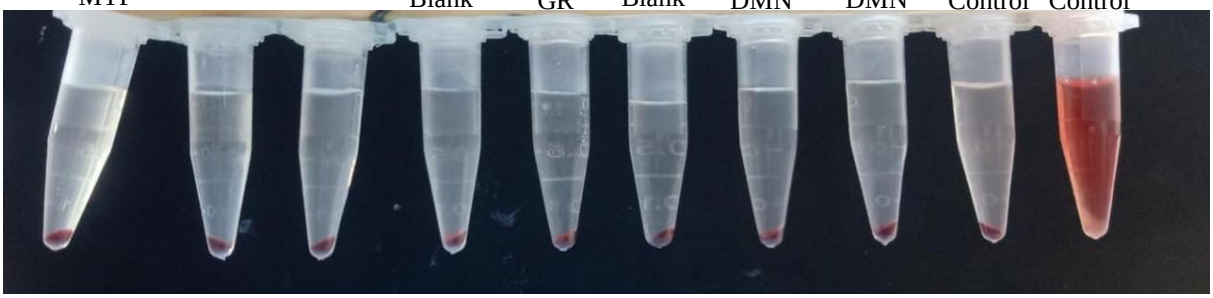

Fig. 8. Hemolysis Result of MP-MTF-GR, MP-MTF-GR-DMN, and their component prepared

Overall, we successfully developed a selective delivery MP of MTF for specific delivery in the hyperglycemic condition. The formulations were able to release MTF based on the concentration of glucose in the media. This could be beneficial to avoid the possibility of hypoglycemic which could be caused by the conventional oral administration. Furthermore, the incorporation of MP into DMN resulted in the formulation possessing adequate mechanical properties and insertion ability. Importantly, the *ex vivo* study showed the selective delivery of the MPs through transdermal administration. In addition, *in vivo* study showed the formula could decrease BGL in diabetic rats. Therefore, the combination approach developed in this study could potentially

become alternative delivery to the oral administration. To refine this concept, pharmacokinetic and pharmacodynamic studies are needed to determine the appropriate patch size given to the patient and the timing of subsequent use in multiple dose administration. In chronic condition like T2DM, it should be noted that the treatment would be done for long period. Therefore, the deposition of polymers in the skin should be considered. Several studies show that PVP and PVA are biocompatible materials.[51–53] Some studies focusing on the application of DMNs containing insulin have shown that the polymers were deposited in the skin. [54,55] However, the deposition of the polymer following long term and repeated application has not been studied. It has been suggested that to avoid the high deposition of the polymer in the skin following the application of DMNs, biodegradable polymers with low molecular weight could be used. [54]

One of the critical points in the development of DMN system is the translation of this technology. It has been suggested that a cooperative collaboration between industry and academia regarding the line scale-up production and manufacturing of DMNs, particularly in pharmacopoeial standards and good manufacturing practice (GMP) guidelines. [56] Additionally, the sterilization issue of products that will be inserted into the skin, such as DMN, is undoubtedly something that needs to be considered. It was shown that even after repeated use on the skin, it did not interfere with the skin barrier function. In addition, the application of polymer-based MN formulas such as DMN and hydrogel-forming MN was found not to stimulate the humoral immune system.[57] Furthermore, it has been reported that the administration of MNs did not cause any microorganism penetration across the skin. Accordingly, it could be estimated that the risk of infection should not be caused by the administration of MN. However, further study is required to investigate the sterility of this combination approach.

With respect to the scaled-up manufacturing of this system, in this study, we used the micro molding method by utilizing centrifugation to remove bubbles and fill microholes perfectly. This method has proven to produce microneedles with perfect physical shape. However, it is undeniable that this fabrication method has limitations for scaled-up manufacturing. Therefore, it is necessary to reconsider the fabrication method for scaled-up manufacturing. One promising method is the fabrication method in the form of a double-penetration female mold (DPFM) combined with the positive pressure microperfusion technique (PPPT), showing that through this system, microneedles can be produced in large quantities, with optimal physical form.[58]

In applying the microneedle, an applicator can ensure that the pressure applied to the microneedle is appropriate so that the needle can be inserted entirely into the stratum corneum. An example of a commonly used applicator is a spring-operated applicator specially designed for MN insertion.[59] In addition, a previous study has provided an innovation in applying microneedle to the stratum corneum by using a pressure-indicating sensor film that would change color when the applied force reaches 30N, and the color would be more concentrated when given a greater force. Furthermore, the minimum pressure required to insert a microneedle array of 1 cm^2 into the stratum corneum is 32 Ncm^{-2}.[57]

4. Conclusion

In this study, MTF was successfully developed in the form of microparticles incorporated with glucose-response polymer using a combination of PBA and gelatin. The results of the physico-chemical evaluation, including entrapment efficiency, particle size, and characterization with various instruments, including FTIR, DSC, and XRD, showed that MTF was completely entrapped in PBA-gelatin polymer without changing its chemical structure. The *in vitro* release profile showed that this formula regulated the release of MTF dependent on glucose levels.

Glucose-response-incorporated MTF microparticles were also successfully delivered through dissolving microneedle with a combination of PVA and PVP as polymers. The results of the physico-chemical evaluation of DMN showed adequate mechanical strength properties and penetration ability and stable DMN against environmental changes, including changes in humidity. *Ex vivo* permeation study of the developed formula shown permeation and release of MTF that occurs depending on glucose levels. Importantly, the combination approach developed in this study showed the significant blood glucose level in diabetic rats compared to other approaches. Thus, the combination of this system can be a solution as an alternative to MTF delivery to reduce gastrointestinal side effects and regulate the on-demand release of MTF, which depends on glucose levels to prevent the risk of hypoglycemia and increase the bioavailability of MTF. This system is promising to be further developed through *in vivo* testing and clinical testing, to increase the therapeutic effect of MTF and reduce its adverse side effects to increase the effectiveness of T2DM therapy.

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Notes

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985 **Table of Content Graphic**

986 Glucose-Responsive Microparticles Loaded Dissolving Microneedles for Selective Delivery of Metformin: A *Proof* of Concept Study

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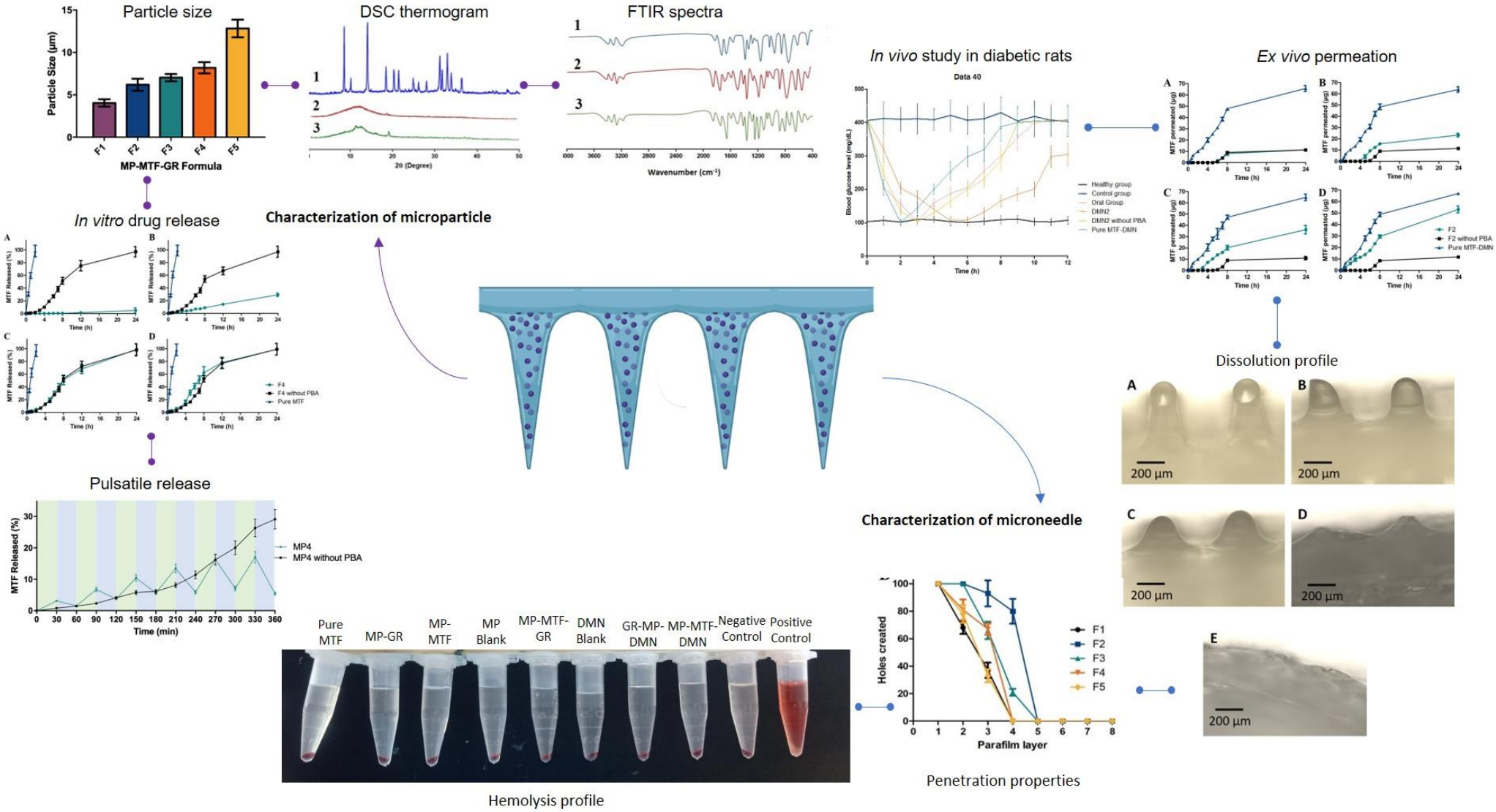

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