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ADVANCED REVIEW

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

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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 and associated organs. Nanotechnology has been exploited as one of the main approaches to improve the lymphatic uptake of drugs. Different nanoparticle approaches utilized for both active and passive targeting of the lymphatic system are discussed here. Specifically, due to the rich abundance of lymphatic capillaries in the dermis, particular attention is given to this route of administration, as intradermal administration could potentially result in higher lymphatic uptake compared to other routes of administration. Recently, progress in microneedle research has attracted particular attention as an alternative for the use of conventional hypodermic injections. The benefits of microneedles, when compared to intradermal injection, are subsequently highlighted. Importantly, microneedles exhibit particular benefit in relation to therapeutic targeting of the lymphatic system, 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 realize the potential extent of this dual-delivery platform, further taking into consideration eventual usability and acceptability in the intended patient end-users.

This article is categorized under:

Therapeutic Approaches and Drug Discovery > Emerging Technologies

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KEYWORDS

dermal lymphatic uptake, lymphatic system, microneedles, nanomedicine, nanoparticles, targeted drug delivery system

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

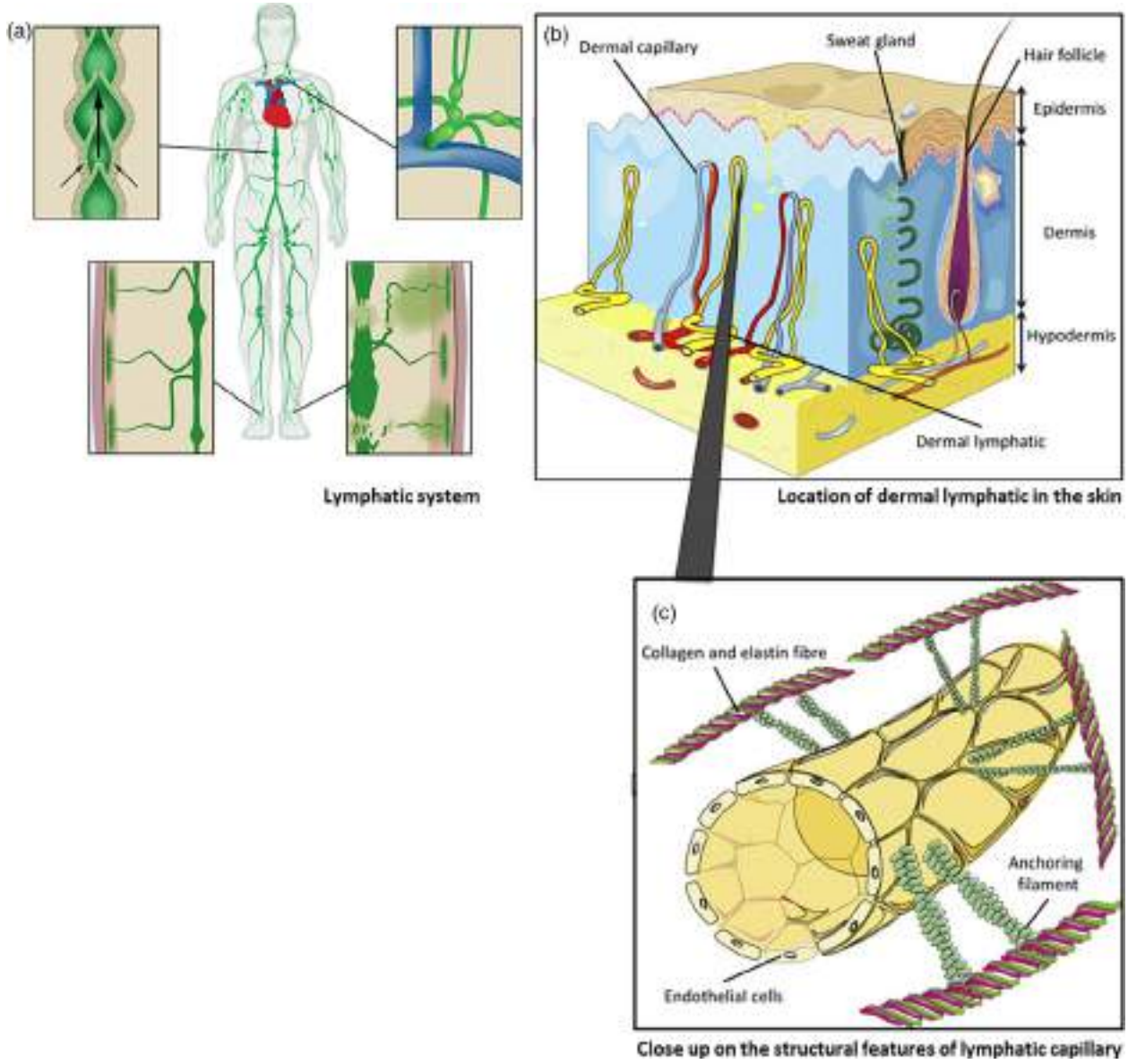


FIGURE 1 Schematic representation of; the lymphatic system throughout the human body (a), the skin structure containing dermal lymphatic network (b), and the detail of dermal lymphatic capillaries showing the vessels anchoring to surrounding collagen and elastin via the anchoring filament (c), respectively.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

interstitial space. The lymphatic system comprises of three main parts, namely, lymphatic vessels (LVs), 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 macromolecule-rich interstitial fluids and immunocompetent cells flow in a unidirectional manner, using open semicircular capillaries of the initial lymphatics from the peripheral tissues into the systemic circulation (Liao & von der Weid, 2015). The extravasated macromolecule-rich fluids (i.e., lymph) are then transported from the lymphatic capillaries (initial ducts) to larger LVs, 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 (2010) proposed that the presence of the endothelial glycocalyx layer (EGL) serves as a small pore system that further disrupts

the oncotic pressures. 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, Li, et al., 2019). With respect to the immune system, the lymphatic capillaries play a major role in the transportation of nonself (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 traveling 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 antigen delivery, antigen presentation, initiation and, regulation of immune responses.

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 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 three classes of lymphatic vessels, namely, lymphatic capillaries (identified as initial LVs), precollecting, 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 & von der Weid, 2015), identified by particular expression of LV endothelial hyaluronan receptor 1 on the surface of LECs (Liao & von der 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).

The LVs are divided into functional sections named lymphangions that are interrelated by semilunar valves that prevent 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 precollecting vessels and, finally, collecting LVs. Precollecting 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 LVs 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, Li, et al., 2019). Lymph comprises electrolytes, immune cells, nutrients, antibodies, lipids, antigens, macromolecules, and lipoproteins.

1.2 | Lymph nodes

The primary lymphoid organs in the body are the lymph nodes (J. Hu, Xu, 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, however, 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 exist in various shapes and sizes, however, 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 fibers, 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 DCs 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, the large lymph vessels covered by overlapping reticular fibers, are located throughout the node. On these reticular fibers, 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 an 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). With particular regard to 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, tumor cells and associated macrophages stimulate lymphangiogenesis in the draining lymph nodes and at the tumor site (A. Alitalo & Detmar, 2012; K. Alitalo, 2011; Proulx et al., 2013; Swartz & Lund, 2012).

Lymphangiogenesis, the formation of new LVs from the pre-existing LVs, is a developmental event that occurs solely during embryogenesis and, is not normally programmed to occur during postnatal development (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 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 & Padera, 2013; Trevaskis, Kaminskas, & Porter, 2015). This consequently, increases 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. This review discusses up-to-date findings regarding the delivery mechanism of nanoparticles and the use of microneedle delivery systems to target the LVs and lymph nodes.

2 | LYMPHATIC DRUG TARGETING

Lymphatic drug targeting can be differentiated into two main categories, namely, passive and active lymphatic targeting. With regard 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 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 relies on the structure of the lymphatic system to achieve drug delivery. Due to nanoparticles' small size, they can be efficiently delivered to the lymphatic system via this pathway. This route of targeting is also recognized 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 moderately 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 by convection. Since the flow of convection is typically delivered from the blood to the lymph, it is assumed that following drug administration from the 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 space 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 and 100 nm seem to favor lymphatic uptake (Ryan, Kaminskas, & Porter, 2014). As an example, ultrafine poly(lactic-co-glycolic acid) (PLGA) nanoparticles of doxycycline (DOX; 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 localization 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. First, 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 LVs by transcytosis (transcellular uptake) promoted by receptors and/or vesicular transfer mechanisms (Miteva et al., 2010). Last, some molecules may also be delivered to the lymphatic system by targeting the 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 of targeting drugs to the APCs in the extracellular matrix can be utilized 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 and 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 recognize 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, have 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 localization of CpG and to produce antitumor immune responses in in the lymph node sinus macrophages (Hu, Li, et al., 2019; Hu, Xu, et al., 2019).

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 through the exploitation of selective receptors and different DC-specific targeted agents. One of the receptors expressed on DC is the mannose receptor, specifically, C-type lectin receptors (CLR). The main characteristic of these 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 preclinical and clinical investigations (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 studies or specifically for the purpose of lymphatic targeting, as highlighted in Table 1.

3 | MICRONEEDLE TECHNOLOGY

3.1 | Definition and development

MN arrays are minimally invasive devices which can bypass 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 (Donnelly et al., 2011). Typically, the MN system comprises of a plurality of microprojections attached to a base support, normally ranging between 25 and 2,000 μm in height (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 have gained particular consideration by researchers in both academia and industry (Larrañeta, Lutton, Woolfson, & Donnelly, 2016; Rodgers et al., 2018). Following continual developments, MN technologies have since been produced by a variety of techniques, comprising different shapes and characteristics, for a broad range of pharmacological agents.

The first-ever MN-based publication was reported in 1995 by Hashimi et al., in which hollow MNs were produced for delivery a bacterial plasmid to nematodes genetic transformation (Hashmi et al., 1995). However, it was not until 1998 that Henry, McAllister, Allen, and Prausnitz (1998) fabricated MNs for true transdermal applications. The first MN arrays were fabricated from silicon, as a result of modern improvements in microelectronic technology and microfabrication. Since then, MN fabrication has steadily improved in recent years with expanded applications, with 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 associated with hypodermic needle and syringe administration can lead to significant suffering in patients, especially in the pediatric group and those who experience needlephobia (Hamilton, 1995; Nir, Paz, Sabo, & Potasman, 2018). The main advantage of MN technology is that the administration does not result in pain, particularly when compared to hypodermic needles. This advantage is due to the small size of the micron-scale projections that penetrate the *SC*, however, avoid stimulation of the pain receptors that dwell in the dermis (Kaushik et al., 2001). Additionally, some types of MN arrays, namely dissolving and hydrogel-forming MNs can potentially avoid the injury caused by needlestick and, eradicate the need of sharps disposal of needles. This is particularly relevant within the UK, as it has

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 three- to six-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 hr, 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)
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 normalized concentration of curcumin in the lymph nodethan that of curcumin solution	(Baek & Cho, 2017)
Doxorubicin loaded LyP-1-conjugated PEGylated liposomes	Subcutaneously injection via foot pad	The conjugated liposomes were able to target lymphatic metastatic tumors and to improve inhibition effect on lymphatic metastatic tumor in comparison with unconjugated liposomes	(Yan et al., 2012)
OVA antigen encapsulated DOTAP-PEG-mannose liposomes	Subcutaneously injection via 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 hr post dose	(Ryan et al., 2013)
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)

(Continues)

TABLE 1 (Continued)

Type of nanoparticles	Route and animal model	Key finding	Reference
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 tissue and the metastatic lymph nodes	(Borrajo et al., 2016)

been reported that approximately more than 100,000 needle stick injuries occur in hospitals each year. Moreover, an evaluation conducted by the World Health Organization reported that unsafe use and inappropriate reuse of needles resulted in approximately 37.6, 39, and 4.4% of hepatitis B, hepatitis C, and HIV cases worldwide, respectively (WHO, 2003).

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 volume 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. (2017), successfully demonstrated that larger MNs patches (16 cm^2) can be self-applied by patients successfully. 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 LVs (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*-vinylpyrollidone). Following administration to the skin, the MNs completely dissolved in the dermal layer. Consequently, the dermal draining lymphatics can be visualized and, by the utilization of a near-infrared camera, the kinetics of the clearance from the application site can be assessed. Interestingly, this approach can also be utilized 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 5 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 to and, formed 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) demonstrated the application of a 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 at the minimum dose required. Within the study, it was shown that lymph drainage pathways from the injection site to LNs were successfully observed using NIR fluorescence imaging when doses of 10 μg or greater of indocyanine green were 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 by other various route, namely, conventional subcutaneous injection, intravenous, and intradermal. Furthermore, 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, then compared the anti-tumor response to that of conventional systemic administration. It was confirmed by NIRF imaging that SOFUSA™ was able to infuse 100 μl/hr 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 tumor 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 removed, forming temporary aqueous conduits in the *SC* part of the skin. As described previously, the first investigation utilizing MN in the field of transdermal drug

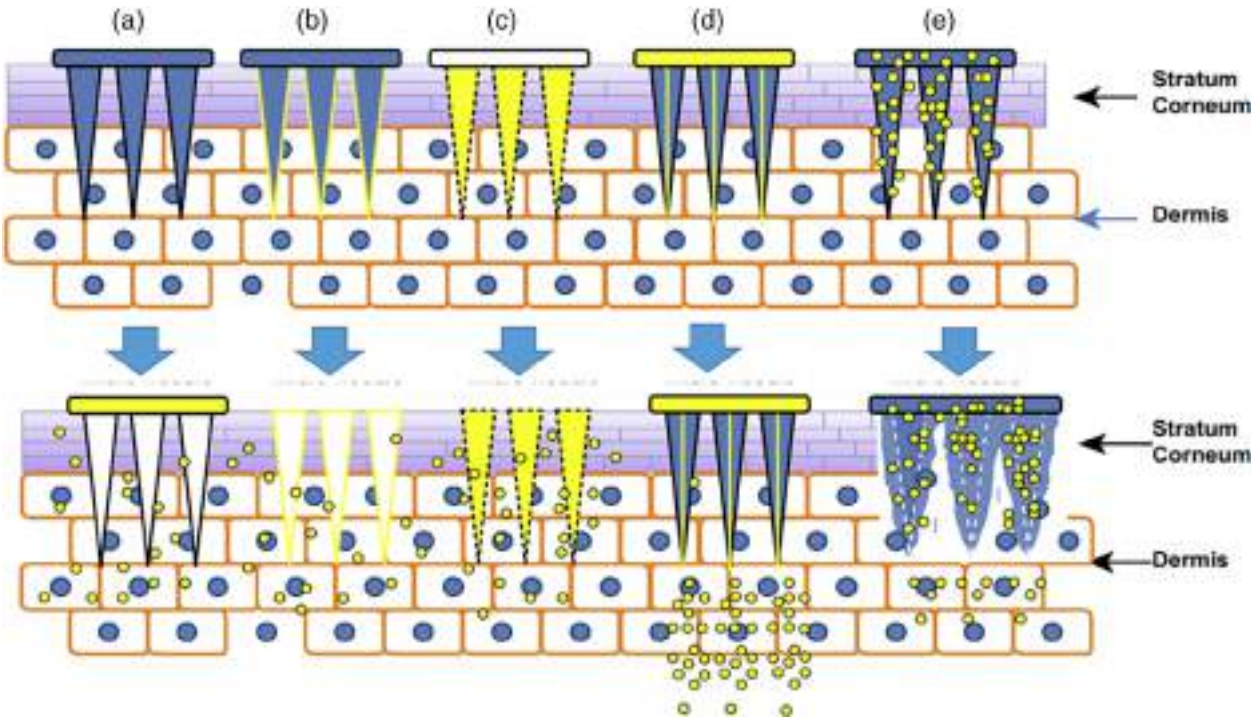


FIGURE 2 Representation illustration of five type MN device. Solid MNs utilized for pre-treatment in the skin and application of drug-loaded reservoir (a), coated MNs for deposition of drug-containing layer into the skin, followed by removal of the MN array (b), dissolving MNs utilized to achieve controlled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

delivery was reported by Henry et al. (1998). This study reported the enhanced delivery of calcein as a model drug, following the application of solid MNs made of silicon using 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 pretreatment 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) has also been extensively studied. In addition, the use of MN roller tools as pretreatment in the skin was found to improve the transdermal penetration of nonsteroidal anti-inflammatory drugs, such as, paracetamol, diclofenac, ketoprofen, and ibuprofen (Stahl, Wohlert, & 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).

Coated MNs are also known as "coat and poke" technique. This approach is prepared by coating a solid MN with 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 MN 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 MN has successfully delivered DNA intradermally, specifically, the MNs tips were coated with DNA solutions (Pearton et al., 2012). In the hereditary skin disorders and cutaneous cancers treatment, appropriate utilization 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, as a consequence, the loading volume of this type of MN is also limited. Therefore, coated MN arrays are more limited to low dose drug molecules (Quinn & Donnelly, 2018).

In the application of Hollow MNs, a liquid formulation containing the drug is injected into the skin through the lumen of the hollow MN needles. Specifically, via the MN bore, continuous delivery of various compounds through the skin can be achieved using this class 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 by 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 simulated interstitial fluid was developed and combined with hollow MNs. This device was able to rapidly extract simulated interstitial fluid within 5 s. In addition, the ability of this device to produce a glucose concentration dependent color 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. (2015) 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 and stimulation of the actomyosin complex increasing paracellular permeability.

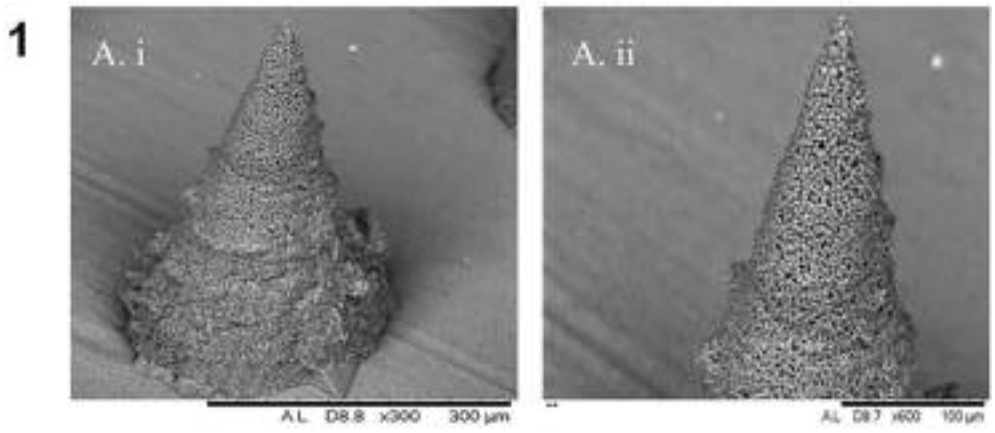
The final and most recent class of MNs are polymeric MNs; this includes both the dissolving MNs and hydrogel-forming subclasses. Dissolving MNs are prepared by micromolding the mixture of soluble matrices and the therapeutic compounds. Commonly, the matrices used in the preparation of dissolving MNs are the combination or the single use of aqueous blend of biocompatible polymers or sugar (Mir, Permana, Ahmed, Khan, & ur Rehman, & Donnelly, 2020; Permana, Mir, Utomo, & Donnelly, 2020; Tuan-Mahmood et al., 2013). Different types of compounds, including small drug molecules, biomacromolecules, and drug nanosuspensions 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, allow the dissolution of the MNs tips. Following this, the drugs contained in the MN needles are then released over time. For 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 order 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 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.

Hydrogel-forming polymers have also been utilized to prepare another type of MNs and, as a result, are 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 and high molecular weight substances (i.e., BSA and insulin) as well as hydrophilic 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 one 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).

3.4 | The combination approach of nanoparticles and microneedles in lymphatic targeting

The first investigation combining the approach of nanoparticle with MNs arrays for improved skin drug delivery was reported in 2003 by McAllister et al. (2003). Specifically, in this work, the effect of various molecular radii of several compounds, namely insulin, calcein, BSA, and nanoparticles upon transdermal delivery utilizing solid MN arrays was investigated. 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 MNs were used, skin permeability in vitro was increased 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 from 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). Second, the penetration rate of the nanoparticles relied on the concentration of the nanoparticles. Vora et al. (2017) investigated PLGA nano- and microparticles of vitamin D_3 incorporated into novel bilayer dissolving MN arrays as an intradermal delivery system (Figure 3a). In this study, MNs were formulated utilizing 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 D_3 nano-microparticles from bilayer MNs exhibited higher drug deposition, with approximately 74.2% of vitamin D_3 delivered after cryostatic skin sectioning, in comparison with needle-free patches (Figure 3b; Vora et al., 2017). Permana, McCrudden, et al. (2019) developed a novel combination approach of nanosuspensions of DOX, albendazole (ABZ), ivermectine (IVM), and dissolving MNs. 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 application.



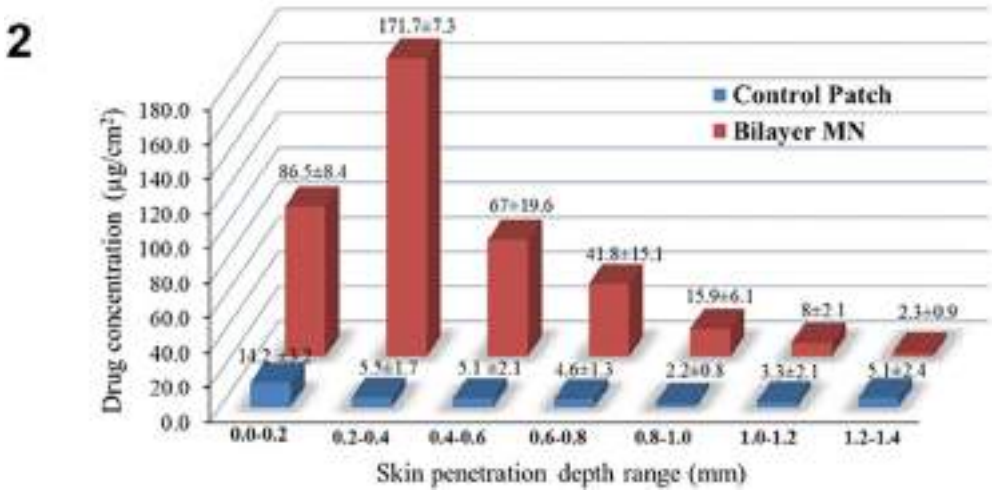


FIGURE 3 Scanning electron micrograph of PLGA nanoparticles-loaded bilayer MNs (a) and the VD_3 concentration detected in different layers of excised neonatal porcine skin after the administration of PLGA nanoparticles-loaded bilayer compared to control patch (b) (Vora et al., 2017). All figures reprinted 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 localized in the lymph nodes in mice (Figure 4a). Interestingly, RhB was still quantified even after 168 hr postapplication (Figure 4b). 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 a 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 4c). Although the mean particle size was greater than 100 nm, numerous particles exhibited 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 antifilariasis drugs, namely 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 enhanced by using this combined method of delivery (Figure 4d). 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. Specifically, the combination of nanoparticles and MN has been used in cancer and vaccination. An investigation performed by Zaric et al. (2013) is one of the examples that successfully utilized 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. Recently, the combination of hyaluronic acid based MNs and doxorubicin formulated into transfersomes, has been developed for treatment of tumor metastasis (Yang, Wu,

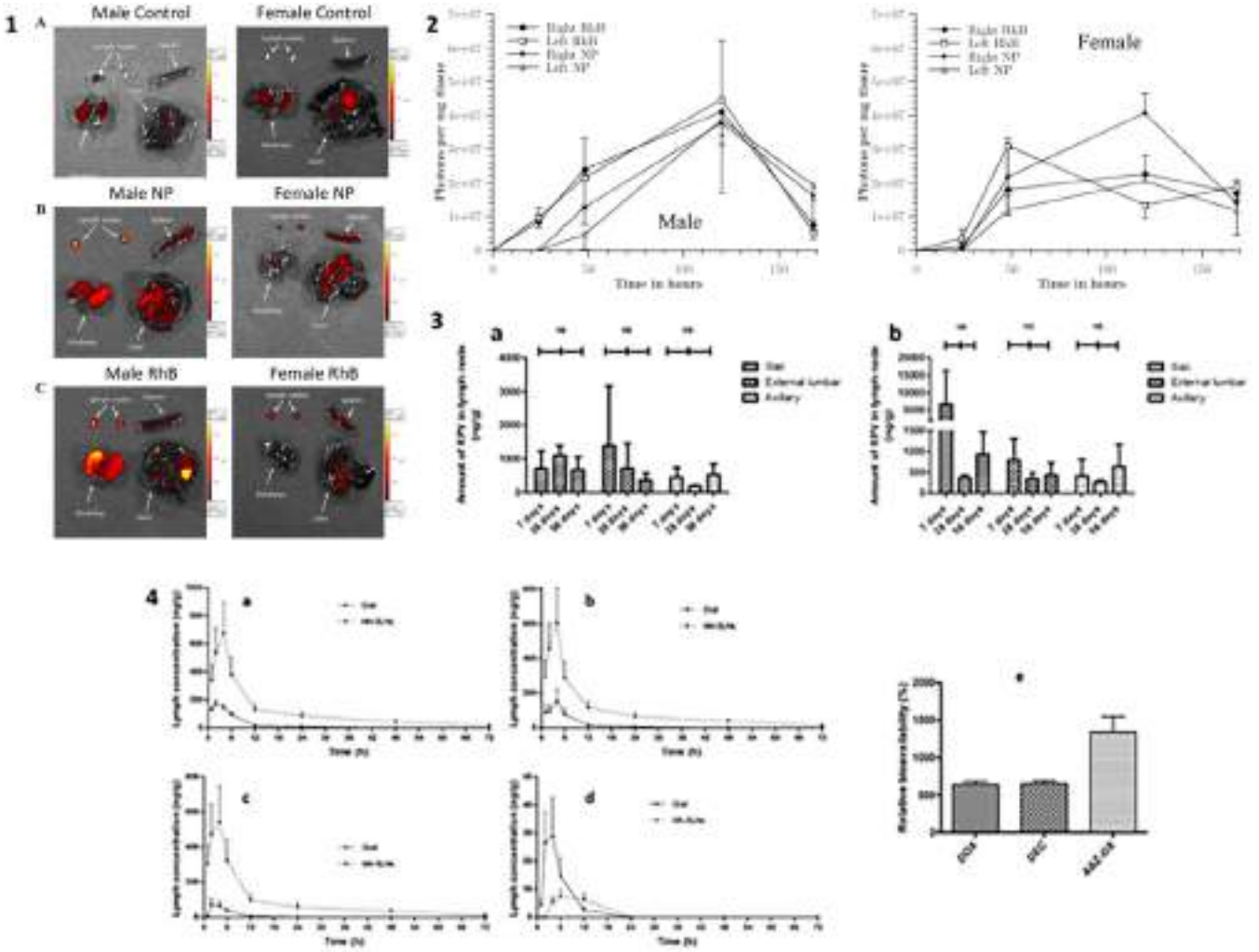


FIGURE 4 In vivo imaging system images of lymph nodes, spleens, livers, and kidneys of male and female mice after 48-hr the administration of different treatment, namely control (no MN treatment) (A), MNs laden with RhB nanoparticles (B) and MNs laden with free-RhB (C) (a). The concentrations of photons 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 (b) (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) (c) (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-OX (E) after the application of drug-loaded SLNs incorporated with MNs in comparison with the oral administration of pure drugs (d) (Permana, Tekko, et al., 2019). All figures are reprinted with permission of the publisher from the references

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, this combination has shown potential as an effective alternative approach for therapy of tumor metastasis using the noninvasive intradermal route.

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

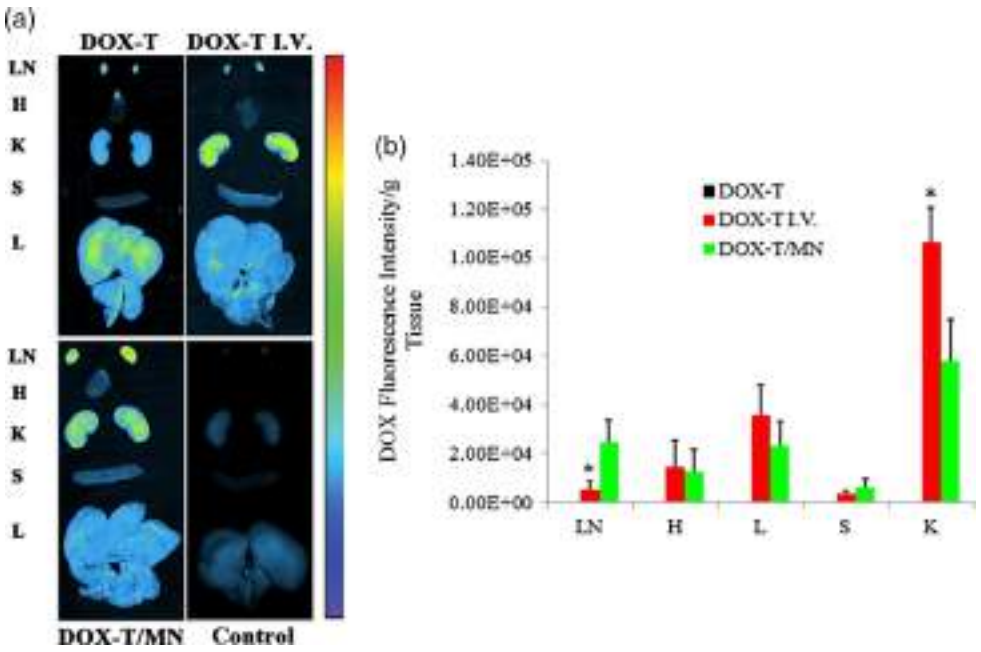


FIGURE 5 In vivo biodistribution (a) and the fluorescence intensity (b) of doxorubicin in lymph nodes (LN), heart (H), kidney (K), spleen (S), and liver (L) of rats following the administration of DOX-T, DOX-T/IV, and DOX-T/MN (Yang et al., 2019). All figures are reprinted with permission of the publisher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

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 an intradermal vaccine delivery device is desirable for potential immunotherapy treatments in the future.

Another study highlighting the success of nanoparticle-MN combination approach has been reported by Li et al. In this study, it was demonstrated that vaccination of mice using dissolving MNs containing chitosan NPs laden with OVA, was able to accumulate OVA-NPs effectively in the peripheral lymph nodes. Consequently, greatly improved immune responses were stipulated by MN-mediated delivery in comparison with those of free OVA (Li, He, Deng, Zhang, & Lin, 2020). Additionally, a study exploring the use of hollow MNs to deliver PLGA-NPs containing OVA showed that, compared to soluble OVA-based vaccine, OVA-loaded NPs yielded faster antibody affinity development kinetics (Niu, Chu, Burton, Hansen, & Panyam, 2019).

3.5 | Future considerations for MN technology

As an emerging drug delivery system, MNs have unique benefits in comparison with other traditional delivery approaches and, as such, enable diverse biomedical applications. The MN-assisted approach may permit not only the desired drug delivery and therapeutic effectiveness, but also doing so with negligible adverse reactions. Numerous scientists have developed and enhanced conventional MN designs in an attempt to achieve the best potential application. Additionally, MNs afford a minimally-invasive, discreet, and pain-free method of delivery, which can be investigated

for both local and systemic treatment (S. Sharma, Hatware, Bhadane, Sindhikar, & Mishra, 2019). As previously documented, MNs can be manufactured utilizing various substances and diverse techniques. Such materials and methods involved in fabrication have resulted in the development of highly efficacious, safe, and stable MNs. Notwithstanding; there are several challenges and obstacles to scale-up MNs from the laboratory to the market for commercialization. In response, vast efforts has been made in order to overcome these challenges and obstacles.

A harmonious collaborative approach between academia and industry toward production-line scale-up and manufacturing of MNs, taking into account the associated production costs, now requires careful consideration. In line with industrial scale-up to the manufacturing of clinically relevant MN products, several regulatory aspects will need to be established, for instance, pharmacopoeial standards and good manufacturing practice (GMP) guidelines. Further issues may lie within the scale up to continuous line manufacturing itself, as this will require an essential and focused strategy, particularly with regard to the considerable and diverse quantity of MN fabrication methods that currently exist in the literature to date (Indermun et al., 2014).

Partners in academia and industry have provided substantial evidence to show appropriate MN usability across various populations (Norman et al., 2014), highlighting significant benefits for patients, the public, and healthcare professionals (Moffatt, Quinn, McCague, & Donnelly, 2020). Moving forward toward clinical progression, regulatory bodies (e.g., the US FDA and the MHRA in the UK) will need to provide minimum standards with respect to MN manufacturing. Additionally, quality control and the possible requisite for a sterile manufacturing process and MN product are aspects that need to be addressed (Lutton et al., 2015). 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 commercialization; therefore, substantial investment from industry and financial stakeholders is required to meet this unmet need.

In recent years, the potential of MN technology for self-administration has been given due consideration, 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 MNs (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 patients 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 recognized 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 with particular regard to this concern.

As the field of MN technology continues to grow, the use of MNs in more varied and diverse purposes has also been evaluated. This is indicated by the utilization of MNs to target different sites of the human body, including dermal lymphatics. Through a careful consideration of the choice of design and composition of the formulations, MNs contribute a unique 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 cell 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 one 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 LVs and nodes, resulting in limited therapeutic penetration in other sections of the lymphatic system. Further consideration of the use of MN in lymphatic delivery must be given to the determination of dose that should be delivered to the lymphatic system in the treatment of such related diseases. Specifically, in hollow MNs, the flow rate of drugs should be investigated in more comprehensive and detailed studies. Importantly, the effect of lymphatic uptake on the pharmacokinetic profiles of drugs must be evaluated. Accordingly, given the promise of this delivery route, further studies regarding utilizing microneedle for lymphatic delivery are now urgently needed.

4 | CONCLUSION

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. Some of these conditions require targeting of chemotherapeutic agents to the lymphatic tissue network and associated organs. However, the use of conventional delivery routes has shown limited success in terms of targeting drugs specifically to the lymphatic system. This Review explored several factors affecting lymphatic delivery, with particular attention given to the size of the therapeutic and the subsequent route of administration.

To increase the site-specific localization of the drugs to the lymphatic system, two main approaches can be employed. The first approach is the utilization 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 order to achieve higher uptake by the dermal lymphatic capillaries particle size is a critical factor. Lymphatic capillaries will absorb formulations containing particles with sizes ranging between 10 and 100 nm. Larger particles will be accumulated in the skin while smaller ones will be absorbed by blood capillaries.

This Review explored the lymphatic uptake of different drugs formulated using a range of nanoparticle approaches. MNs have been used as an alternative approach to the hypodermic needle to administer nanoparticle-based formulations intradermally to target the lymphatic system. These systems have shown higher lymphatic uptake compared to the delivery of free drugs. The use of MN has clear advantages over hypodermic needles. These drug delivery devices can be shelf applied and do not generate sharp wastes. 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 preclinical and clinical MN-based outcomes. Additionally, patient usability, acceptability, and sterility issues must be further evaluated in an attempt to fully realize the potential applications of MNs, so that this novel delivery platform can achieve its ultimate intended patient benefit. Last, in order to achieve such benefits, regulatory approval must be established. Investment by industry will now be essential, though partnerships with organizations 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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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Andi Dian Permana: Investigation; writing-original draft; writing-review and editing. **Firzan Nainu:** Writing-review and editing. **Kurtis Moffatt:** Writing-review and editing. **Eneko Larrañeta:** Conceptualization; writing-review and editing. **Ryan Donnelly:** Conceptualization; supervision; writing-review and editing.

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