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**PENULIS MERUPAKAN PENULIS PERTAMA DAN SELALU
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Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Fwd: NANOMED-644 - "Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery"

1 message

Andi Permana <apermana01@qub.ac.uk>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Tue, Apr 4, 2023 at 3:55 PM

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From: WIREs Nanomedicine and Nanobiotechnology <onbehalf@manuscriptcentral.com>
Sent: Monday, April 6, 2020 4:23:03 PM
To: Eneko Larraneta Landa <e.larraneta@qub.ac.uk>
Cc: Andi Permana <apermana01@qub.ac.uk>; Eneko Larraneta Landa <e.larraneta@qub.ac.uk>; Ryan Donnelly <R.Donnelly@qub.ac.uk>
Subject: NANOMED-644 - "Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery"

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Dear Dr. Larrañeta,

Your manuscript has been successfully submitted online and is presently being given full consideration for publication in WIREs Nanomedicine & Nanobiotechnology.

Manuscript ID: NANOMED-644 (Please include in all future correspondence regarding this submission)
Manuscript Title: "Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery"
CASRAI CRediT Taxonomy: Authors' contribution(s) to the manuscript are attributed as follows:
CRediT Taxonomy

Andi Dian Permana
Investigation-Equal, Writing-original draft-Equal, Writing-review & editing-Equal

Eneko Larrañeta
Conceptualization-Equal, Writing-review & editing-Equal

Ryan Donnelly
Conceptualization-Equal, Supervision-Equal, Writing-review & editing-Equal

I will first complete system and formatting checks and let you know if we need any additional information or files. Once I verify that all the information we need is present, I will notify the editor to begin evaluation and peer review. You can view the status of your manuscript at any time by checking your Author Center after logging into <https://mc.manuscriptcentral.com/nanomed>.

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Thank you for submitting your manuscript to WIREs Nanomedicine & Nanobiotechnology.

4/4/23, 4:09 PM

Universitas Hasanuddin Mail - Fwd: NANOMED-644 - "Recent advances in combination of microneedles and nanomedicines for ly...

Regards,
Edrian Tolentino
WIREs Nanomedicine & Nanobiotechnology Editorial Office

**BUKTI
REVIEW
DARI
REVIEWERS**



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

Fwd: Decision on Manuscript ID NANOMED-644.R1

1 message

Andi Permana <apermana01@qub.ac.uk>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Tue, Apr 4, 2023 at 4:11 PM

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From: Eneko Larraneta Landa <e.larraneta@qub.ac.uk>
Sent: Tuesday, July 21, 2020 2:27:53 AM
To: Ryan Donnelly <R.Donnelly@qub.ac.uk>; Andi Permana <apermana01@qub.ac.uk>
Subject: Fwd: Decision on Manuscript ID NANOMED-644.R1

Hello guys,

Please see below. Reviewer 1 is again asking for more stuff. This is quite annoying. What about having a quick meeting to talk about the required changes?

Regards

Eneko

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From: WIREs Nanomedicine and Nanobiotechnology <onbehalf@manuscriptcentral.com>
Sent: Monday, 20 July 2020, 19:21
To: Eneko Larraneta Landa
Subject: Decision on Manuscript ID NANOMED-644.R1

This message is from an external sender. Please take care when responding, clicking links or opening attachments.

Dear Dr. Larrañeta,

I am writing in regards to Manuscript ID NANOMED-644.R1 entitled "Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery," which you submitted to WIREs Nanomedicine & Nanobiotechnology. The reviewer(s) have suggested some revisions to improve the manuscript. Their comments are included at the bottom of this letter; I invite you to respond to them and revise your manuscript.

Please submit your revision within 6 weeks.

A revised version of your manuscript that takes into account the comments of the reviewer(s) will be reconsidered for publication. Please note that revising your manuscript does not guarantee eventual acceptance, and that your revision will be subject to re-review before a decision is rendered.

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When you submit your revision, you must provide a point-by-point summary of how each of the issues raised by the reviewer(s) were addressed. Please note that the space provided is for plain text only, as the system will not accept any formatting. For each criticism raised, please provide:

1. The criticism from the reviewer.
2. The response and explanation of how the manuscript has been modified in response to the criticism.

IMPORTANT: We have your original files; therefore, please delete the file(s) that you wish to replace and then upload the revised file(s). You will be able to upload both marked and clean versions of your Main Document. Please note that figures must be in PRODUCTION-READY format.

Please do not hesitate to get in touch with us if you have any queries or concerns.

Once again, thank you for submitting your article to WIREs Nanomedicine & Nanobiotechnology. I look forward to receiving your revision.

Sincerely,

Dr. Gareth Williams
Associate Editor, WIREs Nanomedicine & Nanobiotechnology
g.williams@ucl.ac.uk

Reviewer: 1

Comments to the Author(s)

Permana, A.D., Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery.

NANOMED-644.R1

SUMMARY: The revised manuscript was unfortunately, not substantially revised. The English and grammar continue to be poor and non-literal. Most disappointingly, the description of the lymphatic system continues to be nonsensical and could benefit by collaboration with a physiologist with a knowledge of the lymphatics and the immune system. I felt my prior comments were superficially addressed, often incorrectly addressed, and not considered carefully. Many of the confusion sections remain. The following points are again offered.

1. Page 1 Line 57: The description of the lymphatic system is rather poor. The authors cite Levick and Michel but improperly so. Starling's law of 1896 suggested that return of capillary filtrate occurred in the venous capillaries, but Michel and Levick elegantly showed that because of the glycocalyx, virtually no reabsorption occurs in the venous capillaries and instead ALL reabsorption occurs by the lymphatics. The addition made in response to my comment was not appropriate.
2. Page 2 Line 8: The authors describe the lymphatic endothelium presenting antigens, but this is a tertiary effect compared to the mounting of adaptive immune response within lymph nodes. The lymph nodes are part of the lymphatics but a description of their role in the immune system them is not contained. Furthermore, lymph nodes do not filter lymph. This description would be misleading to the novice reader.
3. Figure 1 is a poor choice of figures. There is no blind ended initial lymphatics and the lymphatic vasculature is represented incorrectly as being closed. The lymphatics are open and it is not circulatory. See Figure in O'Donnell, T.F. (PMID: 28214496). I believe the authors do not understand the lymphatic physiology which is the source of questions.
4. Section 1.1 Why don't the authors state that nanoparticles are taken up at the initial lymphatics (that again are not shown in Figure 1). From Section 2.1, It appears that uptake into the initial lymphatics is what the authors refer to as "passive uptake."
5. Section 1.2, line 43. Lymph nodes do not "assemble along the LVs." Instead they are fed by multiple afferent LVs and have one efferent LV. Lymph nodes are not enclosed in connective tissue, but rather adipose tissue.
6. Line 47. Instead of "boles" do the authors mean "basins" ?
7. Page 7. Lymphangiogenesis removes capillary filtrate from the tumor microenvironment thereby reducing regional pressure. The increased interstitial pressure is due to tumor angiogenesis and is NOT due to lymphangiogenesis. The lymphatic serve to remove fluid, thereby reducing interstitial pressure. This is poorly described, misrepresents the literature, and should not be published.
8. On page 8, first full paragraph, the authors describe how substances enter the lymphatics. The initial

lymphatics (again not shown in Figure 1), are the sites where dendritic cells enter the lymphatics. This is the site where nanoparticles (i.e. Technetium radiocolloid in lymphoscintigraphy) are taken up by the lymphatics. This is the site where MN arrays deliver drugs and nanoparticles.

9. The active targeting described on Page 9 is quite confusing. The initial lymphatics are designed to take up macromolecules and cells and by targeting molecules on the lymphatic endothelium, one prohibits transport throughout the lymphatics without the chance to reach the blood circulation. There are no pharmaceuticals that have drug targets on the lymphatic endothelium. If one were to target LYVE-1 with a nanoparticle decorated with HA, for example, then the nanoparticles would likely not make it to the blood stream, but instead be “captured” within the peripheral lymphatics. Is this “active” transport? I would think it is NOT.

10. Table 1 is a listing of drugs in which oral, i.m., s.c. administration results in a biodistribution within the lymphatics. Now in this review, I don’t see how this relates to lymphatic delivery or MN. Biodistribution is different than lymphatic delivery. I am completely confused at what the authors are attempting to convey with this. Are these drugs simply begin cleared through the reticuloendothelial system?

11. The section, “Interstitial administration for lymphatic drug targeting” is confusing. Uptake into the lymphatics is via the initial lymphatics (again not shown in Figure 1) that are beneath the epidermis and surround all organs. The failure to place drugs at the site of the initial lymphatics results in reduced uptake. This is well shown in older lymphoscintigraphy studies that show intradermal versus s.c. administration of radiocolloid is optimal for delivery to draining lymph nodes. The reason why vaccines have greater T and B cell responses when administered intradermally is due to their local exposure to LCs and subsequent uptake by the initial lymphatics as first shown by Cubas (PMID: 19238010). The reason why MN have enhanced lymphatic uptake is because they deposit test vehicle at the site of the initial lymphatics.

12. Page 15: As per my prior review, there is no higher lymphatic flow rate in the dermal lymphatics. The highest flow rates are in the conducting lymphatic vessels draining the watershed. The interstitial pressure does not impact uptake – intradermal injection in the foot of a standing person is as easily taken up as in the dorsum of the hand. This is an open system, not a closed system. The evidence of enhanced uptake from MN or Mantoux injections compared to s.c. or i.m. is simply a matter of depositing the drug at the initial lymphatics.

13. Primary and initial lymphatics are again described, even though primary lymphatics was replaced by initial lymphatics.

14. Finally I offered additional works showing drug delivery to lymphatics had improved efficacy in autoimmune disease and cancer, but instead of the authors integrating that into their writing, they simply added those works in a Table. These works support MN array and provide context for why lymphatic delivery may be advantageous. I felt their cursory inclusion reflected a lack of careful and substantial revision of the manuscript.

**BUKTI
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REVIEW**

RESPONSES TO REVIEWER

Comments to the Author(s)

Permana, A.D., Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery.

NANOMED-644.R1

SUMMARY: The revised manuscript was unfortunately, not substantially revised. The English and grammar continue to be poor and non-literal. Most disappointingly, the description of the lymphatic system continues to be nonsensical and could benefit by collaboration with a physiologist with a knowledge of the lymphatics and the immune system. I felt my prior comments were superficially addressed, often incorrectly addressed, and not considered carefully. Many of the confusion sections remain. The following points are again offered.

1. Page 1 Line 57: The description of the lymphatic system is rather poor. The authors cite Levick and Michel but improperly so. Starling's law of 1896 suggested that return of capillary filtrate occurred in the venous capillaries, but Michel and Levick elegantly showed that because of the glycocalyx, virtually no reabsorption occurs in the venous capillaries and instead ALL reabsorption occurs by the lymphatics. The addition made in response to my comment was not appropriate.

Reviewer comments refer to:

The vital function of lymphatics is to return fluid from peripheral tissues into the systemic circulation. This function has been established by studies in ion channel electrophysiology and human arm investigations (Levick & Michel, 2010).

Authors' response:

We are grateful for reviewer's suggestion. The sentences have been revised accordingly to describe the physiological role of the lymphatic system and also to reflect events occurred in the lymphatic system according to the Revised Starling Principle (RSP) (Michel and Levick, 2010).

The lymphatic system (Figure 1) is a secondary vascular system in the human body (Morfoisse and Noel, 2019). The lymphatic system has an essential role in maintaining tissue homeostasis, supporting immune response and clearance of the interstitial space. The lymphatic system comprises three main parts, namely lymphatic vessels; lymph, which is the fluid contained in those vessels; and lymph nodes (Padera et al., 2016). Physiological function of the lymphatic system starts at the peripheral tissues where macromolecules-rich interstitial fluids and immunocompetent cells flow unidirectionally using open semicircular capillaries of the initial lymphatics from the peripheral tissues into the systemic circulation (Liao and von der Weid, 2015). The extravasated macromolecules-rich fluids (i.e lymphs) are then transported from the lymphatic capillaries (initial ducts) to larger lymphatic vessels prior to the final return into the systemic circulation via the thoracic duct (Liao and von der Weid, 2015). In view of the fluid exchange system in the human body, the classical Starling principle dictates that fluid movements between blood and the surrounding peripheral tissues are governed by differences in the hydrostatic and the oncotic pressure between plasma inside the capillaries and the fluid outside of the vessels (Michel et al., 2020). However, Levick and Michel proposed that the presence of endothelial glycocalyx layer (EGL) serves as a small pore system that further disrupts the oncotic pressures (Levick and Michel, 2010). Hence, the effect of capillary oncotic pressure on transvascular fluid exchange is considerably less than predicted from the original Starling model. This in turn suggesting that virtually all reabsorption occurs in the lymphatics instead of venous capillaries.

2. Page 2 Line 8: The authors describe the lymphatic endothelium presenting antigens, but this is a tertiary effect compared to the mounting of adaptive immune response within lymph nodes. The lymph nodes are part of the lymphatics but a description of their role in the immune system them is not contained. Furthermore, lymph nodes do not filter lymph. This description would be misleading to the novice reader.

Authors' response:

We are grateful for reviewer's suggestion. The sentences have been revised accordingly, as suggested.

The lymphatic system interacts with other systems in the body, including the cardiovascular, digestive and immune systems (D. Hu et al., 2019). With respect to the immune system, the lymphatic capillaries play a major role in the transportation of non-self (foreign) particles, invading microorganisms, as well as immune cells into the regional lymph nodes and other lymphoid organs (Liao and Padera, 2013). Lymph node is a highly compartmentalized microarchitecture that serves as an important location for immunosurveillance and the initiation of the adaptive immune responses (Liao and Padera, 2013). During tissue steady state, peripheral antigen-presenting cells (APCs), such as dendritic cells (DCs), are constantly travelling to the draining lymph node. These APCs are carrying self-antigen to induce self-reactive T cells to undergo clonal depletion, thus maintaining peripheral tolerance (Liao and von der Weid, 2015). However, during microbial infection, invading microorganisms at the peripheral sites are recognized by resident APCs. These APCs are subsequently transported into the lymph nodes and/or lymphoid organs via the initial lymphatics to interact with naïve lymphocytes. Clonal expansion and differentiation of the activated lymphocytes into effector and memory cells denote the successful induction of the adaptive immune responses (Liao and Padera, 2013; Liao and von der Weid, 2015). Overall, lymphatic system plays a significant role in the antigen delivery, antigen presentation, initiation, and regulation of immune responses.

3. Figure 1 is a poor choice of figures. There is no blind ended initial lymphatics and the lymphatic vasculature is represented incorrectly as being closed. The lymphatics are open and it is not circulatory. See Figure in O'Donnell, T.F..(PMID: 28214496). I believe the authors do not understand the lymphatic physiology which is the source of questions.

Authors' response:

We are grateful for reviewer's suggestion. The figure has been revised accordingly, as suggested.

4. Section 1.1 Why don't the authors state that nanoparticles are taken up at the initial lymphatics (that again are not shown in Figure 1). From Section 2.1, It appears that uptake into the initial lymphatics is what the authors refer to as "passive uptake."

Authors' response:

We are grateful for reviewer's suggestion. We are very thankful to the Reviewer for the comment. We have mentioned this in our manuscript.

It has been reported that small nanoparticles with sizes between 10–100 nm are passively accumulated in the lymph nodes after being taken up by the lymphatics and to further target lymph node (Schudel et al., 2019).

5. Section 1.2, line 43. Lymph nodes do not "assemble along the LVs." Instead they are fed by multiple afferent LVs and have one efferent LV. Lymph nodes are not enclosed in connective tissue, but rather adipose tissue.

Reviewer comments refer to:

The primary lymphoid organs in the body are the lymph nodes, which assemble alongside the LVs of the body (J. Hu et al., 2019; Marieb & Hoehn, 2013). There are hundreds of these small organs but, as they are typically enclosed in connective tissue, they are not ordinarily observed. Large groups of lymph nodes occur close to the surface of the body in the area of cervical, axillary and inguinal, where the collecting LVs gather to form boles (Liptak & Boston, 2019; Marieb & Hoehn, 2013).

Authors' response:

We are grateful for reviewer's suggestion. The sentences have been revised accordingly, as suggested.

The primary lymphoid organs in the body are the lymph nodes (J. Hu et al., 2019; Marieb and Hoehn, 2013). Physiologically, a lymph node is fed by multiple afferent LVs and have one efferent LV (Betterman and Harvey, 2016; Liao and von der Weid, 2015). There are hundreds of these small lymphoid organs but, as they are typically enclosed

in the adipose tissue (Betterman and Harvey, 2016), they are not ordinarily observed. Large groups of lymph nodes occur close to the surface of the body in the area of cervical, axillary and inguinal, where the collecting LVs gather to form basins (Liptak and Boston, 2019; Marieb and Hoehn, 2013).

6. Line 47. Instead of “boles” do the authors mean “basins” ?

Authors’ response:

We are grateful for reviewer’s suggestion. The word has been revised accordingly, as suggested.

7. Page 7. Lymphangiogenesis removes capillary filtrate from the tumor microenvironment thereby reducing regional pressure. The increased interstitial pressure is due to tumor angiogenesis and is NOT due to lymphangiogenesis. The lymphatic serve to remove fluid, thereby reducing interstitial pressure. This is poorly described, misrepresents the literature, and should not be published.

Reviewer comments refer to:

In cancer, infection and inflammatory diseases, metastatic distribution usually occurs through LVs to the sentinel lymph node. Furthermore, by producing pro-inflammatory and lymphangiogenic factors, tumour cells and associated macrophages stimulate lymphangiogenesis in the draining lymph nodes and at the tumour site (Alitalo, 2011; Proulx et al., 2013; Swartz & Lund, 2012). Lymphangiogenesis and lymph vessel dilation, which further increase the interstitial pressure, change the flow of the lymph from tumours and, consequently, alter immunity (Proulx et al., 2013; Swartz & Lund, 2012). The increase of lymph flow in the interstitial space could be beneficial for improved lymphatic targeting. Therefore, a delivery system which can deliver drugs to the interstitial space would be of great benefit to improving treatment of lymph-related diseases. Microneedles are an emerging delivery system which can efficiently deliver drugs to this site.

Authors' response:

We are grateful for reviewer's suggestion. The sentences have been revised accordingly, as suggested.

Lymphangiogenesis, formation of new lymphatic vessels from the pre-existing lymphatic vessels, is a restricted developmental event largely occurs during embryogenesis but is not normally programmed during postnatal condition (Christiansen and Detmar, 2011). Lymphangiogenesis has been suggested to play a role in the progression of human cancer metastasis (Christiansen and Detmar, 2011). During cancer progression, uncontrolled proliferation of tumor cells and tumor angiogenesis induces the elevation of interstitial pressure which subsequently stimulates lymphangiogenesis to increase the removal of fluid from the peripheral tissues (Liao and Padera, 2013; Trevisan et al., 2015). which in turn shall provide a beneficial factor for the lymphatic targeting approach. Therefore, a delivery system which can deliver drugs to the interstitial space would be of great benefit to improving treatment of lymph-related diseases. Microneedles are an emerging delivery system which can efficiently deliver drugs to this site.

8. On page 8, first full paragraph, the authors describe how substances enter the lymphatics. The initial lymphatics (again not shown in Figure 1), are the sites where dendritic cells enter the lymphatics. This is the site where nanoparticles (i.e. Technetium radiocolloid in lymphoscintigraphy) are taken up by the lymphatics. This is the site where MN arrays deliver drugs and nanoparticles.

Authors' response:

We are very thankful to the Reviewer for the comment. Indeed, we agreed that DCs are able to deliver the particles to the initial lymphatic both active and passive way, as explained in our manuscript.

"Alternatively, some molecules may also be delivered to the lymphatic system by targeting the antigen presenting cells (APCs) and subsequent APCs movement into the

lymph nodes. Generally, the APCs such as DCs take up the antigens in the extracellular matrix in the dermis (Trevaskis et al., 2015). Afterward, APCs permeate into the lymph and move to lymph nodes where they introduce antigen to effector cells (lymphocytes). Accordingly, the strategies of targeting the drugs to the APCs in the extracellular matrix can be utilised to deliver the drugs to the draining lymph nodes (Reddy et al., 2007)”

“As discussed previously, intradermal administration is preferable due to the high amount of DC in the dermis (Trevaskis et al., 2015). Cubas et al. (2009) have compared lymphatic trafficking and immune response following immunization by different routes. They found that intradermal immunization was able to show the greatest level of LN association for the longest period of time, which associated to the strongest humoral and cellular immune responses. Furthermore, this administration also showed the greatest population of germinal center and activated B cells, resulting in greater antibody levels and antigen-specific cytotoxic T lymphocyte responses (Cubas et al., 2009). In addition to DC, Langerhans cells (LC) are another of the APCs that are contained in the epidermis. Like DC, LC can migrate to draining lymph nodes (Johnston et al., 2000). Intradermal injection can directly deliver the drugs to the dermis. However, the application of MNs may potentially deliver the drug particles to both the epidermis and dermis (Pamornpathomkul et al., 2018; Vora et al., 2017), resulting in the higher possibility of drugs to be taken by LC and DC compared to other route of administrations.

As explained, the drug uptake *via* DC is able to promote lymphatic targeting. Therefore, the active delivery of drugs to the lymphatic has also been developed by the use of selective receptors and different DC-specific targeted agents. One of the receptors expressed on DC is mannose receptor, C-type lectin receptors (CLR). The main characteristic of this receptors is the ability in carbohydrate recognition (Tacken et al., 2007). Targeting a cancer antigen (e.g. MUC1) to this receptor by directly reacting with mannose without the use of any type of delivery approach has exhibited significant cancer therapy in numerous investigations both preclinically and clinically (Karanikas et al., 1997; Tacken et al., 2007).

9. The active targeting described on Page 9 is quite confusing. The initial lymphatics are designed to take up macromolecules and cells and by targeting molecules on the lymphatic endothelium, one prohibits transport throughout the lymphatics without the chance to reach the blood circulation. There are no pharmaceuticals that have drug targets on the lymphatic endothelium. If one were to target LYVE-1 with a nanoparticle decorated with hyaluronan (HA), for example, then the nanoparticles would likely not make it to the blood stream, but instead be “captured” within the peripheral lymphatics. Is this “active” transport? I would think it is NOT.

Authors’ response:

We would like to offer our gratitude to the Reviewer for the constructive comment. Indeed, after careful checking, we decided to remove the referred explanation from our manuscript to improve the clarity.

10. Table 1 is a listing of drugs in which oral, i.m., s.c. administration results in a biodistribution within the lymphatics. Now in this review, I don’t see how this relates to lymphatic delivery or MN. Biodistribution is different than lymphatic delivery. I am completely confused at what the authors are attempting to convey with this. Are these drugs simply being cleared through the reticuloendothelial system?

Authors’ response:

We thank the Reviewer for the comment. We have extensively discussed the application of nanotechnology for lymphatic delivery in our manuscript. To support this, therefore, we made Table 1 to show the successful approach of nanoparticles to deliver the drugs to the lymphatic system, either observed from biodistribution study or from lymphatic delivery purpose. As per comment of Reviewer, we have revised the term “lymphatic delivery” in this part.

11. The section, “Interstitial administration for lymphatic drug targeting” is confusing. Uptake into the lymphatics is via the initial lymphatics (again not shown in Figure 1) that are beneath the epidermis and surround all organs. The failure to place drugs at the

site of the initial lymphatics results in reduced uptake. This is well shown in older lymphoscintigraphy studies that show intradermal versus s.c. administration of radiocolloid is optimal for delivery to draining lymph nodes. The reason why vaccines have greater T and B cell responses when administered intradermally is due to their local exposure to LCs and subsequent uptake by the initial lymphatics as first shown by Cubas (PMID: 19238010). The reason why MN have enhanced lymphatic uptake is because they deposit test vehicle at the site of the initial lymphatics.

Authors' response:

We are very thankful to the Reviewer for the comment. Indeed, we agreed that the intradermal administration results in greater immune responses due to the presence of APC, as explained previously in our manuscript. We have now included the explanation from paper suggested by the Reviewer, combined with our previous explanation.

Explanation about the lymphatic uptake *via* DCs

“Alternatively, some molecules may also be delivered to the lymphatic system by targeting the antigen presenting cells (APCs) and subsequent APCs movement into the lymph nodes. Generally, the APCs such as DCs take up the antigens in the extracellular matrix in the dermis (Trevaskis et al., 2015). Afterward, APCs permeate into the lymph and move to lymph nodes where they transfer antigen to effector cells (lymphocytes). Accordingly, the strategies of targeting the drugs to the APCs in the extracellular matrix can be utilised to deliver the drugs to the draining lymph nodes (Reddy et al., 2007)”

Explanation about the advantages of MNs for lymphatic delivery *via* DCs and LCs

“As discussed previously, intradermal administration is preferable due to the high amount of DC in the dermis (Trevaskis et al., 2015). Cubas et al. (2009) have compared lymphatic trafficking and immune response following immunization by different routes. They found that intradermal immunization was able to show the greatest level of LN association for the longest period of time, which associated to the strongest humoral and cellular immune responses. Furthermore, this administration also showed the

greatest population of germinal center and activated B cells, resulting in greater antibody levels and antigen-specific cytotoxic T lymphocyte responses (Cubas et al., 2009). In addition to DC, Langerhans cells (LC) are another of the APCs that are contained in the epidermis. Like DC, LC can migrate to draining lymph nodes (Johnston et al., 2000). Intradermal injection can directly deliver the drugs to the dermis. However, the application of MNs may potentially deliver the drug particles to both the epidermis and dermis (Pamornpathomkul et al., 2018; Vora et al., 2017), resulting in the higher possibility of drugs to be taken by LC and DC compared to other route of administrations.

12. Page 15: As per my prior review, there is no higher lymphatic flow rate in the dermal lymphatics. The highest flow rates are in the conducting lymphatic vessels draining the watershed. The interstitial pressure does not impact uptake – intradermal injection in the foot of a standing person is as easily taken up as in the dorsum of the hand. This is an open system, not a closed system. The evidence of enhanced uptake from MN or Mantoux injections compared to s.c. or i.m. is simply a matter of depositing the drug at the initial lymphatics.

Authors' response:

We thank the Reviewer for valuable response. Finally, after re-reading the main concept of the lymphatic delivery, we agreed with the Reviewer comments and finally removed the theory of “high lymphatic flow in the dermal” in our manuscript.

13. Primary and initial lymphatics are again described, even though primary lymphatics was replaced by initial lymphatics.

Authors' response:

We are grateful for reviewer's suggestion. The referred word “primary lymphatics” has been revised accordingly to “initial lymphatics”, as suggested.

14. Finally I offered additional works showing drug delivery to lymphatics had improved efficacy in autoimmune disease and cancer, but instead of the authors integrating that

into their writing, they simply added those works in a Table. These works support MN array and provide context for why lymphatic delivery may be advantageous. I felt their cursory inclusion reflected a lack of careful and substantial revision of the manuscript.

Authors' response:

We thank the Reviewer for valuable response. Following the reviewer's comment, we have extensively explained the works showing drug delivery to lymphatics had improved efficacy in autoimmune disease and cancer, according to the study suggested previously by the Reviewer as follows:

"Several studies have described the application of MNs, showing improved efficacy in autoimmune disease and cancer. Sevick-Muraca et al (2008) have demonstrated the application of single hollow MN device to deliver a near-infrared (NIR) fluorophore, indocyanine green for noninvasive optical imaging of LNs in breast cancer patients undergoing sentinel lymph node mapping (SLNM) at the minimum dose required. They have shown that lymph drainage pathways from the injection site to LNs were successfully observed when 10 µg or higher of indocyanine green dosages were delivered, visualized using NIR fluorescence imaging. Accordingly, this approach was found to be feasible for LN molecular imaging (Sevick-Muraca et al., 2008). Furthermore, Aldrich et al (2017) developed nanotopography (SOFUSA™) delivery for intradermal lymphatic delivery etanercept. In their study, they compared the pharmacokinetic profiles of etanercept following the administration *via* nanotopography and the administration various route, namely conventional subcutaneous injection, intravenous and intradermal. Furthermore, the lymphatic uptake was also evaluated in LNs. The results revealed that compared to untreated control, ID, and SC administration, the delivery of etanercept using SOFUSA™ resulted in improved lymphatic pumping and considerably reduced swelling in a progressive rat model of collagen-induced arthritis. Importantly, the nanotopography device was found to enable the highest retention in draining LNs in comparison with other administration routes (Aldrich et al., 2017). The application of nanotopography for lymphatic delivery has also been developed by Kwon et al. (2019). In their study, they used SOFUSA™ nanotopographical microneedle-array device for lymphatic delivery of cytotoxic T-

lymphocyte-associated antigen 4 (CTLA-4) and indocyanine green and compared anti-tumor response in comparison systemic administration. They showed that NIRF imaging exhibited that SOFUSATM was able to infuse 100 µL/h of indocyanine green into the epidermal spaces, where the initial lymphatics take up the agent allowing imaging of the impulsion of indocyanine green -laden lymph into the brachial LNs. Compared to conventional systemic administration, lymphatic infusion of anti-CTLA-4 could potentially lead to the inhibition of tumour growth. Finally, in clinical studies, nanotopographic infusion was able to deliver indocyanine green to the axilla and inguinal LNs of healthy human volunteers (Kwon et al., 2019).”

REFERENCES:

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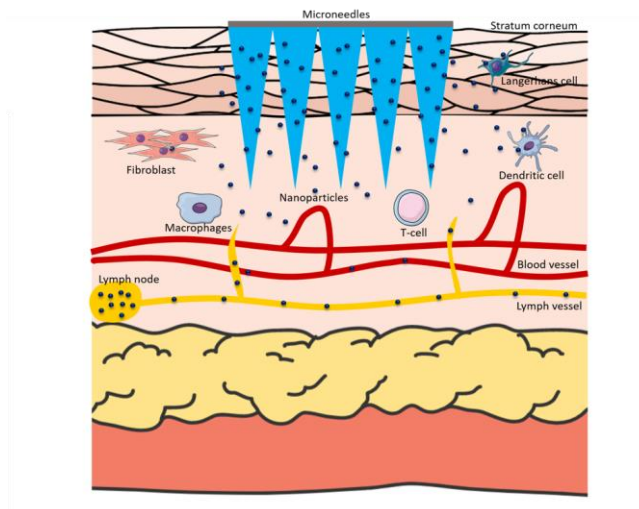
Article Title:

Recent advances in combination of microneedles and nanomedicines for lymphatic targeted drug delivery

Article Type:

○ ADVANCED REVIEW

Graphical/Visual Abstract and Caption



Nanoparticles ~~have~~ been investigated as a potential approach enhancing the lymphatic uptake of drugs. ~~Through~~ combination with microneedle delivery systems, this approach could potentially ~~be~~ be-useful in the

| passive and active delivery of nanoparticles to the lymphatic system via dermal lymphatic capillaries.

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Abstract

Numerous diseases have been reported to affect the lymphatic system. As such, several strategies have been developed to deliver chemotherapeutics to this specific network of tissues e-lymphatic system and associated organs. Nanotechnology has been exploited as one of the main approaches that could potentially improve the lymphatic uptake of drugs. ~~Different-Different~~ nanoparticle approaches utilised for both active and passive targeting of to target the lymphatic system -both actively and passively- are discussed here. Specifically, due to the rich abundance of dermal lymphatic capillaries in the skin dermis, particular attention is given to the route of administration, as exploring that intradermal administration could potentially result in higher lymphatic uptake compared to other route of administration routes. Recently, the progress in the progress in microneedle research has attracted particular the attention as an alternative for of scientist to replace the use of conventional hypodermic injections. The benefits of microneedles, when compared to intradermal injection, are subsequently provided highlighted. Importantly, several microneedles exhibit particular benefit in relation to therapeutic targeting of the lymphatic system advantages of microneedles in lymphatic targeting, especially when combined with nanoparticles, which are further discussed. However, despite the apparent benefits provided by this combination approach, further comprehensive preclinical and clinical studies are now necessary to realise the extent of the potential of this dual-delivery platform, further taking into consideration the application in the clinical study of this field must be further developed to enhance eventual the benefit, usability and acceptability in the intended patient end-users patients.

Keywords: Dermal lymphatic uptake, lymphatic system, targeted drug delivery system, microneedles, nanomedicine, nanoparticles

1. THE LYMPHATIC SYSTEM

The lymphatic system (Figure 1) is a secondary vascular system in the human body (Morfoisse & Noel, 2019), which has an essential role in maintaining tissue homeostasis, supporting the immune response and clearance of the interstitial space. The lymphatic system comprises of three main parts, namely: lymphatic vessels, lymph which is the fluid contained in those vessels, and lymph nodes (Padera, Meijer, & Munn, 2016). Physiological function of the lymphatic system starts at the peripheral tissues where macromolecules-rich interstitial fluids and immunocompetent cells flow in a unidirectional manner using open semi-circular capillaries of the initial lymphatics from the peripheral tissues into the systemic circulation (Liao & von der Weid, 2015). The extravasated macromolecules-rich fluids (i.e. lymph) are then transported from the lymphatic capillaries (initial ducts) to larger lymphatic vessels, prior to the final return into the systemic circulation via the thoracic duct (Liao & von der Weid, 2015). In relation to the fluid exchange system in the human body, the classical Starling principle dictates that fluid movements between blood and the surrounding peripheral tissues are governed by differences in the hydrostatic and the oncotic pressure between plasma inside the capillaries and the fluid outside of the vessels (Michel, Woodcock, & Curry, 2020). However, Levick and Michel proposed that the presence of endothelial glycocalyx layer (EGL) serves as a small pore system that further disrupts the oncotic pressures (Levick & Michel, 2010). Hence, the effect of capillary oncotic pressure on transvascular fluid exchange is considerably less than that originally predicted from the original Starling model. This in turn suggests that practically all reabsorption occurs in the lymphatics instead of venous capillaries.

The lymphatic system consists of a network of tissues that interacts with other systems in the body, including the cardiovascular, digestive and immune systems (D. Hu et al., 2019). With respect to the immune system, the lymphatic capillaries play a major role in the transportation of non-self (foreign) particles, invading microorganisms, as well as immune cells into the regional lymph nodes and other lymphoid organs (Liao & Padera, 2013). The lymph node is a highly compartmentalized microarchitecture that serves as an important location for immunosurveillance and the initiation of the adaptive immune responses (Liao & Padera, 2013). During tissue steady state, peripheral antigen-presenting cells (APCs), such as dendritic cells (DCs), are constantly travelling to the draining lymph nodes. These APCs are carrying self-antigen that induce self-reactive T cells to undergo clonal depletion, thus maintaining peripheral tolerance (Liao & von der Weid, 2015). However, during microbial infection, invading microorganisms at the peripheral sites are recognized by resident APCs. These APCs are subsequently transported into the lymph nodes and/or lymphoid organs via the initial lymphatics that then interact with naïve lymphocytes. Clonal expansion and differentiation of the activated lymphocytes into effector and memory cells denote the successful induction of the adaptive immune responses (Liao & Padera, 2013; Liao & von der Weid, 2015). Overall, the lymphatic system plays a significant role in the antigen delivery, antigen presentation, initiation, and regulation of immune responses.

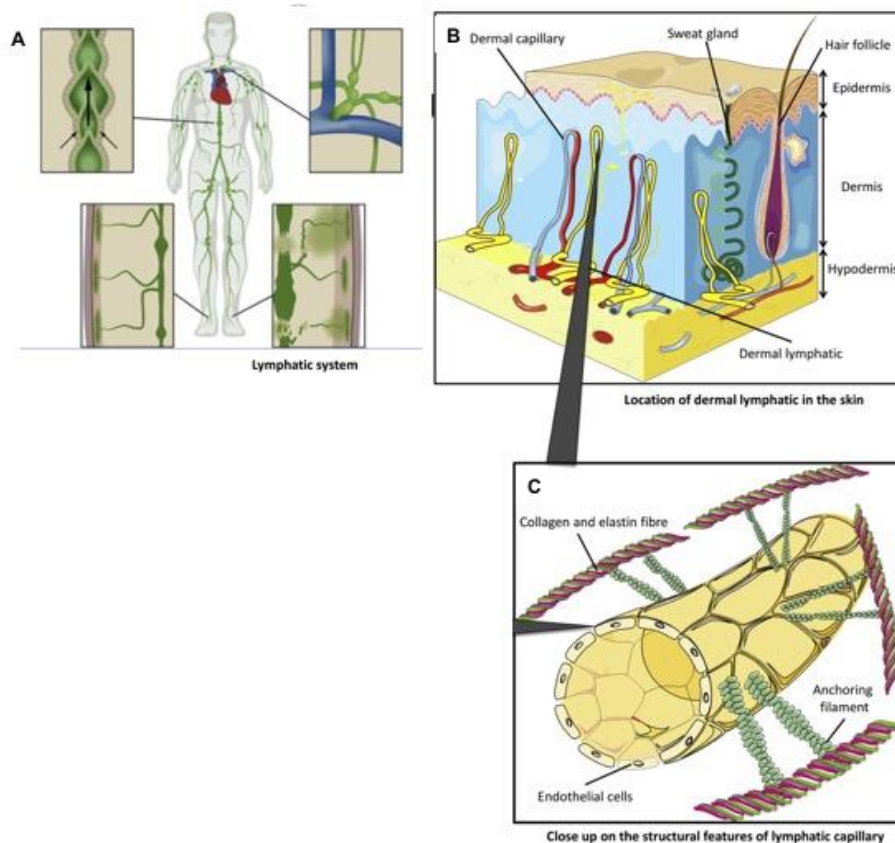


Figure 1. Schematic representation of the lymphatic system throughout the human body (A), the skin structure containing dermal lymphatic network (B), and the detail of dermal lymphatic capillaries showing the vessels anchoring to surrounding collagen and elastin via the anchoring filament (C), respectively. O'Donnell, Rasmussen, & Sevic-Muraca, 2017; Sabri et al., 2020). The figure is reproduced with permission of the publisher from the reference.

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1.1. Lymphatic vessels and lymph

The lymphatic vessels (LVs) form and direct a drainage system from the interstitial spaces to the thoracic conduit, as well as the right lymphatic trunk (Liao & von der Weid, 2015). The LVs are the primary channel for soluble antigens and antigen-presenting cells (APCs) to the lymph nodes responsible for the initiation of immune responses (K. Alitalo, 2011). According to the molecular types, physical appearance and role, there are three classes of the lymphatic vessels, namely: lymphatic capillaries (identified as initial LVs), pre-collecting and collecting LVs.

Initial LVs produce the “blind” ends of the vascular system of the lymphatics. The main function of these capillaries is the absorption of the interstitial fluid. Initial LVs consist of lymphatic endothelial cells (LECs), a specific thin film with shapes similar to an oak leaf, creating cell junctions which resemble a discontinuous button pattern (Liao & Weid, 2015), identified by particular expression of LV endothelial hyaluronan receptor 1 on the surface of LECs (Liao & Weid, 2015). They provide coverage of mural cells and do not have, or have limited basement membranes. As a result, due to these features, the interstitial fluids are able to penetrate initial LVs easily. Additionally, the transfer of immune cells and the diffusion of macromolecules is permitted via these capillaries. These button-like cell junctions show an discontinuous outline of the bond proteins of vascular endothelial cadherin and the adhesion molecule of platelet endothelial cell, developing overlying flaps amongst adjacent endothelial cells (Liao & von der Weid, 2015), and are assumed to act as main valves (Liao & von der Weid, 2015). This main valve structure is essential to avoid fluid leakage into the interstitial space from the initial LVs. In order to avoid the collapse of the vessels and keep the main lymphatic valves accessible, the adjacent extracellular matrix by fastening filaments support and are attached to LECs in lymphatic capillaries (Leak & Burke, 2004; Schulte-Merker, Sabine, & Petrova, 2011).

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The LVs are divided into functional sections named lymphangions that are interrelated by semi-lunar valves that avoid the lymph flowing back into the lumen of the lymphangions. Synergistic performance of these two kinds of valves, combined with regular spasm and relaxation of the smooth muscular layer of the lymphangions (regulated by the autonomic nervous system) generates a peristaltic movement (Mendoza & Schmid-Schönbein, 2003). Therefore, it results in the movement of lymph from one lymphangion to other lymphangions (Von Der Weid & Zawieja, 2004). The lymphangions subsequently join into afferent LVs, draining into the subcapsular sinus of several lymph nodes located throughout the body (Pal & Ramsey, 2011).

After the lymph extends its pathway from the initial LVs, it focalises toward pre-collecting and, finally, collecting lymphatic vessels. Pre-collecting LVs comprise small lymphatic muscle cells (LMCs) coverage, a combination of initial LVs and collecting LVs, and small valves. In contrast to initial LVs, the collecting LVs are identified by extended LECs, that are interconnected by constricted junctions having a shape like zipper and bounded by an continuous basement membrane (Wiig & Swartz, 2012) to avoid leakage of lymph throughout transportation (Baluk et al., 2007; Yao, Baluk, Srinivasan, Oliver, & McDonald, 2012). Additionally, collecting lymphatic vessels are sheltered by a muscular layer with LMCs, which sporadically contracts to provide the movement of lymph (Von der Weid, 2013; Von Der Weid & Zawieja, 2004) and comprises luminal regulators that are critical for avoiding backflow of lymph (D. Hu et al., 2019). Lymph comprises electrolytes, immune cells, nutrients, antibodies, lipids, antigens, macromolecules and lipoproteins.

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1.2. Lymph nodes

The primary lymphoid organs in the body are the lymph nodes (J. Hu et al., 2019; Marieb & Hoehn, 2013). Physiologically, a lymph node is fed by multiple afferent LVs and drained by one efferent LV (Betterman & Harvey, 2016; Liao & von der Weid, 2015). There are hundreds of these small lymphoid organs but, as they are typically enclosed in the adipose tissue (Betterman & Harvey, 2016), they are not ordinarily observed. Large groups of lymph nodes occur close to the surface of the body in the cervical, axillary and inguinal regions, where the collecting LVs gather to form basins (Liptak & Boston, 2019; Marieb & Hoehn, 2013).

Lymph nodes have various shapes and sizes, but the majority are bean-like in shape and their diameter is smaller than 1.5 cm (Mohseni et al., 2014). Specifically, a thick fibrous capsule envelops each node, from which connective membrane components, known

as trabeculae, spread inward to split the lymph node to different sections. The inner assembly of the lymph nodes of reticular fibres, known as stroma, maintains the substantial lymphocytes concentration in the lymph nodes (Marieb & Hoehn, 2013).

A lymph node possesses two histologically-separate sections, the cortex and the medulla (Hood, 2017). The thin layer of the cortex comprises of tightly packed follicles, containing separated B-cells. These follicles are encapsulated by dendritic cells and are bound the inner part of the cortex, mainly housing the transport of T-cells. The T-cells perform their roles as observation agents by constantly circulating in the blood, lymph nodes, and the lymph (Marieb & Hoehn, 2013). Thin internal expansions from the soft tissue of the cortical lymphoid, medullary cords, comprise lymphocytes and cells of plasma. Lymph sinuses, large lymph vessels covered by overlapping reticular fibres, are located throughout the node. On these reticular fibres, various macrophages dwell and phagocytise unknown substances in the lymph because macrophages passages by in the lymph sinuses (Bellomo, Gentek, Bajénoff, & Baratin, 2018; Marieb & Hoehn, 2013).

An increasing appreciation of the effect that the lymphatic system has on a variety of physiological functions, has led to the understanding of the importance of this system within a wide range of diseases. These include lymphedema (Ozcinar et al., 2012), cancer and metastases (Ozcinar et al., 2012), metabolic disorders, for example, obesity (Escobedo & Oliver, 2017) and atherosclerosis (Milasan, Smaani, & Martel, 2019), infection, for example, HIV (Patricia et al., 2018) and filariasis (Jeremiah, Aboltins, & Stanley, 2011; Taylor, Hoerauf, & Bockarie, 2010). In cancer, infection and inflammatory diseases, metastatic distribution usually occurs through LVs, extending to the sentinel lymph node. Furthermore, by producing pro-inflammatory and lymphangiogenic factors, tumour cells and associated macrophages stimulate lymphangiogenesis in the draining lymph nodes and at the tumour site (A. Alitalo & Detmar, 2012; K. Alitalo, 2011; Proulx et al., 2013; Swartz & Lund, 2012).

Lymphangiogenesis, the formation of new lymphatic vessels from the pre-existing lymphatic vessels, is a developmental event that occurs restrictively during embryogenesis and, is not normally programmed to occur during postnatal condition (Christiansen & Detmar, 2011). Lymphangiogenesis has been suggested to play a role in the progression of human cancer metastasis (Christiansen & Detmar, 2011). During cancer progression, uncontrolled proliferation of tumour cells and tumour angiogenesis induces the elevation of interstitial pressure which subsequently stimulates lymphangiogenesis to increase the removal of fluid from the peripheral tissues (Liao & Padera, 2013; Trevaskis, Kaminskis, & Porter, 2015). This subsequently increased the flow of lymph in the interstitial space, which in turn, provides a beneficial factor for the lymphatic targeting approach. Therefore, a delivery system that is capable of delivering drugs to the interstitial space would be of great benefit to improving treatment of lymph-related diseases. Microneedles are an emerging delivery system that can efficiently deliver drugs to this target site.

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2. LYMPHATIC DRUG TARGETING

Lymphatic drug targeting can be differentiated into two main categories, namely passive and active lymphatic targeting. With regards to passive lymphatic targeting, several nanocarrier drug delivery systems, including nanocapsules, nanosuspensions, solid lipid nanoparticles (SLNs), liposomes and dendrimers have been developed to target the lymphatic network through this mechanism. In contrast to relying on passive physiological targeting mechanisms, active lymphatic targeting can be achieved by increasing the affinity between the receptors situated within the lymphatic tissue network and the nanoparticles. Moreover, lymphatic targeting can also be achieved by cell-mediated uptake, by targeting the dendritic cells (DCs) and Langerhans cells, which in turn take up the particles, transferring them to the lymphatic systems.

2.1 Passive lymphatic targeting

In passive lymphatic targeting, the delivery approach used relies on the structure of the lymphatic system in order to achieve drug delivery. Due to nanoparticles' size, they can be efficiently delivered to the lymphatic system via this pathway. This route of targeting is also recognised as a result of the enhanced permeation and retention effect (Clemons et al., 2018). The main characteristics of vascular capillaries are the formation of close-fitting junctions formed by each endothelial cell and the appearance of a complete basement membrane. In contrast, as described in the previous section, the initial lymphatic capillaries have limited smooth muscle, do not have complete basement membrane and display extensive junctions of interendothelial with button-shaped and small fastening filaments, bound to a fibrous scleroprotein in the adjacent network. Small molecules and moderate sized molecules (~16-20 kDa), which are less than 10 nm, easily penetrate both the endothelium of the blood and the lymphatic capillary (Supersaxo, Hein, & Steffen, 1990). Nevertheless, because the flow of the lymph via lymph vessels is around 100-500 fold slower than that of the flow of the blood via vascular vessels, these moderate sized substances and small sized substances are predominantly taken up by the blood capillaries from the interstitial space (McCrudden et al., 2018; Trevaskis et al., 2015).

Principally, there are two transport mechanisms involved in the uptake of substances across the endothelium into the blood capillaries, namely paracellular or transcellular transport. Generally, molecules are transported from interstitial space through convection and diffusion. The diffusion process is rapid for smaller substances and, therefore, the primary mechanism of interstitial transport is convection. Since the flow of convective is typically delivered from the blood to the lymph, it is assumed that following the drug administration via interstitial site, macromolecules possessing molecular weights of 20-30 kDa and particles >10 nm are not likely absorbed by the blood. Instead, these substances are most likely to be distributed to the lymphatic capillaries (Charman, McLennan, Edwards, & Porter, 2001; Supersaxo et al., 1990). The delivery of larger substances (more than 100 nm in diameter) via the interstitial is eventually restricted by the dimensions of the water channels that provide the tubing for the interstitial shift. These water channels are characteristically approximately 100 nm in diameter. Therefore, substances possessing diameters between 10–100 nm seem to favour lymphatic uptake (Ryan, Kaminskas, & Porter, 2014). As an example, ultrafine poly(lactic-co-glycolic acid) (PLGA) nanoparticles of doxycycline (95.43 ± 0.8 nm) have been developed for passive lymphatic targeting. This study showed that the subcutaneous injection of the nanoparticles was able to enhance the localisation of drugs in the lymph node compared to the administration of free drug solutions (Singh et al., 2016).

There are three primary main mechanisms for macromolecules and particles (10-100 nm) to enter the lymphatic system. Firstly, intake of these molecules into the lymphatics from the interstitial space may occur via passive diffusion (paracellular uptake) through the intercellular intersections within LECs (Dixon, 2010; Lim et al., 2013). Alternatively, these molecules may also enter lymphatic vessels by transcytosis (transcellular uptake) promoted by receptors and/or vesicular transfer mechanisms (Miteva et al., 2010). Lastly, some molecules may also be delivered to the lymphatic system by targeting the antigen presenting cells (APCs) and subsequent APCs movement into the lymph nodes. Generally, the APCs such as DCs take up the antigens in the extracellular matrix (Trevaskis et al., 2015). Afterward, APCs permeate into the lymph and move to lymph nodes where they transfer antigen to effector cells (lymphocytes). Accordingly, the strategies

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of targeting the drugs to the APCs in the extracellular matrix can be utilised to deliver the drugs to the draining lymph nodes (Reddy et al., 2007). It has further been reported that small nanoparticles with sizes between 10–100 nm are passively accumulated in the lymph nodes after being taken up by the lymphatics and to further target lymph node (Schudel, Francis, & Thomas, 2019).

2.2 Active lymphatic targeting

Active lymphatic targeting occurs when a targeting ligand is attached to a molecule, specifically to target the lymphatic system. In this process, the molecules are able to recognise the target cells via ligand-receptor interactions, and, therefore, the molecules may enter the target (Clemons et al., 2018; X. Y. Zhang & Lu, 2014). A recent study highlighted the use of active lymphatic targeting to deliver a nucleic acid (CpG oligodeoxynucleotides) to lymph nodes. In this study, an approach based on cationic agarose (C-agarose) and CpG oligodeoxynucleotides was developed. Siglec-1 on the surface of lymph node sinus macrophages, which have excellent specificity for targeting lymph nodes, has been reported to have a strong affinity with C-agarose. The results of this study showed that administration of C-agarose+CpG gel subcutaneously were able to improve the localisation of CpG and to produce antitumor immune responses in the lymph node sinus macrophages (Hu et al., 2019).

In order to stimulate interaction with LECs and subsequent uptake through the lymphatic endothelium, macromolecules or carriers derivatised with targeting ligand compounds binding targets that are expressed on LECs, have also been developed. For example, Sharma et al. (2020) demonstrated the use of hyaluronan, a ligand for lymph vascular endothelial receptor (LYVE-1) to target the lymphatic vasculature. After intradermal injection of hyaluronan conjugated to NIR-783 dye, the lymphatic vessels were more visible for longer periods compared to intradermal injection of indocyanine green without hyaluronan, due to the binding of HA-NIR to LYVE-1 present on the LEC (R. Sharma et al., 2020).

Additionally, the use of hyaluronic acid (Jiang et al., 2008) and LyP-1 (Laakkonen, Porkka, Hoffman, & Additionally, the use of hyaluronic acid (Jiang et al., 2008) and LyP-1 (Laakkonen, Porkka, Hoffman, & Additionally, the use of hyaluronic acid (Jiang et al., 2008) and LyP-1 (Laakkonen, Porkka, Hoffman, & Additionally, the use of hyaluronic acid (Jiang et al., 2008) and LyP-1 (Laakkonen, Porkka, Hoffman, & Additionally, the use of hyaluronic acid (Jiang et al., 2008) and LyP-1 (Laakkonen, Porkka, Hoffman, & Ruoslahti, 2002) have also been developed for use within active lymphatic targeting. In these approaches, hyaluronic acid was used to target lymphatic vessel endothelial hyaluronic acid receptor 1 on LECs and LyP-1 administered via IV, was used to target p32 on tumours. Additionally, it has been reported that LECs also secrete chemoattractants (for example, chemokines (Card, Yu, & Swartz, 2014)) that stimulate the immune and tumour cells adhesion and transferring to lymph nodes within LVs (Trevaskis et al., 2015). Accordingly, the delivery of compounds to the lymphatic system may also be achieved by approaches that have a strong affinity with these agents.

As explained in Section 2.1, drug uptake via DC is capable of promoting lymphatic targeting. Therefore, the active delivery of drugs to the lymphatic has also been developed by the use of selective receptors and different DC-specific targeted agents. One of the receptors expressed on DC is mannose receptor, C-type lectin receptors (CLR). The main characteristic of this receptors is the ability of carbohydrate recognition (Tacken, De Vries, Torensma, & Figdor, 2007). Targeting a cancer antigen (e.g. MUC1) to this receptor by directly reacting with mannose, without the use of any type of delivery approach, has exhibited significant enhancements in cancer therapy in numerous investigations both preclinically and clinically (Karanikas et al., 1997; Tacken et al., 2007). The aforementioned studies have clearly shown the use of nanoparticles in both passive and active lymphatic targeting. Additionally, several studies shown the successful approach of nanoparticles to deliver the drugs to the lymphatic system, whether observed within biodistribution

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studies or specifically for the purpose of lymphatic targeting, as highlighted in Table 1.

Table 1. A list of studies investigating the use of nanoparticle in lymphatic targeting

Type of nanoparticles	Route and animal model	Key finding	Reference
Rilpivirine nanosuspension	Intramuscular injections in rats and dogs	The drug concentrations in the lymph nodes draining the intramuscular injection site exceeded the plasma concentrations by over 100-fold 1 month after administration, while the concentrations in the lymphoid tissues decreased to 3- to 6-fold the plasma concentrations beyond 3 months.	(Van 't Klooster et al., 2010)
Xeplion® IM LAI PP nano-/microsuspension (PP-LAI)	Intramuscular injection in rats	High concentrations of the compound were detected in medial iliac lymph node and regional popliteal lymph node from day 1 onward, indicating lymphatic accumulation of the compound in the lymph nodes	(Darville et al., 2016)
Puerarin nanosuspension	Oral administration in rats	Drugs were accumulated in lymphoid tissue.	(Zhou et al., 2014)
Tenofovir (TFV), lopinavir (LPV) and ritonavir (RTV) nanosuspension	Subcutaneous injection in rats	After 24 h, TFV, LPV and RTV drug levels in the lymph nodes were 79.6-, 162.7- and 44.3-fold higher than those in blood.	(Kraft et al., 2017)
Mitoxantrone SLNs	Subcutaneous injection to Human MCF-7 breast cancer in nude mice and animal model of P388 lymph node metastases in Kunming mice	The accumulation of drugs in axillary lymph nodes was approximately two-times greater following subcutaneous injection of SLNs when compared to the injection of drug solution with the same route of administration	(Lu, Xiong, Yang, Yin, & Chao, 2006)

Type of nanoparticles	Route and animal model	Key finding	Reference
Methotrexate SLNs	Oral delivery to Albino rats	SLNs enhanced the oral bioavailability of MTX and increased its concentration in the lymphatic region.	(Paliwal et al., 2009)
Lopinavir SLNs	Oral delivery in Male Wistar rats	SLN increased the cumulative percentage dose of lopinavir secreted in lymph which was 4.91-fold higher when compared to a pure drug in MC solution	(Aji Alex, Chacko, Jose, & Souto, 2011)
Curcumin SLNs	Oral delivery to healthy Male Sprague Dawley rats	SLN exhibited a significantly higher normalised concentration of curcumin in the lymph node than that of curcumin solution	(Baek & Cho, 2017)
Doxorubicin loaded LyP-1-conjugated PEGylated liposomes	Subcutaneously injection <i>via</i> foot pad	The conjugated liposomes were able to target lymphatic metastatic tumours and to improve inhibition effect on lymphatic metastatic tumour in comparison with unconjugated liposomes	(Yan et al., 2012)
OVA antigen encapsulated DOTAP-PEG-mannose liposomes	Subcutaneously injection <i>via</i> foot pad	The liposome with mannose effectively improved the accumulation of OVA in draining lymph nodes and the spleen and enhanced the uptake by resident antigen-presenting cells, compared to conventional liposome	(C. Wang et al., 2014)
Methotrexate-conjugated PEGylated dendrimers	Subcutaneously injection	The higher lymphatic absorption was shown after SC administration of dendrimers constructed using lower molecular weight linkers	(Ryan et al., 2017)
Doxorubicin loaded PEGylated polylysine dendrimers	Intravenous and subcutaneous injection	The dendrimer improved the recovery of doxorubicin in the lymph by up to 685 times and 3.7 times in comparison with the solution and liposomal, respectively after 30 h post dose	(Ryan et al., 2013)

Type of nanoparticles	Route and animal model	Key finding	Reference
Docetaxel loaded Polyaminoacid nanocapsules	Subcutaneously injection in the interscapular region	The nanocapsules with sizes of 100 nm accumulated faster in the lymph nodes, compared to the nanocapsules with sizes of 200 nm.	(Abellan-Pose et al., 2016)
Docetaxel-loaded polyglutamic acid-PEG nanocapsules	Intravenously in the tail vein or subcutaneously in the interscapular region	The nanocapsule formulations were able to deliver the drug to both, the ~~tumor~~ <u>tumour</u> tissue and the metastatic lymph nodes.	(Borrajó et al., 2016)

3. MICRONEEDLE TECHNOLOGY

3.1 Definition and development

MN arrays are minimally invasive devices which can by-pass the *stratum corneum*, the principal skin barrier to topically-applied drugs, without pain and, as such, are envisioned for use within drug delivery, particularly intradermal and transdermal (R. F Donnelly et al., 2011). Typically, This the MN system comprises of a plurality of microprojections which are attached to a base support, typically normally ranging between 25 and 2000 µm in height (R. F Donnelly et al., 2010; Hirobe et al., 2013). MN technologies were first proposed as a drug delivery device in the 1970s (Gerstel & Place, 1976), however, it is only in recent years that improved dermal and transdermal MN-mediated drug delivery approaches MN approaches for improved both dermal and transdermal drug delivery have gained remarkable consideration by researchers in academia and industry (Larrañeta, Lutton, Woolfson, & Donnelly, 2016; Rodgers et al., 2018). MN technologies were first proposed as a drug delivery device in the 1970s (Gerstel & Place, 1976). Following continual their developments, MN technologies have been produced by a range variety of techniques, comprising different shapes and characteristics, for a broad range of in a broad range of pharmacological agents sicochemical, and shapes characteristics.

The first-ever publication on MNs was reported in 1995 by Hashimi et al., who in which produced hollow MNs were produced to for delivery a bacterial plasmid to for nematodes genetic transformation (Hashimi et al., 1995). However Nevertheless, it was not until 1998 that Henry et al. (1998) performed initial development and the first fabricated MNs for true transdermal applications emerged. The first MN arrays were fabricated from silicon, and appeared as a result direct outcome of modern improvements in in microelectronic technology and microfabrication (Henry, McAllister, Allen, & Prausnitz, 1998). In recent years, MN fabrication has steadily developed improved with expanded applications, consistently with further further advances in polymer chemistry and microengineering, encouraging innovative system designs. Notable progress has been made in terms of dermal and transdermal drug delivery. MN arrays have been manufactured from glass (Ayittey, Walker, Rice, & De Tombe, 2009), metal (Ullah, Kim, & Kim, 2018), silicon (Wei-Ze et al., 2010), ceramics (Ita, 2018) and polymers (R. F Donnelly et al., 2011; Pamornpathomkul et al., 2018) in a plethora of designs, with each type presenting their own benefits and limitations.

3.2 Benefits of microneedles

The pain related to hypodermic needles can lead to significant suffering in patients, especially in the paediatric group and those who experience needlephobia the patients having phobia with needles (Hamilton, 1995; Nir, Paz, Sabo, & Potasman, 2018). The main advantage of MN technology is that the administration of this device does not result in pain when compared to hypodermic needles. This advantage is associated attributed to with the small size with of the micron-scale projections that penetrate the SC, but avoid without stimulation of touching th the pain receptors that dwell in the dermis (Kaushik et al., 2001). Additionally, some types of MN arrays, namely dissolving and hydrogel-forming MNs, MNs can potentially avoid the injury caused by needlestick and, eradicate the need of sharps disposal of needles. This is particularly crucial especially critical within as in the UK, as it has been reported that approximately more than 100,000 needle stick injuries caused occur by needlestick have occurred in hospitals each year. Moreover, an evaluation performed conducted by the World Health Organization reported that unsafe use utilisation and inappropriate reuse of needles of needles resulted in approximately 37.6%, 39%, and 4.4% of hepatitis B, hepatitis C and HIV cases worldwide, respectively (WHO, 2003).

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Despite the fact that the effective systemic delivery of chemotherapeutics can be achieved by the use of hypodermic needles, this method is painful and invasive. As discussed, when compared to hypodermic injection, the use of MNs results in reduced injury in injection site and quicker time of healing process (Bariya, Gohel, Mehta, & Sharma, 2012). Additionally, numerous *in vivo* investigations, both in animal models (Matriano et al., 2002) and humans (Wei-Ze et al., 2010), have revealed that MN penetration produces negligible bleeding.

Specifically, with respect to the volume of drugs that can be delivered, intradermal injection can only inject around 0.1 mL per injection site and the application cannot be repeated in the same place (Cederholm, Evers, & Löfström, 1991; Ren, Li, Smith, DeTolla, & Furth, 2002). Therefore, this administration would not be able to deliver the required dose for high dose drugs. However, MNs can deliver higher doses of drugs intradermally by using a larger size of MN patches. Recently, Ripolin et al., successfully demonstrated that larger MNs patches (16 cm²) can be self-applied by patients successfully (Ripolin et al., 2017). This study highlighted the potential for the MN-mediated delivery of high dose drugs using MNs.

In terms of lymphatic targeting, the MN-assisted approach has been applied to investigate the effect upon lymphatic uptake for numerous compounds. Compared to subcutaneous injection, the application of a MN device was able to alter the absorption kinetics of etanercept, somatropin and insulin in the lymphatic vessels (Harvey et al., 2011). In addition, this delivery system has also been used for the imaging of the lymphatic system. In this study, MNs containing a near-infrared dye (indocyanine green) were prepared from the aqueous blend of poly(N-vinylpyrrolidone). Following administration to the skin, the MNs completely dissolved in the dermal layer. Consequently, the dermal draining lymphatics can be visualised and, by the utilisation of a near-infrared camera, the kinetics of the clearance from the application site can be assessed. Interestingly, this approach can also be utilised for quantitative determination of the function of dermal lymphatics in numerous dermal circumstances and assessments of treatment-response (Brambilla, Proulx, Marschalkova, Detmar, & Leroux, 2016). Furthermore, the comparison between MN devices and subcutaneous injection of amphiphilic peptide antigen and amphiphilic CpG adjuvants (amph-vaccines) has been investigated (An & Liu, 2017). In this study, the MN transdermal approach was capable of delivering the amphiphilic vaccines efficiently into lymphatics and draining lymph nodes. Following five minutes of dissolving MN administration into the highly lymphatic-perfused dermis layer of the skin, amphiphilic vaccines released from dissolving MNs. Following this, the vaccines were attached and form a complex with endogenous albumin, and effectively drain into draining lymph nodes, resulting in enhanced lymphatic delivery. As discussed previously, intradermal administration is preferable due to the high amount of DC situated within the dermis. In addition to DC, Langerhans cells (LC) are another of the APCs that are contained in the epidermis. Like DC, LC can migrate to draining lymph nodes (Johnston, Halliday, & King, 2000). Intradermal injection can directly deliver the drugs to the dermis. Therefore, there is a reduced possibility of LC to uptake the drugs. However, the application of MNs may potentially deliver the drug particles to both the epidermis and dermis (Pamornpathomkul et al., 2018; Vora et al., 2017), resulting in the higher possibility of drugs to be taken-up by LC and DC, compared to other route of administrations.

Several studies have described the application of MNs, showing improved efficacy in autoimmune disease and cancer. Sevick-Muraca *et al.* (2008) have demonstrated the application of single hollow MN device to deliver a near-infrared (NIR) fluorophore, indocyanine green, for non-invasive optical imaging of LNs in breast cancer patients undergoing sentinel lymph node mapping (SLNM) at the minimum dose required. Within the study, it was demonstrated that lymph drainage pathways from the injection site to LNs were successfully observed using NIR fluorescence imaging when 10 µg or higher of indocyanine green dosages was delivered

Accordingly, this approach was found to be suitable for LN molecular imaging (Sevick-Muraca et al., 2008). Furthermore, Aldrich *et al.* (2017) developed nanotopography (SOFUSA™) delivery for intradermal lymphatic delivery of Etanercept. The authors compared the pharmacokinetic profiles of etanercept following administration via nanotopography and the administration various route, namely conventional subcutaneous injection, intravenous and intradermal. Furthermore, the lymphatic uptake was also evaluated in LNs. The results revealed that compared to the untreated control, ID, and SC administration, the delivery of etanercept using SOFUSA™ resulted in improved lymphatic pumping and considerably reduced swelling in a progressive rat model of collagen-induced arthritis. Importantly, the nanotopography device was found to produce the highest retention in draining LNs in comparison with other administration routes (Aldrich et al., 2017). The application of nanotopography for lymphatic delivery has also been developed by Kwon *et al.* (2019). In this study, the authors used the SOFUSA™ nanotopographical MN-array device for lymphatic delivery of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and indocyanine green and compared the anti-tumour response to that of conventional systemic administration. It was confirmed by NIRF imaging that SOFUSA™ was able to infuse 100 µL/h of indocyanine green into the epidermal spaces. Following which, the initial lymphatics took up the agent allowing imaging of the impulsion of indocyanine green-laden lymph into the brachial LNs. In comparison to conventional systemic administration, lymphatic infusion of anti-CTLA-4 could potentially lead to the inhibition of tumour growth. Finally, in clinical studies, nanotopographic infusion was able to deliver indocyanine green to the axillary and inguinal LNs of healthy human volunteers (Kwon *et al.*, 2019).

3.3 Types of delivery approach

In general, there are five types of MNS, including solid MNs, coated MNs, hollow MNs and polymeric MNs (including dissolving MNs and hydrogel-forming MNs) (Figure 2). Solid MNs are also known as 'poke with patch' method. In the application, solid MNs are inserted to the skin manually and then are removed, forming temporary aqueous conduits in the SC part of the skin. As described previously, the first investigation utilising MN in the field of transdermal drug delivery was reported by Henry *et al.* (Henry et al., 1998). This study reported the enhanced delivery of calcein as a model drug following the administration of solid MNs made of silicon applying the 'poke and patch' strategy. In recent years, this approach has been broadly employed, particularly using metal and silicon MNs. Additionally, the application of metal solid MNs for the delivery of different substances, such as macromolecules, including insulin and bovine serum albumin (BSA) has been reported by McAllister *et al.* (2003). The effect of skin pre-treatment using solid polymeric (Mikolajewska et al., 2010) and silicon MN arrays (Ryan F. Donnelly et al., 2009) focusing on the delivery of photosensitising agents (5- aminolevulinic acid, 5-aminolevulinic acid methyl ester and meso- tetra(N-methyl-4-pyridyl)porphine tetratosylate) have also been extensively studied. In addition, the use of MN roller tools as pre-treatment in the skin was found to improve the transdermal penetration of non-steroidal anti-inflammatory drugs, such as paracetamol, diclofenac, ketoprofen, ibuprofen (Stahl, Wohler, & Kietzmann, 2012). Furthermore, in some investigations, solid MNs were combined with other penetration enhancement approaches, including sonophoresis or iontophoresis. Importantly, their combination was reported to exhibit the improvement of delivery of macromolecular drugs transdermally (Han & Das, 2013; X. M. Wu, Todo, & Sugibayashi, 2007).

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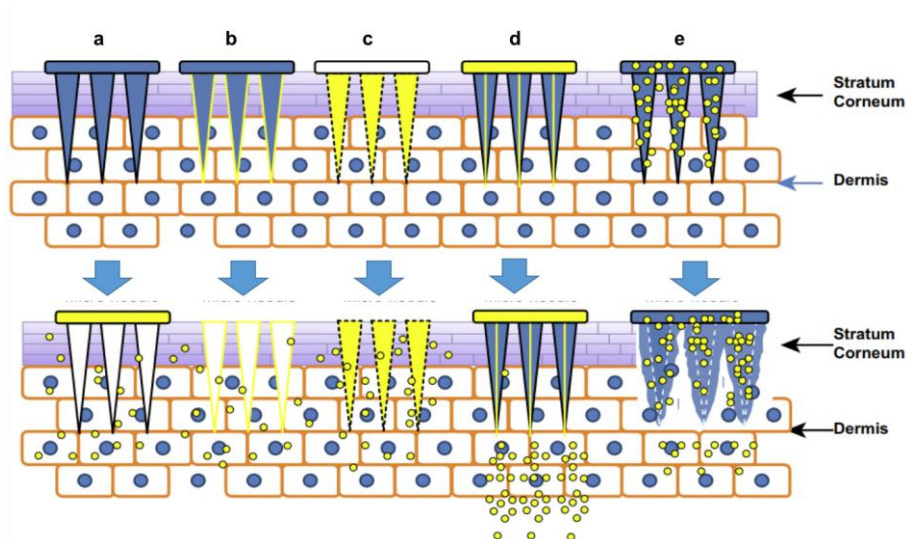


Figure 2. Representation illustration of five type MN device. Solid MNs utilised for pre-treatment in the skin and application of drug-loaded reservoir (a), coated MNs for deposition of drug-containing layer into the skin, followed by removal of the MN array (b), dissolving MNs utilised to achieve controlled or rapid delivery of drug (c), hollow MNs utilised to create holes as conduits in the skin and permit delivery of a liquid drug *via* the hole created (d) and hydrogel-forming MNs utilised to deliver drugs from a drug reservoir *via* the hydrogel matrix (e). Taken from (M. Sharma, 2019) (1). The figure is reproduced with permission of the publisher from the reference.

Coated MNs are also known as ‘coat and poke’ technique. This approach is prepared by coating a solid MN using a liquid formulation of drug prior to its administration in the skin. Through this formulation, following insertion of the MN array into the skin, the coating laden upon the MNs dissolves and drug accumulates in the skin. This type of MNs has been applied in improvement of transdermal penetration of a broad variety of substances, including desmopressin (Cormier et al., 2004), parathyroid hormone (Daddona, Matriano, Mandema, & Maa, 2011), salmon calcitonin (Tas et al., 2012), and fluorescein sodium (Chen, Qiu, Zhang, Yang, & Gao, 2015). Also, this type of MNs have successfully delivered DNA intradermally, specifically, MNs tips were coated with DNA solutions (Pearton et al., 2012). In the hereditary skin disorders and cutaneous cancers treatment, appropriate utilisation of this approach may be an effective and efficient choice. However, due to the small size of the needles and the fact that capacity of liquid drugs is limited to what can be coating onto the surface of the MN arrays, subsequently, the loading volume of this type of MN is also limited. Therefore, coated MN arrays are more limited to low dose drug molecules (Quinn and Donnelly, 2018).

In the application of Hollow MNs, a liquid formulation containing the drug is injected into the skin by the hollow MN needles. Specifically, via the MN bore, continuous delivery of various compounds through the skin can be achieved using this sort of MNs (Larrañeta, Lutton, et al., 2016). Recently, delivery of insulin has gained particular interest by this

outlet, and, consequently, has become one of the most commonly delivered compounds utilising hollow MNs (Davis, Martanto, Allen, & Prausnitz, 2005; Roxhed, Samel, Nordquist, Griss, & Stemme, 2008; P. M. Wang, Cornwell, Hill, & Prausnitz, 2006). Additionally, intradermal delivery of smaller compounds, such as sulforhodamine has also been performed using this type of MNs (Martanto et al., 2006). The monitoring of blood glucose has also been performed using this approach by Nicholas et al. (2018). In this study, a paper back plate inserted with a colorimetric system for the fast visual determination of glucose in the simulated interstitial fluid was developed and combined with hollow MNs. This device was able to rapidly extract simulated interstitial fluid within five seconds. In addition, the ability of this device to produce a glucose concentration dependent colour change within 30 s was established (Nicholas et al., 2018). The use of hollow MNs in conjunction with nanotopography technology for transdermal delivery of Etanercept was developed by Walsh et al. This study was conducted in both rats and rabbits and, the results showed that the transdermal delivery of etanercept was significantly improved due to modification of the tight junction proteins, followed by phosphorylation of myosin light chains (MLC) and, stimulation of the actomyosin complex increasing paracellular permeability (Walsh et al., 2015).

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The final and, most recent class of MNs are polymeric MNs; this includes both the dissolving MNs and hydrogel-forming subclasses. Dissolving MNs are prepared by micromoulding the mixture of soluble matrices and the therapeutic compounds. Commonly, the matrices used in the preparation of dissolving MNs are the combination or the single use of aqueous blend of biocompatible polymers or sugar, (Mir et al., 2020; Permana, Mir, Utomo, & Donnelly, 2020; Tuan-Mahmood et al., 2013). Different types of compounds, including small drug molecules, biomacromolecules and a drug nanosuspension have been successfully delivered using these types of MN arrays (Ryan F. Donnelly & Larrañeta, 2018; Larrañeta, Stewart, et al., 2016; Mc Crudden et al., 2019). When the MNs are inserted into the skin, the MNs imbibe the skin's interstitial fluid and, thus, allows the dissolution of the MNs tips. Following this, the drugs contained in the MN needles are then released over time. In the dissolving MNs, the release kinetics of the drug in the formulations are mainly governed by the dissolution rate and time of the polymers used, as well as the hydrophilicity of the drug itself. Consequently, in to achieve drug delivery in a controllable sustained manner, the composition of polymer used to prepare the MN array and, the process of MN fabrication should be taken into account and modified, depending on the desired release properties (Larrañeta, Lutton, et al., 2016). The delivery of various drugs and biopharmaceutical compounds, including low molecular drugs such as ibuprofen, both transdermally and intradermally have been improved using this delivery approach (McCrudden et al., 2014). Additionally, high molecular drugs such as cyclosporine A (Jeong, Kim, Kim, & Park, 2018) and insulin (Yu et al., 2017) have also been delivered by this type of MN system. In addition to this, proteins (Liu et al., 2019; Mönkäre et al., 2015), DNA (Bernelin-Cottet et al., 2019; Cole et al., 2018) and vaccines (Bernelin-Cottet et al., 2019; Zhu et al., 2019) have also been successfully delivered using this approach.

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Hydrogel-forming polymers have also been utilised to prepare another type of MNs, as a result, now commonly known as hydrogel-forming MN. In the formulation, cross-linked polymeric hydrogels, free of drug, are used to prepare the MNs by forming a solid baseplate. A drug containing reservoir is then attached to this baseplate (Ryan F. Donnelly et al., 2012). Following hydrogel-forming MN insertion into the skin, the tips rapidly take up interstitial fluid from the tissue. Subsequently, diffusion of the drug from the reservoir via the swollen inserted MN arrays into the skin occurs. This delivery approach has been applied to effectively deliver both high dose and low dose drugs, as well as the rapid delivery of proteins (Ryan F. Donnelly et al., 2014). The percutaneous delivery of a broad variety of compounds, including drugs with high molecular weight substances (i.e. BSA and insulin) as well as hydrophilic

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small molecular weight compounds (i.e. caffeine, theophylline, metronidazole and methylene blue) have been enhanced using this type of MN (Ryan F. Donnelly et al., 2012). Moreover, this approach has also been applied for the minimally invasive extraction and subsequent quantification of drugs compounds and glucose from the skin, in both in vitro and in vivo studies

In this study, caffeine, theophylline and glucose were successfully extracted from *in vitro* receptor chamber using hydrogel-forming MN arrays. The hydrogel-formulation consisted of aqueous blends of 11.1% w/w of hydrolysed poly(methyl-vinyl ether-co-maleic anhydride) and 5.6% w/w of poly(ethylene glycol) 10 kDa. Importantly, this approach demonstrated that the hydrogel-forming MNs were able to extract theophylline from rats' systemic circulation, as well as caffeine and glucose from human volunteers (Caffarel-Salvador et al., 2015). The majority of hydrogel-forming MN arrays described in the literature are made predominantly of a combination of poly(methyl-vinyl ether-co-maleic anhydride) and poly(ethylene glycol) accomplished by a cross-linking reaction. It is important to note that the use of this type of MN arrays has been shown to be safe when applied repeatedly into animals and human volunteers (Al-Kasasbeh et al., 2020; Vicente-Perez et al., 2017).

4. The combination approach of nanoparticles and microneedles in lymphatic targeting

The first investigation combining nanoparticle approach with MNs arrays for improved skin drug delivery was reported in 2003 by McAllister *et al.* Specifically, in this work, the effect of various molecular radii of several compounds, namely insulin, calcein, BSA and nanoparticles on their transdermal delivery utilising solid MN arrays was investigated (McAllister et al., 2003). Additionally, two different types of poly(styrene) latex nanoparticles with diameters of 25 and 50 nm were used as model nanoparticles. In this study, when solid microneedles were used, skin permeability was increased in vitro by orders of magnitude for macromolecules and nanoparticles. Additionally, Zhang *et al.* (2010) evaluated the use of solid silicon MN arrays in the transdermal delivery of coumarin 6-loaded PLGA nanoparticles. There were two key findings of this study. Primarily, following MN application, a significant amount of nanoparticles were detected in the epidermis layer rather than in the dermis layer of the skin (W. Zhang et al., 2010). Secondly, the penetration rate of the nanoparticles relied on the concentration of the nanoparticles. Vora *et al.* (2017) investigated PLGA nano- and microparticles of vitamin D₃ incorporated into novel bilayer dissolving MN arrays as an intradermal delivery system (Figure 3.1). In this study, MNs were formulated utilising 20% w/v of PVP gel by a simple centrifugation method. The authors found that the MN formulations exhibited excellent mechanical strength and insertion ability (into a skin simulant model and excised neonatal porcine skin). In addition, *ex vivo* intradermal neonatal porcine skin deposition of vitamin D₃ nano-microparticles from bilayer MNs exhibited higher drug deposition with approximately 74.2% of vitamin D₃ delivered after cryostatic skin sectioning, in comparison with free-needle patches (Figure 3.2) (Vora et al., 2017). Permana *et al.* (2019) developed a novel combination approach of nanosuspensions of doxycycline (DOX), albendazole (ABZ) and ivermectine (IVM) and dissolving MNs (Permana, McCrudden, & Donnelly, 2019). In this study, the incorporation of nanosuspension into dissolving MNs enhanced the delivery of the three drugs into neonatal porcine skin *in vitro*, particularly within the dermis layer, compared with the administration of the nanodispersions without MNs. Importantly, this study was the first dermatokinetic study investigating the skin profile of drugs in the epidermis and dermis following MN applications.

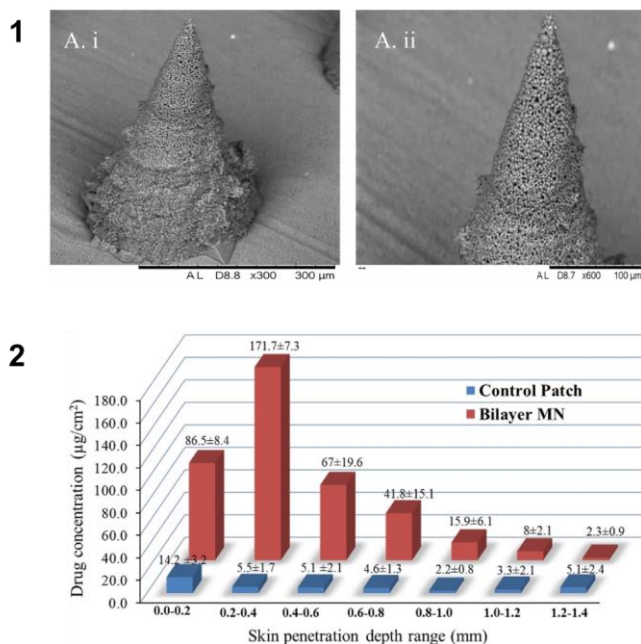


Figure 3. Scanning electron micrograph of PLGA nanoparticles-loaded bilayer MNs (1) and the VD₃ concentration detected in different layers of excised neonatal porcine skin after the administration of PLGA nanoparticles-loaded bilayer compared to control patch (2) (Vora et al., 2017). All figures all reproduced with permission of the publisher from the reference.

With respect to the combination of nanoparticles and MNs for lymphatic targeting, Kennedy *et al.* developed PLGA nanoparticles (69.3 ± 4.6 nm) in combination with dissolving MNs using **Rhodamine B (RhB)** as a model compound. The application of combination system exhibited that RhB was localised in the lymph nodes in mice (Figure 4.1). Interestingly, RhB was still quantified even after 168 h post-application (Figure 4.2). Furthermore, after MN application of RhB nanoparticles, RhB **demonstrated substantial accumulation** in the lymph nodes, **when compared to the administration of free RhB** (Kennedy *et al.*, 2017). In **additional studies**, the combination of rilpivirine (RPV) nanosuspension (> 200 nm) and dissolving MNs in rats **was evaluated, and it was demonstrated** that RPV was still **quantifiable** after 56 days in the lymph nodes **following MN application** (Figure 4.3). Although the mean particle size was greater than 100 nm, there were numerous particles with sizes below 100 nm and, therefore, these particles could reach the lymph node (McCrudden et al., 2018). Specifically, MN devices have also been employed to facilitate dermal delivery of SLNs of antifilaria drugs, namely doxycycline (DOX), diethylcarbamazine (DEC) and albendazole (ABZ) with sizes of < 100 nm to target the lymph nodes, the infection site of filarial nematodes in the lymphatic filariasis (Permana, Tekko, et al., 2019). **In in vivo studies in rats, through passive transport mechanisms,** the lymph node relative bioavailabilities of DOX, DEC and ABZ were **found to be** enhanced by using this **combined method of delivery** (Figure 4.4). Interestingly, compared to conventional oral administration, the biodistributions of DOX, DEC and ABZ in the liver, kidney and spleen were reduced **following** the administration of MNs containing drug-loaded SLNs.

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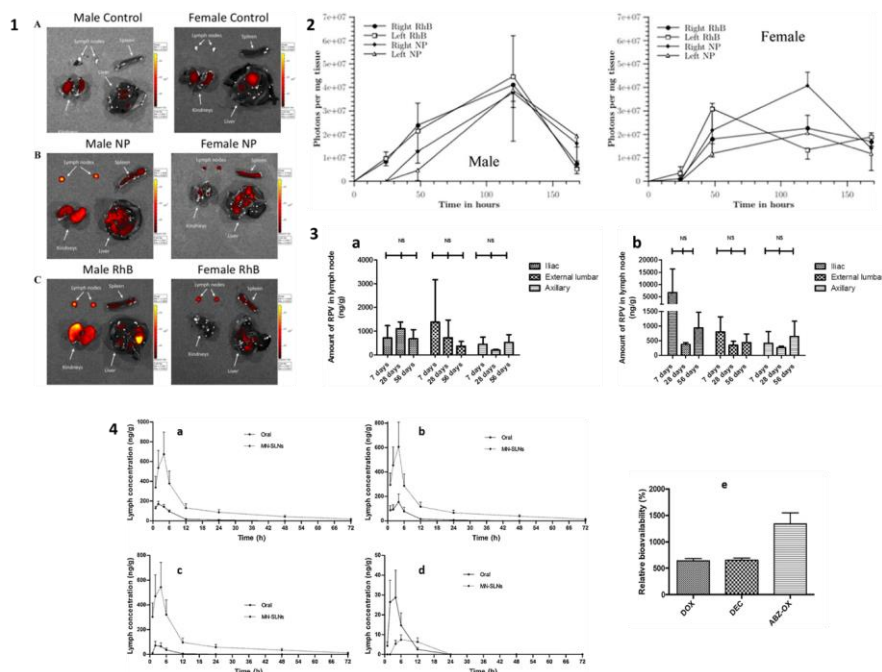


Figure 4. *In vivo* imaging system images of lymph nodes, spleens, livers and kidneys of male and female mice after 48-h the administration of different treatment, namely control (no MN treatment) (A), MNs laden with RhB nanoparticles (B) and MNs laden with free-RhB (C) (1). The concentrations of photons of RhB delivered per mass of tissue in the superficial parotid lymph nodes following the administration of MNs laden with RhB nanoparticles and MNs laden with free-RhB (2) (Kennedy et al., 2017). The amount of rilpivirine detected in excised lymph nodes in rats treated for 7, 28 or 56 days after the administration of MN containing [rilpivirine](#) nanosuspension (a) and intramuscular injection of rilpivirine (b) (3) (McCrudden et al., 2018). The [concentration of DOX](#) (a), DEC (b), ABZ-sulfoxide (c) and ABZ-sulfone (d) after oral treatment of drug suspensions and drug-loaded SLNs incorporated with MNs. The lymphatic relative bioavailability of DOX, DEC and ABZ-DOX (e) after the application of drug-loaded SLNs incorporated with MNs in comparison with the oral administration of pure drugs (Permana, Tekko, et al., 2019). All figures are reproduced with permission of the publisher from the references.

Specifically, the combination of nanoparticles and [MN](#) has been used in cancer and vaccination. An investigation performed by Zaric *et al.* is one of the examples [that successfully](#) utilised this approach in vaccination. In [this](#) study, ovalbumin (OVA)-loaded PLGA nanoparticles were delivered intradermally *via* dissolving MNs. Following administration, the nanoparticles were taken up by skin-resident DC. Following this, the DCs migrated to cutaneous draining lymph nodes and released OVA. Finally, significant expansion of antigen-specific T cells was then stimulated (Zaric *et al.*, 2013). Recently, the combination of [hyaluronic acid based MNs](#) and doxorubicin formulated into transfersomes_s has been developed for treatment of tumor metastasis (Yang, Wu, Zhou, Chen, & Kong, 2019). This study [showed](#) that the accumulation of doxorubicin in lymph nodes was statistically higher after the administration of the MNs laden with doxorubicin transfersome (DOX-T/MN), in comparison with [both](#) intravenous injection (DOX-T/IV) and epidermal diffusion of doxorubicin solution (DOX-T) (Figure 5). Therefore,

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this combination has shown potential as an effective alternative approach for therapy of tumour metastasis using the non-invasive intradermal route.

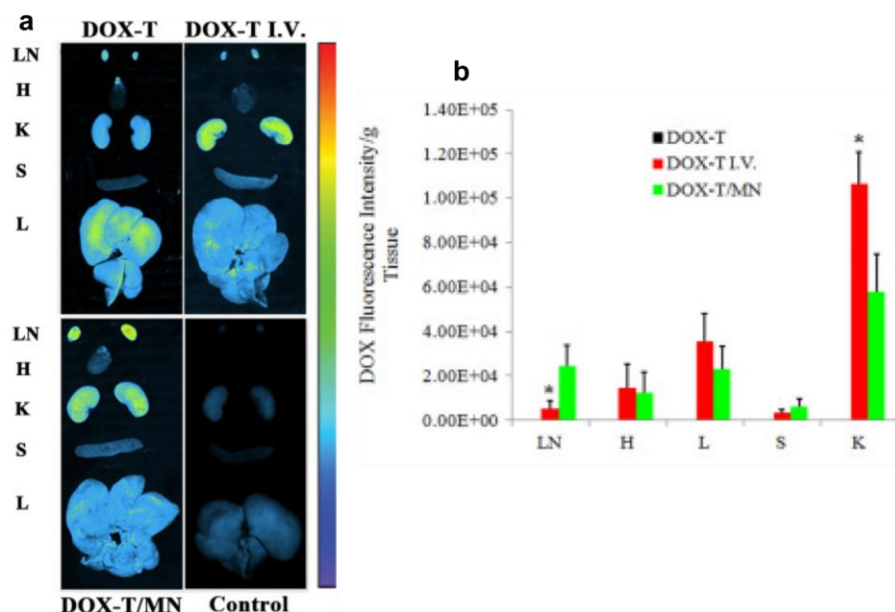


Figure 5. *In vivo* biodistribution (a) and the fluorescence intensity (b) of doxorubicin in lymph nodes (LN), heart (H), kidney (K), spleen (S) and liver (L) of rats following the administration of DOX-T, DOX-T/IV, and DOX-T/MN (Yang et al., 2019). All figures are reproduced with permission of the publisher from the references.

In an additional study, OVA and an adjuvant, CpG oligodeoxynucleotides (CpG), were encapsulated into chitosan nanoparticles, which were further incorporated into dissolving MNs prepared from PVP (Zhilin, Yingju, Deng, & Zhang, 2019). In this study, the positively charged chitosan formed nanocomplexes with negatively charged OVA and CpG, leading to a high encapsulation efficiency. Following insertion into the skin, the MNs released OVA and the adjuvant. In *in vivo* studies in mice, chitosan NPs efficiently accumulated in peripheral lymph nodes following the administration by dissolving MNs, subsequently, promoting significantly improved immune responses in comparison to that expressed by administration of free OVA. Moreover, compared to subcutaneous injection, the MN administration was shown to achieve a comparable immunogenicity level in a less invasive manner, indicating a more efficient method of administration afforded by MNs. As mentioned previously, a different type of nanocarrier, the transfersome, with anionic and cationic counterparts has been investigated as a carrier of OVA to be delivered by dissolving MNs (X. Wu *et al.*, 2019). The results showed that the anionic transfersome (SAT) exhibited higher cellular uptake *via* DC2.4 compared to the cationic transfersome (SCT). However, SCT promoted stronger escape capabilities from endocytic sections, enabling antigen processing, and essentially, produced greater accumulations in lymph nodes in comparison with the anionic SAT. Such an improved antigen-specific immune response achieved through

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Owing to the innovative and novel nature of the device, the lack of regulation of the field of MN research continues to be a major obstacle to the availability of a MN product on the market. Particular regulatory guidelines regarding patient use are required, also taking into consideration several factors including packaging, waste, ease of application, reassurance of correct application and additional safety concerns (Quinn, Larrañeta, & Donnelly, 2016). In addition to this, the lack of investment is also a considerable barrier to successful MN commercialisation, therefore, substantial investment from industry and financial stakeholders is required to achieve this unmet need.

In recent years, a significant consideration regarding MN technology is its potential for self-administration, in particular, the need for a device to ensure appropriate application. Furthermore, the use of applicators has been highlighted in several studies in the application of MN (Leone et al., 2018; Park, Choi, Seo, Choy, & Prausnitz, 2010; Sausse Lhernould & Delchambre, 2011). It has been reported that, with respect to polymeric MNs, several studies have exhibited that MN arrays can be easily applied by most of patient without the requirement of trained personnel. However, in an attempt to determine the variability in application technique in different patients and in their level of confidence to self-apply, further comprehensive investigations now require to be conducted. While it is recognised by some, that the use of an applicator device is desirable in order to achieve reproducible and reliable application forces in different patients, the use of applicators in MNs administration would in turn increase associated commercial costs (Lee et al., 2015). Alternatively, a pressure-indicating film has been developed and could be a more affordable choice to instil patient confidence in the application of MNs (Vicente-Pérez et al., 2016). In addition, the opinions of the users of MN should be assessed particularly in this concern.

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As the field of MN technology continues to grow, the use of MNs in more varied and diverse purposes have also been evaluated. This is indicated by the utilisation of MNs to target different sites of the human body, including dermal lymphatics. Through a careful consideration of choice of design and composition of the formulations, MNs contribute a unique delivery approach to deliver chemical substances to the initial lymphatics in the dermis, which house a broad range of immune cells. Accordingly, due to the potential of such immune cells targeting, this delivery approach could potentially open new opportunities for cancer therapy and vaccination. However, it is essential to take into account that the amount of drug capable of being incorporated into MNs is typically within the μg to mg range, which presents as potential limitation. Another difficulty in lymphatic targeting is related to the physiology of the lymphatic system itself. This system is not like blood flow system, which only flow in one-way direction, and, therefore, the compounds administered to this system will possibly lead to the accumulations of the drugs in the upper part of the lymphatic vessels and nodes but limited therapeutic penetration in other sections of the lymphatic system.

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Conclusion

As a secondary vascular system in the body, the lymphatic system plays a crucial role in tissue homeostasis, immune regulation and clearance of the interstitial space. However, these essential functions are often hampered by diseases that affect the lymphatic system. As a neoplastic disease develops and general survival declines with progressive lymphatic involvement, there is a clear interest in targeting chemotherapeutic agents specifically to the lymphatic tissue network and associated organs. However, the use of conventional delivery routes has yet to show significant benefit in terms of targeted delivery the drugs specifically within

the lymphatic system. This Review explored several factors affecting lymphatic delivery, with particular attention given to the particle size of the therapeutic and, the subsequent route of administration.

To increase the site-specific localisation of the drugs to the lymphatic system, two main approaches can be employed. The first approach is the utilisation of a suitable delivery system. Another approach is the route of the administration of the drugs themselves. Due to the high density of the lymphatic capillaries located in the dermis, the administration of drug delivery systems intradermally has shown higher lymphatic uptake compared to other routes, namely, that of subcutaneous and intravenous injections. In addition, owing to the characteristics of dermal lymphatic capillaries, they readily absorb particles ranging in the sizes of 10–100 nm following intradermal administration, whilst blood capillaries generally take up particles having diameters < 10 nm. In contrast, particles possessing diameters > 100 nm mainly accumulate in the skin, remaining locally to where the particles were administered. This Review explored the lymphatic uptake of different drugs formulated using a range of nanoparticle approaches. As an alternative approach to the hypodermic needle, dissolving microneedles (MN) are a delivery device, which can by-pass the stratum corneum, the primary skin barrier, without causing pain or bleeding. Accordingly, MN have been proposed as innovative drug delivery systems for intradermal administration. This system comprises of needles with length of less than 1 mm, which pierces as deeply as the skin's dermis. Importantly, a greater surface area of the skin can be easily covered through the use of MN patches in comparison with a single-site administration intradermal injection. As such, by further increasing the size of MN patch, will in turn result in delivery even higher amounts of drug compared to a standard intradermal injection. Using nanoformulations that necessitate efficient lymphatic uptake as delivery choices for chemotherapeutic agents could potentially be beneficial for treating numerous diseases of the lymphatic system. This Review discussed the significant benefits of the combination of nanoparticles delivered using various MNs for lymphatic targeting, showing their higher lymphatic uptake compared to the delivery of free drugs. The studies explored in this Review are promising. Nevertheless, in order to achieve further progress, a focused and strategic collaboration between academia and industry is now urgently required to drive the technology from "bench to bedside" through generation of more comprehensive pre-clinical and clinical MN-based outcomes. Additionally, patient usability, acceptability and sterility issues must be further evaluated in an attempt to fully realise the potential applications of MNs, so that this novel delivery platform can achieve its ultimate intended patient benefit. Lastly, in order to achieve such benefits, regulatory approval must be established. Investment by industry will now be essential, though partnerships with organisations concerned with global health, to strive to provide practical and appropriate solutions to address such issues, primarily those affecting developing countries.

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