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Smart micro/nanoneedles for gene delivery

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ABSTRACT

Gene therapy, a transformative field in biomedicine, holds immense promise for treating a wide range of diseases. Smart micro/nanoneedles (MNs/NNs) emerged as non-viral carriers, offering safety and reduced immunogenicity, in addition to precise and controlled gene delivery, and represent promising platforms for gene delivery. They can also be tailored to respond to specific biological or environmental triggers, enabling precise targeting and localized delivery, ensuring that therapeutic genes reach their intended destination. Despite significant progress, challenges persist in scalable manufacturing, biocompatibility and safety, and genetic cargo stability. The incorporation of artificial intelligence (AI) into design and predictive modelling offers promising and cost-effective solutions to these limitations. This review provides a comprehensive analysis of MN/NN fabrication methodologies that enable structural and functional customization, while highlighting the interdisciplinary nature of MN/NN technologies and their transformative role in the future of gene therapy.

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Introduction

Gene therapy is a promising field that holds great potential for treating various diseases caused by genetic abnormalities, including genetic disorders (1-3), cancer (4-6), and viral infections (7). The goal of gene therapy as a medical approach is to regulate, add, delete, correct, or replace a defective or missing gene in a patient's cells, which can restore the normal function of the affected cells or tissues and therefore, treat or prevent disease (8). Based on previous findings of transformation (9) and transduction, and on the ability of phages to transfer genetic material from one bacterium to another (10), Temin concluded through his experiments that genetic information could transfer from DNA to RNA and vice versa using the Rous sarcoma virus (RSV) (11). Later, using his discoveries and those of many others, the first gene therapy trial with a therapeutic purpose was conducted on two children suffering from adenosine deaminase deficiency (ADA-SCID). Their white blood cells were extracted and altered *ex vivo* to express the normal gene for making adenosine deaminase (12). After this trial, subsequent gene transfer trials were initiated for various diseases and despite the unsatisfactory results of the first studies, gene therapy persisted in its efforts (13). After the completion of the Human Genome Project, which further fueled interest in personalized medicine and disease-associated genes, RNA interference (RNAi) and small interfering RNA (siRNA) gained prominence as gene-silencing tools (8, 14). Later, gene-editing tools such as CRISPR-Cas9 (15-17) and zinc finger nuclease (ZFN) (18) revolutionized gene therapy. Additionally, mRNA vaccines and gene therapies based on individual genetic variations have gained attention in recent years.

Gene delivery is a critical aspect of gene therapy, which is used to deliver genetic material to target cells or tissues in a patient's body through various methods. However, since genetic material is negatively charged and high in molecular weight, they face two major barriers: the first is the stratum corneum (SC), and the second is the cytoplasm of viable cells; therefore, successful delivery of genes to target cells remains a major challenge (13, 19, 20).

Gene delivery can be achieved through various methods, including viral and non-viral vectors and physical methods. Viral vectors, such as adenovirus (21), retrovirus (22), and lentivirus (23), are often used in gene delivery because they can efficiently deliver genes to target cells (8, 24). However, viral vectors can elicit immune responses (25, 26), and there is a risk of insertional mutagenesis, in which the virus integrates into the host genome and disrupts normal gene function (27). Non-viral vectors, such as liposomes (28), polyethyleneimine (PEI) (29), and nanoparticles (NPs)

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(30), offer a safer alternative but are less efficient for gene delivery (26, 31). Physical methods of gene delivery include electroporation (32, 33), jet injection (34), and gene gun (35), which involve the physical introduction of genes into cells (31, 36). These gene delivery methods often suffer from low efficiency, toxicity, and lack of target specificity (37).

Furthermore, the skin and its underlying tissues contain abundant APCs, such as dendritic cells, which can activate the immune system (38). Therefore, DNA and RNA vaccines have revolutionized disease prevention by leveraging the body's immune system to recognize and combat pathogens. However, these vaccines face several challenges, including stability, storage requirements, and targeted delivery, which impact their effectiveness and practicality (36). Since the conventional method of using a hypodermic needle to overcome the skin barrier has limitations, such as pain induction and inadequate APC targeting, new platforms are being investigated (39, 40). Advances in nanotechnology-based carriers, such as nanoparticles, liposomes, and noisomes, have significantly enhanced site-specific delivery at the cellular and tissue levels (41).

To progress towards developed gene transfer strategies, MNs and NNs have emerged as promising solutions for targeted and efficient gene delivery and offer several advantages over traditional gene delivery methods (42, 43). For example, MNs/NNs can be painlessly administered by patients themselves, which eliminates the need for trained medical personnel and reduces healthcare costs (44, 45). Moreover, MNs/NNs can be easily transported and stored, often without the need for a cold chain, which is particularly important for gene therapies that require frequent administration or vaccination (40, 46, 47). They can be designed to penetrate the skin or mucosal barriers and deliver the genetic material directly to the underlying cells or tissues, which can be beneficial, especially in DNA vaccines (48). NNs are one-dimensional nanostructures up to 100 nm that are fabricated from metal/metal oxides. One key difference between MNs and NNs is the ability of NNs to penetrate the cytoplasmic membrane and achieve direct intracellular delivery (49, 50).

In this review article, we provide a comprehensive overview of smart MNs/NNs and their current states in gene delivery, including their design, fabrication, and potential applications. Our review aims to provide valuable insights into the latest developments in this field, highlighting the advantages and limitations of MNs/NNs for gene therapy. We would also briefly discuss the role of AI as a promising tool for designing and fabricating smart MNs/NNs (51).

Methods

The current comprehensive literature search was conducted by searching major data bases (PubMed, Scopus, and Web of Science databases) focusing on the last decade. The following keywords and Boolean operators (AND, OR) were used in various combinations: "Microneedle; Nanoneedle; Gene delivery; Carrier; Biomedicine. Titles and abstracts were initially screened for relevance, followed by full-text evaluation. Key data, including their design, fabrication, potential applications in gene delivery, and limitations, were extracted.

Fundamentals of Smart MNs/NNs

Unlike conventional MNs/NNs, smart MNs/NNs can be designed to target specific cells or tissues, thereby reducing off-target effects and improving therapeutic outcomes. One of the key advantages of smart MNs/NNs is their ability to respond to environmental changes such as pH (48, 52), temperature (53), or other physiological parameters, enabling the release of genes only in response to specific stimuli, which further enhances the specificity and efficiency of gene delivery (48). Moreover, smart MNs/NNs can be coated with various materials to improve the stability of the gene payload, increase cellular uptake, or reduce immune responses (54). Additionally, smart MNs/NNs can be designed to be biodegradable using polymers such as polyvinyl alcohol (PVA), which eliminates the need for device removal after gene delivery (30, 55). Smart MNs/NNs have shown promising results in preclinical studies for various applications, including genetic vaccination (48), cancer gene therapy (30), and regenerative medicine (56). Smart MNs/NNs also offer hope for treating genetic disorders caused by gene mutations, especially those that affect the skin. By precisely targeting affected cells, they can correct or replace faulty genes in monogenic disorders (57, 58). For complex genetic disorders MNs/NNs can deliver siRNAs that modulate multiple pathways by silencing reporter genes such as luciferase/GFP, which impacts CD44 pathways responsible for skin conditions such as cancer and psoriasis (59).

Fabrication techniques for MNs synthesis

The performance and functionality of the smart MNs/NNs depend primarily on their materials, sizes, shapes, and functions, which are determined by the fabrication techniques (60, 61). Therefore, choosing the appropriate fabrication technique is crucial for achieving the desired gene delivery outcome. Different fabrication techniques have distinct advantages and disadvantages, such as cost, complexity, throughput, quality, and biocompatibility (61, 62). MNs' fabrication techniques can be broadly classified into three categories: subtractive, formative, and additive manufacturing, as shown in Table 1.

Subtractive manufacturing

Subtractive manufacturing is a technique that involves removing the excised part of the bulk substrate to form the desired needle geometry. It is also known as machining or material-removal process. Subtractive manufacturing can be performed by various tools and machines, such as milling cutters, lathes, grinders, and drilling machines. This technique can produce MNs with high accuracy and precision, reduce the amount of waste and be exploited with a wide range of materials. However, the high cost, limited design flexibility, and environmental impact of this technique have posed significant challenges for mass production (49, 63).

Photolithography with selective etching

Photolithography with selective etching is a process that uses light to pattern a photoresist layer on a substrate, and then etches away the exposed or unexposed regions to form the needle geometry. The substrate, such as silicone, is coated with a thin layer of photosensitive material, called photoresist, which can change its solubility or adhesion when exposed to light (42). A photomask, a transparent plate with a pattern of opaque areas, is placed over the photoresist layer and illuminated using a light source, such as ultraviolet (UV) light. Depending on the type of photoresist, the

Table 1. A comparative summary of microneedles (MNs) fabrication techniques, highlighting their functional advantages and associated limitations

MNs fabrication techniques		Advantages	Disadvantages	Ref.
Subtractive manufacturing	Photolithography with selective etching	MNs with high aspect ratio, sharp tips and uniform sizes and shapes	Costley and time-consuming process	(60)
	Machining with chemical etching	Low cost and simple process	Low accuracy and poor surface quality	(63)
	Laser machining	High accuracy and resolution resulting in MNs/NNs with various shapes	Poor surface quality and arced needle tips as a result of high heat generation	(64, 65)
	Wire electrical discharge machine (EDM)	Fabricating high-aspect ratio MNs of any material regardless of their mechanical properties	Complex and costly process of optimizing parameters such as voltage, frequency, etc.	(59)
	Machining with micro-milling	Fabricating MNs with complex and customized shapes	Complex process of optimizing parameters.	(63)
Additive manufacturing	3D printing	Fabricating complex and customized shapes with high efficiency	Limited material selection and a loss of cargo during polymerization	(63, 66)
	Drawing lithography	Creating high-aspect ratio and flexible MNs that are minimally invasive when penetrating biological barriers	Weak mechanical strength of the needles and limited shape control	(67, 68)
Formative manufacturing	Molding	Suitable for mass production and a variety of materials	Losing the cargo to the base or back of the needle	(67)

exposed areas (positive photoresist) or the unexposed areas (negative photoresist) (60, 63) become more soluble or less adhesive to the substrate. Then, a developer solution, such as sodium hydroxide (NaOH) or tetramethylammonium hydroxide (TMAH), is used to dissolve or detach the soluble or less adhesive areas of the photoresist, leaving behind the desired pattern on the substrate. An etchant solution, such as hydrofluoric acid (HF) (62) or potassium hydroxide (KOH) (42), is used to remove the substrate material from areas not covered by the photoresist, creating the needle geometry (60). At last, a stripper solution, such as acetone or sulfuric acid (H_2SO_4), is used to remove the remaining photoresist from the substrate, leaving behind MNs with high aspect ratio, sharp tips, and uniform sizes and shapes (49, 69). The needle tips can be further sharpened, especially in NNs using reactive-ion etching (RIE) (60). The etching process can be even modified using reactions such as BOSCH to determine the tip length (42).

Machining with chemical etching

In this technique, a chemical solution dissolves the unwanted material from the workpiece, leaving the desired shape. The workpiece, such as metal, is coated with a masking material, such as wax, paint, or tape, which protects the areas that are not to be etched. The workpiece is then immersed in an etchant solution, such as NaOH (70) or KOH (71), which reacts with the exposed material and removes it by chemical reaction (72). At the final stage, the workpiece is rinsed with water or another solvent to remove the etchant solution and the masking material. Despite its low cost and simple process, this technique has limitations, such as low accuracy and poor surface quality, which need to be addressed (63). Results from a study indicated that although increasing the concentration of the etchant and the duration of etching can enhance MN/NN features, they can also cause them to be more brittle as more material from needle bodies is removed (72).

Laser machining

This process uses a high-powered laser beam to vaporize or melt the material from the workpiece, creating a precise cut or hole and therefore resulting in MNs with high accuracy and resolution (49). This technique can be classified into laser ablation and laser cutting. In laser ablation, a pulsed laser beam is used to ablate a substrate in a specific sequence, creating 3D MNs with hollow or solid structures that can release their cargo upon insertion into the skin (49, 63, 73). Conversely, in laser cutting, a continuous or pulsed infrared laser beam is used to cut a metal foil into 2D MNs with various shapes, such as conical and rectangular, which can be coated with genetic material and transferred to the skin upon insertion (64, 74, 75). However, the high heat generation can cause poor surface quality and arced needle tips (65). This issue often manifests itself when silicone is exploited. Due to its low thermal conductivity, a structurally and mechanically weakened zone called the heat-affected zone (HAZ) is formed, resulting in microcracks and ultimately poor surface quality (65).

Wire electrical discharge machine (EDM)

This method employs electrical discharge between the two electrodes to shape the conductive substrate into desired MNs. These rapid spark discharges can generate extreme heat reaching 8000–12000 °C, vaporizing the material at the point of discharge (76). Therefore, it could fabricate high aspect ratio MNs of any material, regardless of their hardness or other mechanical properties. For instance, MNs of 700 µm length and 200 µm base width were prepared via EDM, coated with siRNA using the pipette reservoir method, and were then used to treat skin conditions caused by aberrant gene expression (59). However, both electrical and non-electrical process parameters have a great influence

on the MN performance. Optimizing these parameters, such as voltage, frequency, pulse on-time and off-time, discharge energy, dielectric fluid, and flushing pressure, can be complex and costly (76).

Machining with micro-milling

This process uses a rotating tool with multiple cutting edges to remove material from the workpiece by advancing it in a direction parallel to the axis of rotation. This technique is used to create MNs with complex, customized shapes, such as helical, spiral, or branched. Since feed rate, cutting depth, and spindle speed are of great importance when using this method, more effort is needed to optimize the machine's properties (49, 63). To further improve the process, tool path strategies programmed in computer-aided manufacturing (CAM) can be exploited. For instance, García-López *et al.* used a CAM micro-milling process to manufacture AISI 316L conical stainless-steel needle arrays with a 1 mm base diameter and 1 mm height. Additionally, the effect of lubrication on the surface finish and geometrical accuracy was evaluated. The results showed that although needle height was barely affected, the needle tip diameter was significantly reduced when lubricant was used. An increased surface roughness was also observed in the absence of lubricant (77).

Additive manufacturing

Additive manufacturing can produce three-dimensional needles by adding successive layers of material according to a digital model. Additive manufacturing can create complex and customized shapes, but also has limitations in terms of speed, cost, and quality (49).

3D printing

In this method, a digital model of the needle is first designed and then printed layer by layer as MNs. There are different methods of 3D printing, such as fused deposition modeling (FDM), selective laser melting (SLM), digital light processing (DLP), two-photon polymerization (TPP), or stereolithography (SLA) (72). Among these, SLA is the most common, in which a laser irradiation source is used to cause polymerization of the photopolymer resin, resulting in the design of the MNs (49). Similar to the principle of this method but with higher efficiency, DLP exploits a mirror array and a light bulb to achieve the same results as the polymer. However, the material selection is limited, and a loss of cargo activity during polymerization is probable (63). In one study, a combination of SLP and DLP was employed, which involved preparing the 3D model in design software and then deconstructing it into cross-sectional images. These images were projected onto a digital micromirror device, generating UV light patterns that solidified the photocurable solution in a sequential, layer-by-layer fashion, ultimately resulting in a three-dimensional MN array (66).

Drawing methods

Drawing lithography is a technique that utilizes the tensile properties of viscous fluids to form MN structures. A viscous polymer droplet is contacted and stretched to create the needle shape. This method is particularly useful for creating high-aspect-ratio MNs, which are beneficial for penetrating biological barriers with minimal invasiveness (67). However, the mechanical strength of the needles produced by this method can be weak, and the shape control is somewhat limited (49, 63). In one study, a HEM was constructed using this technique. An electrode was attached to a syringe pump as the drawing system to elongate MNs from 2D glassy maltose, which was later separated from the maltose surface to create dissolving MNs (68).

Soft lithography, on the other hand, involves creating molds from soft elastomeric materials, such as polydimethylsiloxane (PDMS). The process allows the replication of intricate patterns and structures at the micro- and nanoscale. In the context of smart MNs, soft lithography can be used to fabricate flexible needles that are less likely to cause tissue damage upon insertion. This flexibility is advantageous for patient comfort and can be critical for the successful delivery of genes to target cells (49).

Both techniques are integral to the advancement of gene delivery systems, offering unique benefits that can be leveraged depending on the specific requirements of the gene therapy application.

Formative manufacturing

Formative fabrication methods primarily focus on shaping MNs by deforming a material using a negative mold into the desired structure without adding or removing material. Formative manufacturing can produce large quantities of needles with low cost and high quality, but also has limitations in terms of flexibility and complexity (49, 63, 78).

Molding

Molding is one of the most common formative methods, which is broadly categorized into micro-casting and microinjection molding. In micro-casting, a liquid or semi-liquid material is cast into a silicone or PDMS mold with the negative shape of the MNs via vacuum or centrifugation (60, 79). Once the material solidifies, it retains the shape of the mold. This method is suitable for mass production and can be used with a variety of materials, including polymers and biodegradable substances (46, 47, 80). One challenge of this method is losing cargo to the base or the back of the needle, which can result in a considerable amount of waste. To overcome this limitation, a two-step loading method is employed to ensure the genetic cargo is concentrated in MN tips. The first step is to load a combination of the genetic cargo and the minimum amount of polymer necessary to stabilize it, followed by the second step, which involves the loading of polymer only as the base (67).

Microinjection, on the other hand, exploits metal and polymer powders and injects them into the micro-molds using a microinjection machine. The high temperature during sintering results in bonds between the metal powders and ultimately makes porous metal MNs, but can also degrade the nucleic acid payload (81). The porosity and pore dimensions can be adjusted using factors such as powder composition and sintering parameters (49, 65).

Synthesis approaches for NNs

The interconnection between MNs and NNs lies in their shared goal of creating minimally invasive devices for gene delivery applications, yet they diverge in scale, complexity, and fabrication techniques. NNs fabrication techniques produce needles with diameters ranging from a few to tens of nanometers, which can bridge the inside and outside of cells and deliver or sense genetic materials at the intracellular level (50). The fabrication techniques can be broadly categorized into two approaches: bottom-up and top-down, as shown in Table 2 (49).

Bottom-up approaches

The bottom-up approach in NNs fabrication for gene delivery is a process that builds structures from the atomic or

Table 2. A summary of nanoneedles (NNs) fabrication techniques and their technical challenges, along with their key benefits

NNs fabrication techniques		Advantages	Disadvantages	Ref.
Bottom-up approaches	Self-assembly	Cost-effective method of fabricating highly ordered nano-structures with precise geometries without the need for external templates	Difficulty to isolate individually and therefore not fully practical	(82)
	Chemical vapor deposition (CVD)	Precise control over needle geometry	Poor surface morphology due to layer-by-layer composition	(82)
	Atomic layer deposition (ALD)	More accuracy and structure control as well as precise thickness control over the deposited layer	Complex and time-consuming process with the need of repeated exposures to separate precursors	(82)
Top-down approaches	Dry and wet etching	Integrating NNs into larger, more complex systems	Complex and energy-consuming process	(82)

molecular level. This method relies on the natural tendency of atoms and molecules to self-organize through chemical or physical forces to form the desired nanostructures without leading to any waste (49, 82).

Self-assembly

This bottom-up approach involves the spontaneous organization of individual molecules into well-defined, stable structures through non-covalent interactions, such as hydrogen bonding, van der Waals forces, and π-π interactions. Self-assembly allows for the creation of highly ordered nanostructures with precise geometries and functionalities (82). These nanostructures can be designed to have sharp tips and high aspect ratios, which are essential for penetrating cell membranes and delivering genetic material directly into cells. One of the key advantages of self-assembly is its ability to create complex nanoscale structures without the need for external templates or sophisticated equipment, making it a cost-effective and versatile method for fabricating nanoneedles (49). However, because of the said complexity and difficulty in isolating individually, this method is still not fully practical (82).

Chemical vapor deposition (CVD)/ atomic layer deposition (ALD)

This process, also known as vapor-liquid-solid (VLS), involves depositing a thin film of material onto a substrate through the chemical reactions of gaseous precursors such as ethylene and helium (83). This process usually requires metal NPs as catalysts, which have a great impact on needle density and diameter. CVD allows for the creation of NNs with uniform properties with precise control over their geometry by varying the chemical gas composition, catalyst size, reaction time and heating temperature (49, 83, 84). Carbon hollow NNs were fabricated using template-based CVD and used for transfecting cells with plasmid DNA. These arrays can carry a payload ranging from 0.66 kDa to 3600 kDa, indicating their broad application range and lack of limitations when carrying large DNA cargos (83). However, the CVD process can result in poor surface morphology due to the layer-by-layer composition. Using radio frequency for generating heat has also greatly improved this method by affecting the morphology and crystallinity of the NNs (82).

ALD uses the same principle for the sequential reaction of two precursors with the surface of a substrate in a self-limiting manner, which results in hollow NNs. The key advantage of ALD is the precise thickness control over the deposited layer. Moreover, compared with CVD, ALD is more accurate and offers greater structural control as well as more uniform film formation, making it a better candidate for NNs used in gene delivery (82). However, the process is more complex and time-consuming, since it requires repeated exposures to separate precursors (61).

Top-down approaches

Top-down approaches in the fabrication of smart NNs for gene delivery involve sculpting materials into the desired nanoscale structures using various subtractive or pattern-transfer techniques, unlike bottom-up methods that build structures atom by atom (82). These methods are similar to those in subtractive manufacturing, including different dry (60, 84) and wet etching techniques, with differences in scale and precision (49). For instance, metal-assisted chemical etching (MACE) was used as a wet etching process, employing Ag particles to form porous NNs for the delivery of VEGF plasmid DNA, resulting in neovascularization of the target tissue (62). Furthermore, RIE, a dry-etching technique, coupled with electron cyclotron resonance microwave plasma chemical vapor deposition (MPCVD), was exploited to fabricate diamond NNs that could deliver DNA lipoplexes to neurons with at least 8-fold improvement in transfection efficiency (84).

Additionally, RIE can be used as a dry etching method that exploits high-energy gas ions to form NNs of desired shapes (49).

Both approaches have unique advantages and are often used in conjunction to create NNs with the desired properties for specific gene delivery applications. The bottom-up approach is particularly useful for creating NNs with functionalized surfaces for targeted delivery, while the top-down approach is beneficial for integrating NNs into larger, more complex systems.

Design considerations in gene delivery

The selection of materials for the fabrication of smart MNs/NNs is a pivotal aspect of gene delivery systems. The efficacy, safety, and overall success of gene therapy hinge on the properties of the exploited material (85). These materials must ensure biocompatibility, precise control over gene release, and minimal invasiveness, while also being amenable to mass production and quality control. Materials must be chosen to avoid adverse immune responses, inflammation, and toxicity, which could compromise the therapeutic efficacy and patient safety (63, 86).

In addition, the design of smart MNs/NNs involves the integration of various functionalities to enhance gene delivery efficiency. These functionalities include cellular

targeting, payload protection and release, tissue penetration, and intracellular delivery (87).

Cellular targeted MNs/NNs

Cellular targeting is a crucial aspect of gene delivery, ensuring the precise delivery of therapeutic genes to target cells or tissues. It involves using various strategies to guide gene carriers or smart MNs/NNs to specific cell types in a controlled manner, enhancing therapeutic efficacy while minimizing off-target effects. This is especially important in cancer gene therapy, where the genetic material needs to be selectively delivered to cancer cells (88). Cellular targeting approaches can be broadly categorized into two main strategies: passive targeting and active targeting.

Passive targeting relies on the natural characteristics of cells or tissues to accumulate smart MNs/NNs. This approach takes advantage of physiological properties, such as the leaky vasculature of tumor tissues and inflammation-associated features, including the tumor's lower pH and overexpression of enzymes (49, 89, 90), to enhance the accumulation of gene carriers at the desired target sites. For instance, in cancer gene therapy, gene carriers may accumulate preferentially in tumor tissues due to overexpression of hyaluronidase, which facilitates their penetration by degrading the extracellular matrix and increasing vascular permeability and is also correlated with the stage of cancer (91). Passive targeting is beneficial when the target cells or tissues exhibit distinctive characteristics that can be exploited to enhance accumulation, thereby increasing local gene delivery efficiency and decreasing exposure to healthy tissues.

Active targeting involves the incorporation of specific ligands on the surface of smart MNs/NNs to selectively bind to cell-surface receptors or antigens present on the target cells (92). This strategy enables precise targeting of cells expressing the specific receptor or antigen, facilitating the delivery of therapeutic genes to the desired sites (49, 93). By utilizing active targeting, smart MNs/NNs can bypass non-target cells and tissues, thereby reducing potential off-target effects and enhancing the therapeutic index of gene delivery. Various targeting ligands and antibodies have been explored for active targeting, including peptides, aptamers, antibodies, and small molecules (38). Table 3 summarizes the types of cellular targeting strategies by smart MNs/NNs.

pH-responsive materials

Polymers such as poly(acrylic acid) (PAA) (93), poly(methacrylic acid) (PMAA), and poly(N-2-methacryloyloxyethyl pyrrolidone) (PNMP) (47) consist of hydrophilic, ionically functional polymer chains and undergo conformational changes in response to changes in pH. When exposed to an acidic pH, such as the acidic microenvironment found in tumors, the polymers can undergo swelling or dissolution, leading to the release of encapsulated genes (51, 90, 91). Aside from responding to lower pH values, MN patches could be engineered to respond to physiological pH. As an example, a 2017 study used a DNA vaccine encoding an Alzheimer antigenic determinant was coated onto an MN with a polyelectrolyte multilayer and heparin. The charge reversal triggering layer was composed of oligo(sulfamethazine)-b-poly(ethylene glycol)-b-poly(amino urethane) (OSM-b-PEG-b-PAEU) and exhibited positive charge at the low pH 4.03 and negative charge at the physiological pH. The negative charge of the copolymer combined with heparin caused an electrostatic repulsion, which facilitated the release of the DNA vaccines encoding an Alzheimer antigen. This vaccine was transfected into RAW 264.7 macrophage cells for the *in vitro* test and injected into BALB/c mice for the *in vivo* experiment. The SEM images showed complete disassembly when immersed in phosphate-buffered saline (PBS) with a pH of 7.4, whereas in citric acid-NaOH buffer at pH 4.03, which is the process pH, cargo release was inhibited. In addition, the expression of antigen and humoral responses via MNs delivery compared to subcutaneous (SC) delivery with hypodermic needles was significantly higher, which demonstrated a potential platform for the treatment of Alzheimer's disease (52). A similar strategy was exploited in another study to improve the delivery efficiency of the p53 expression plasmid, a suppressor gene of cancer cells in cancer gene therapy. PH-responsive polyelectrolyte multilayers (PEM) were applied to the surface of polycaprolactone (PCL) MNs using a layer-by-layer (LbL) assembly technique. The PEM consisted of two components: transition layers (composed of poly-L-lysine-dimethylacetamide/polyethyleneimine (PLL-DMA-PEI)) and gene-loaded layers. When inserted, the negatively charged PLL-DMA would transform into a positively charged form due to the weakly acidic environment. As a result, the transition layers would rapidly collapse, facilitating the efficient release of outer DNA. To perform an *in vivo* tumor inhibition test, human oral epidermoid carcinoma cells were injected subcutaneously

Table 3. Summary of cellular targeting strategies employed in smart micro/nanoneedles (MNs/NNs), describing how specific materials interact with targeting signals to activate stimuli-responsive mechanisms, along with therapeutic advantages and limitations

Cellular targeting	Materials	Targeting signal	Mechanism	Advantages and limitations	Ref.
Passive targeting	PAA, PMAA, PNMP	Changes in pH	Undergoing conformational changes such as swelling or dissolution leading to the release of the encapsulated gene	Enhancing gene delivery efficacy as a result of improved gene delivery with reduced systemic side effects	(91, 93)
	PLGA, HA, PNIPAM	Changes in temperature	Changes in solubility or gelation in response to temperature variations	Sustained gene release by forming a reservoir, triggered release upon exposure to targeted local hyperthermia	(53, 94, 95)
Active targeting	PNMP, photochromic compounds or light-absorbing metal/metal oxide NPs	Exposure to light	Structural changes or cleavage upon exposure to specific wavelengths of light resulting in reversable changes in their physical properties	Achieving spatial and temporal control over gene delivery by selectively illuminating the target area	(47, 90)
	Metal NPs and carbon-based nanomaterials	Electrical stimuli	Undergoing changes in physical properties in respond to electric fields or current	Achieving on-demand gene delivery and intracellular transfection through the application of electrical signals leading to spatiotemporal control over gene release	(68, 96)

PMAA: Poly(methacrylic acid); PNMP: Poly(N-2-methacryloyloxyethyl pyrrolidone); PLGA: Poly(d,l-lactic-co-glycolic acid); HA: Hyaluronic acid; PNIPAM: Poly(N-isopropylacrylamide)

into BALB/c nude mice, and the mice were then divided into four groups. Then their tumor size and body weight were tracked for 21 days and the tumor inhibition efficacy was calculated. The results further validate the theory, with the efficacy of intravenous injection and (model DNA/PEI) MN being only 30.5% and 46.4%, respectively. In comparison, the efficacy of (PLL-DMA/PEI) MN was calculated to be as high as 90.1% (89). This pH responsiveness enables tumor-specific gene delivery, enhancing the efficacy of gene therapy while minimizing systemic side effects.

Temperature-responsive materials

These are another class of stimuli-responsive materials utilized in smart MNs/NNs that exhibit changes in their physical properties, such as solubility or gelation, in response to temperature variations (95). This property was exploited in a tri-block polymer consisting of poly lactic-co-glycolic/polyethylene glycol/poly lactic-co-glycolic (PLGA-PEG-PLGA), which is in liquid form at room temperature but forms a gel when exposed to higher physiological temperature and therefore forms a reservoir for sustained gene release in MNs for the delivery of plasmid deoxyribonucleic acid (94). In one study, incorporating temperature-responsive polymers, such as hyaluronic acid (HA), into the needle design achieved gene release upon exposure to local hyperthermia. A derivative of indocyanine green (IR820), an approved near-infrared (NIR) dye, was used as a photothermal agent to absorb NIR light upon exposure, thereby precisely controlling the release of the p53 gene at the target site (53). Following the same principle, poly(N-isopropylacrylamide) (PNIPAM) was incorporated as a thermosensitive polymer into the separable back layer of an MN patch containing a polymer-encapsulated DNA vaccine to induce both humoral and cellular immune responses against COVID-19. PNIPAM, a polymer with a lower critical solution temperature (LCST) of 14-16 °C, is insoluble in water at room temperature but if the injection site is cooled to 14 °C or below for several minutes, the cap would be hydrophilic enough to be separated. The separable capacity of the MN patches was tested *in vitro* by incubation in a water bath (which showed complete detachment after 3 min. It was then further confirmed in an *in vivo* experiment in C57BL/6 mice. (95).

Light-responsive materials

Light-responsive materials, known as photo-responsive polymers, typically incorporate light-sensitive moieties, such as photochromic compounds or light-absorbing metal/metal oxide NPs, into their structure (90). These moieties undergo reversible photochemical or photophysical transformations upon exposure to specific wavelengths of light, leading to structural changes or cleavage (47, 91). A photo-responsive and pH-responsive polymer, Poly(o-Nitro-benzyl-methacrylate-*co*-Methyl-methacrylate-*co*-Poly(ethylene-glycol)methacrylate) (PNMP), was exploited as the release layer to deliver pDNA encoding luciferase and poly-1 transfecting agent on cue. Initially, PNMP is soluble in organic matter. However, upon short-term exposure to ultraviolet, the o-nitrobenzyl groups are cleaved, resulting in the formation of UV-PNMP, which is soluble in water above pH 6.5. The release profile of UV-PNMP MNs immersed in PBS with a pH of 7.4 demonstrates a significant loss of DNA cargo compared to the non-irradiated MNs. Furthermore, for *in vivo* experiments, pGag, a plasmid encoding the model HIV antigen SIV-gag, was coated onto MN patches with a UV-PNMP release layer and administered to C57Bl/6-MHC II-GFP mice and was then compared with control mice injected intradermally (ID), IM with or without electroporation, and MNs with a non-irradiated PNPM layer. The results demonstrated a robust immune response via expansion of Gag-reactive T cells in UV-PNMP MN-treated mice compared to IM- and ID-treated groups. However, the response was ablated in mice injected with non-irradiated MNs, which shows the importance of the photo-sensitive release layer (47). Low-melting-point moieties can also be exploited for on-demand release via liquefaction when exposed to an external NIR source. These properties can be exploited to achieve spatial and temporal control over gene delivery by selectively illuminating the target area (90).

Electro-responsive materials

These materials, such as metal NPs and carbon-based nanomaterials, utilize electrical signals to trigger gene release from smart MNs/NNs. These materials can respond to electrical stimuli, such as electric fields or current, by undergoing changes in their physical properties, therefore enabling spatiotemporal control over gene release (90). This responsiveness was harnessed to achieve on-demand gene delivery via the application of electrical signals.

For instance, a hybrid electro-microneedle (HEM) was designed as a monolithic hybrid assembly of a dissolving maltose MN and an electrode, and used anomalously to achieve stepwise-aligned cutaneous permeation, cutaneous release, and intracellular transfection of p2CMVmIL-12 to successfully treat B16F10 subcutaneous tumors in a mouse model. The model was tested in situ using murine epidermal cells and pCMV-Gluc as a reporter gene and compared with a negative control without electrical pulses and a positive control using a hypodermic needle and electrode-tweezers. The bioluminescence intensity achieved by the positive control and HEM model was similar and significantly greater than that of the negative control, which shows the potential of HEM for overcoming the limitation of dissolving MNs without integrating electroporation. To evaluate the efficacy of *in vivo* gene transfer, p2CMVmIL-12, a a pro-inflammatory cytokine, was injected into melanoma tumor-bearing C57BL/6 mice and analyzed for tumoral expression of IL-12 and overall tumor progression and survival rate. As expected, the expression of IL-12 was considerably greater in HEM and the positive control compared to the negative control. Additionally, the HEM model was the most effective at tumor repression with a 10-15 day delay in tumor growth and 25% survival after 45 days (68).

In another study, a novel flexible MN array electrode (MNAE) chip was designed, featuring a transparent parylene film as a flexible substrate for electrodes, along with silicone MN arrays. The MNAE, consisting of the RFP plasmid and Cy5-labelled siRNA, was then injected into mice to evaluate both DNA and siRNA transfection efficiency. The results from both experiments showed evenly distributed fluorescence at an optimum voltage of 35 V, which is generally harmless to humans. Tumor fluorescence in un-electroporated and electroporated mice with a higher voltage (45 V) was significantly decreased, suggesting the important role of MNAE in genetic cargo delivery and in slowing their elimination with minimum tissue damage (96).

Payload protection of gene in MMNs/NNs structures

Payload protection refers to the strategies employed to safeguard the therapeutic cargo or genetic material (payload) during the gene delivery process. The aim is to protect the payload from degradation, premature release,

or clearance before it reaches the target cells, thus ensuring its stability and efficacy (58, 97-99). Several approaches are utilized to provide payload protection during gene delivery, including encapsulation of the gene into different delivery systems, protective coatings and complexation methods.

Various types of gene carriers in MNs/NNs structures

The carriers can form stable complexes with the genetic cargo, which is referred to as gene delivery complexes (85). After successful cellular uptake and intracellular trafficking, the carrier molecules should appropriately release the genetic material, ensuring its availability for gene expression or other functional activities. This release can be triggered by factors such as changes in pH, enzymatic degradation, or intracellular signals (87).

Various types of carriers or vehicles can be used for encapsulation in gene delivery, which are divided into viral (100) and non-viral vectors (79). A summary of these carriers is provided in Table 4.

Viral vectors

Viral vectors are genetically modified viruses that can be used as gene delivery carriers. They can encapsulate the payload within their viral capsids. Viral vectors, such

Table 4. Application of various types of gene delivery carriers incorporated into micro/nanoneedles (MNs/NNs) structures, outlining loading strategies, experimental models and therapeutic outcomes

Methods	Type of gene delivery carrier	Type of MNs/NNs	Delivery complex	Experimental model *in vitro/in vivo*	Results	Ref.
Viral vectors	AAV	PVA MN patch	AAV containing the luciferase coding sequence (AAV-LUC) / AAV-VEGF	*In vitro* assessment of transfection capacity in HEK 293 cells via flow cytometry and fluorescence microscopy *In vivo* evaluation of gene transfer, distribution of gene expression and cardiac function and adverse remodeling improvement in a rat model of MI using echocardiography	*In vitro* test showed a transduction efficacy of 97.2% and *in vivo* treatment demonstrated an even distribution of gene expression in contrast to DI. Furthermore, MN-AAV-VEGF showed a higher EF improvement, greater FS and the smallest LVIDs and LVIDd which indicated functional neovascularization and ameliorated cardiac function	(101)
Non-viral vectors	lipoplexes	Solid silicone MN patch / MN + EP / hollow MNs	Lipofectamine 2000 complexed with plasmid DNA encoding ovalbumin (pOVA)	*In vitro* assessment of transfection efficacy in HeLa cells at various weight ratios via ELISA kit *In vivo* experiment and evaluation of immunization in BALB/c mice	Results demonstrated an increase in particle size and zeta potential when weight ratios were increased with the optimal being 200 nm at Lipofectamine 2000:pOVA 2:1. As a result, cellular uptake and overall immune response was improved	(36)
		Silicone MN arrays	liposome-encapsulated CXCL1-specific siRNA combined with Fluvax® 2012® (Liposomes consisting of a mixture of DOTAP, DOPE, cholesterol, PEG2000-C16Cer)	*In vivo* evaluation of delivery efficiency of fluorescent-labelled DNA encapsulated in liposomes in BALB/c mice using scanning electron microscopy *In vivo* assessment of gene expression in BALB/c mice when administrated with liposome encapsulated siRNA targeting CXCL1 with Fluvax 2012® via RT-PCR	Results indicated a delivery efficiency of approximately 34% 2 min post application. It also demonstrated a detectable amount of cargo at deposition site which shows a locally targeted delivery. Furthermore, CXCL1 mRNA expression in skin was reduced by approximately 4-fold which shows a successful knockdown of the gene	(28)
	Polyplexes	HA, dextran and PVP dissolving MNs	STAT3 siRNA complexed with PEI	*In vitro* test for cellular uptake efficiency in B16F10 melanoma cells using CLSM. *In vivo* evaluation of antitumor efficiency when administrated to B16F10 melanoma tumor-bearing mice	Results from CLSM showed almost 100% cellular uptake of the polyplex compared with naked siRNA which was 1%. Furthermore, the results demonstrated a silencing of STAT3 gene up to 60% which resulted in a dose-dependent reduction in tumor volume	(104)
	Hybrid NPs	PLGA MN array	A PEM consisting of plasmid DNA encoding firefly luciferase, PBAE and PLGA coated with DOPC and DOPG	*In vivo* evaluation of gene delivery and gene expression in C57B1/6 mice via CLSM and whole animal bioluminescence imaging	Results indicated a burst release with higher intensity when the hybrid NPs containing PEM and pLUC coated with DOPC and DOPG were administrated.	(79)
	Hybrid complexes	PVP / PVA dissolvable MNs	Plasmid DNA encoding luciferase complexed with RALA at various N:P ratios	*In vitro* test of transfection efficiency in NCTC 929 murine fibroblast cells Evaluating DNA stability via gel electrophoresis	Results indicated an improvement in transfection efficiency when increasing N:P ratio with the highest being 43.69% at the ratio of 12. Furthermore, DNA conformation was preserved and protected from degradation in higher N:P ratios compared with lower ratios and naked pDNA.	(85)

PVA: Polyvinyl alcohol; AAV: Adeno-associated viruses; MNs/NNs: Micro/nanoneedles; PVA: Polyvinyl alcohol; LUC: luciferase; EF: Ejection fraction; DI: Direct injection; MI: Myocardial infarction; LVIDd: Left ventricular diastolic inner diameter; LVIDs: Smallest ventricular systolic inner diameter; FS: Fractional shortening; pOVA: Plasmid DNA; CLSM: Confocal laser scanning microscope encoding ovalbumin; PEM: Polyelectrolyte multilayer; DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPG: Dioleoylphosphatidylglycerol; NPs: Nanoparticles; PEI: Polyethyleneimine; PLGA: Poly(d,l-lactic-co-glycolic acid) ; PBAE: Poly (β- amino ester)

as adenoviruses, adeno-associated viruses (AAVs) (21), or lentiviruses (23) possess natural abilities to infect target cells and deliver genetic material. They can be modified to remove their pathogenic properties while retaining their payload-encapsulation capabilities. Viral vectors offer efficient protection of the payload, high transduction efficiency, and the potential for long-term gene expression (24, 75). Shi *et al.* used phase-transition MNs to deliver an AAV containing the luciferase coding sequence (MN-AAV-LUC) to the rat heart. At first, a PVA MN patch, 6 mm in diameter and containing approximately 45 needle tips with base widths of 334 μm and heights of 850 μm, was fabricated and coated with the vector. The swelling capacity of the needles was then measured, yielding an 8.3-fold swelling ratio. The AAV release profile of the MNs was evaluated by real-time polymerase chain reaction (PCR) of supernatant collected after incubation, indicating a burst release followed by a slower release profile over 24 hr, which shows a unique kinetic profile with predictable accuracy. To ensure AAV binding, fluorescent fluorescein isothiocyanate (FITC)–labeled AAV (MN-FITC-AAV) was used and showed a strong fluorescence signal compared to the control MNs without FITC-AAV. Furthermore, the released vectors were tested for their infectious capacity *in vitro* using human embryonic kidney (HEK) 293 cells by flow cytometry and fluorescence microscopy, which showed a relative transduction efficiency of 97.2%. *In vivo* gene transfer and distribution of green fluorescent protein (GFP) reporter gene expression were also traced using a non-invasive small-animal bioluminescence imaging system post MN-AAV-GFP administration. In contrast to the direct injection (DI) control group, the distribution of gene expression was not confined to the injection site and was therefore considered even. Next, cardiac function improvement and adverse remodeling, functional neovascularization, and activated signaling pathways were evaluated in a rat model of myocardial infarction (MI) after administration of MNs coated with AAV-VEGF. Variations in cardiac function were measured using echocardiography 4 weeks post-administration and compared with the DI control group. Ejection fraction (EF) improvement (36.10±5.25%) was higher in the MN-AAV-VEGF group compared with the DI-AAV-VEGF (30.29±2.10%). The MN-AAV-VEGF group also showed greater fractional shortening (FS) than the DI group (18.28±2.97% versus 15.04±1.05%). Furthermore, the MN-AAV-VEGF group also had the smallest ventricular systolic inner diameter (LVIDs) and left ventricular diastolic inner diameter (LVIDd) values (LVIDd, 9.12±1.09 mm versus 10.55±0.69 mm, P=0.0179; LVIDs, 7.59±1.01 mm versus 9.33±0.81 mm, P=0.0048). The results indicated improved cardiac function, reduced scar size, and elevated myocardial perfusion as a result of successful MN-mediated, AAV-encapsulated genetic cargo delivery (101).

Non-viral vectors

While viral vectors have been widely used for gene delivery due to their natural ability to efficiently enter cells and deliver genetic material, they also come with several disadvantages that limit their application, including immunogenicity, limited packaging capacity, genotoxicity, and limited tissue targeting. These disadvantages associated with viral vectors highlight the need for alternative gene delivery strategies, such as non-viral vectors, to overcome these limitations and enhance the safety and efficacy of gene-based therapies (24, 25, 54).

Lipoplexes

Lipoplexes are non-viral complexes formed between genetic material and lipids (102). Lipids can be cationic lipids, such as liposomes or lipid NPs, which contain positively charged headgroups, often derived from quaternary ammonium compounds and interact with negatively charged genetic material through electrostatic interactions (93), which results in the formation of lipoplexes through a self-assembly process (36). In an aqueous environment, cationic lipids and genetic material come together, leading to the condensation of the genetic material within the lipid bilayer. This condensation leads to the formation of NPs with a core-shell structure, in which the genetic material is encapsulated within the core (64, 84, 93).

To evaluate the effect of lipoplexes on improving skin immunization, transfection efficacy, and serum stability of the genetic cargo, different weight ratios of Lipofectamine 2000 complexed with plasmid DNA encoding ovalbumin (pOVA) were tested both *in vitro* and *in vivo*. Three types of patches, including a 5 ’ 5 silicone solid MN patch, an MN+EP patch, and hollow MNs were prepared. pOVA complexed with Lipofectamine 2000 at different weight ratios of 1, 2, 3, 4, and 5 were evaluated by photon correlation spectroscopy for their particle size and zeta potential. By increasing the weight ratio from 1 to 5, both zeta potential and particle size were also increased from -10 to 60 mV and 300 to 600 nm, respectively. The complexes were then tested in human cervical carcinoma cells (HeLa) at various weight ratios (0.5 μg/well of pOVA) for their transfection efficiency using a highly sensitive ELISA kit for OVA. Results indicated that for optimal transfection efficacy, particle size should be approximately 200nm, which can be achieved by Lipofectamine 2000:pOVA at a weight ratio of 2. Furthermore, to test the serum stability of the cargo using gel electrophoresis, complexes containing 0.25μg of pOVA were incubated with 10% serum from female BALB/c mice at 37 °C for six hours and compared with naked pOVAs. For the *in vivo* test, female BALB/c mice were injected subcutaneously in the neck region with 100 μL of various pOVA formulations (100 μg/mL of pOVA). Blood samples were collected on day 0 and day 21 at the end of the study and analyzed for OVA-specific serum IgG antibody using the standard ELISA protocol. The results from the transfection study in human cervical carcinoma cell (HeLa) lines and immunization study in female BALB/c mice indicated that although lipid NPs could, in theory, improve overall cellular uptake and immune response, their size and zeta potential, which are both determined by their weight ratio, play an important role in their DNA binding ability and interaction with negative cell membranes. Furthermore, the results also demonstrate significantly increased immune responses compared to naked pOVA, which is attributed to its protection against endonuclease. However, regarding serum stability, Lipofectamine lipoplexes were destabilized in 10% BALB/c mouse serum, which lead to pOVA leakage and a slightly decreased efficacy (36).

Another example of exploiting lipoplexes is in thyroid-associated ophthalmopathy (TAO), an autoimmune disease that accelerates the production of adipose tissue. Patients suffering from this condition would often undergo surgeries, which are accompanied by pain and a lengthy recovery period. A recent study demonstrated via sampling of TAO

patients, that adipocyte phospholipase A2 (AdPLA) was up-regulated in their orbital adipose tissue. AdPLA, encoded by the Pla2g16 gene, regulates adipocyte lipolysis and therefore has a critical role in the development of not only obesity but also TAO. Thus, silencing AdPLA via gene knockdown using minimally invasive devices, such as MN, seems like an attractive alternative. Liu *et al*. employed this approach and achieved considerable knockdown results in both *in vitro* and *in vivo* settings (57). The AdPLA siRNA was complexed with Lipofectamine 2000 and compared with a nonsense siRNA as the negative control for its transfection efficiency in Human preadipocyte (HPA-s) and mouse fibroblast ST3-L cells, which showed a significant knockdown after 24h. Furthermore, Oil red O staining was performed in the cell lines to evaluate the regulatory role of AdPLA in lipid accumulation, which was also significantly reduced at days 3 and 5 post-transfection. For the *in vivo* experiment, a solid MN array and an injectable MN that was connected to a micro-syringe were used. However, the MN patch resulted in a 50% suppression rate, which is considerably lower than the 70% suppression observed with the injectable MN, which may be due to many manufacturing and gene stability challenges that will be discussed later (57).

Liposomes are spherical vesicles composed of lipid bilayers. They can be used as carriers for encapsulating genetic material (8, 102). Liposomes can be formed by thin-film hydration, which involves dissolving lipids in organic solvents and then forming a thin lipid film by solvent evaporation (93, 103). This film can be rehydrated with aqueous solutions, leading to liposome formation (28). Genetic material can be loaded into the liposome's aqueous core or incorporated into the lipid bilayer (8). Liposomes offer several advantages, including biocompatibility, easy synthesis, versatility in modifying their surface properties, and the ability to carry a wide range of payload sizes (103). For instance, liposome-encapsulated CXCL1-specific siRNA was dry-coated onto silicone micro-projection arrays using the hydration of a freeze-dried matrix (HFDM) and used to silence CXCL1 mRNA transcription. Firstly, Liposomes consisting of a mixture of DOTAP, DOPE, cholesterol, PEG2000-C16Cer (at a ratio of 9.2:3.7:1:7.1 mg respectively, dissolved in tertiary-butanol) were prepared and added to siRNA at a concentration of 80 µg/ml in sucrose (55.5 mg/mL) at a ratio of 1:1. The liposome-encapsulated siRNA was then combined with Fluvax® 2012® mixed with MC at a ratio of 2:1:1 respectively and dry-coated onto silicone micro-projection arrays via the steady steam nitrogen gas method (4'4 mm silicone wafer, 110 µm in length and 40 µm in diameter). Coating quality was evaluated by scanning electron microscopy and was found to be evenly distributed (60 ng of siRNA per array). To test the delivery efficiency, fluorescent-labelled DNA encapsulated in liposomes was loaded on MNs and applied to the ventral side of the ear pinnae of female BALB/c mice ear skin for 30 seconds, 2 min, and 10 min. The optical density (OD) of coated but unused micro-projection arrays was then compared with the residue of those applied to mice and showed a delivery efficiency of 34% ±17% 2 min post-application. The results from confocal microscopy indicated that the deposition rate both laterally through the epidermis and vertically within the dermis reached its peak 2 min post-application. However, after 10 min, a detectable amount of the cargo remained at the deposition site, which shows a targeted distribution throughout the local dermal and epidermal skin sites. To further test the *in vivo* application of the arrays, liposome-encapsulated siRNA targeting CXCL1 with Fluvax 2012® was loaded and co-administered to mice and evaluated for CXCL1 mRNA using RT-PCR. CXCL1, a key regulator of neutrophil migration in the skin, is up-regulated after Fluvax injection. Therefore, when combined with CXCL1-specific siRNA, CXCL1 mRNA expression in skin was reduced by approximately 4-fold compared to control, which led to a 2.7-fold reduction in CXCL1 protein production, as determined by western blot and densitometric analysis. These results clearly show a functional knockdown of CXCL1 chemokine in skin (28).

Furthermore, the efficiency of liposomes can be improved through structural modifications. For example, Liang *et al*. adjusted liposomes' flexibility by varying the lipid concentration and compared their efficiency in facilitating the internalization of 6-carboxy-fluorescein (FAM)-GAPDH siRNA in L929 cells using sponge Haliclona sp. Spicules (SHS) as a natural MN array. Results from confocal microscopy show a higher GAPDH knockdown of 41.09% ± 5.14% when lipid concentration was 0.05% compared to the original 1% (103).

Polymeric NPs

Polyplexes, as a subclass of polymeric NPs, are commonly used non-viral vectors that protect and deliver genetic material into target cells. The polyplex structure consists of the genetic material condensed or compacted within the polymer matrix, forming NPs or microparticles (85, 99). Cationic polymers play a central role in polyplex formation. These polymers have positively charged groups, such as amino groups, that can interact with the negatively charged phosphate backbone of the genetic material through electrostatic interactions (79). The condensation process results in the compaction of the genetic material, protecting it from enzymatic degradation and facilitating cellular uptake (93). Examples of commonly used cationic polymers in gene delivery include (PEI) (52), PLL (54), PAA (93), poly (β- amino ester) (PBAE) (47, 79), and chitosan (104).

Pan *et al*. conducted research to test whether delivering STAT3 siRNA complexed with PEI via dissolving MNs could effectively silence the STAT3 gene, therefore, inhibit further development of melanoma. The dissolving MNs, 650 µm in height and 300 µm in base, were fabricated from HA, dextran, and PVP and loaded with STAT3 siRNA/PEI complex. They were then tested *in vitro* for cellular uptake efficiency in B16F10 melanoma cells using a confocal laser scanning microscope (CLSM). Cellular uptake of the siRNA/PEI complex after 4 hr of incubation reached almost 100%, which is significantly higher than that of naked siRNA (about 1%). Furthermore, following transfection, the polyplex silenced STAT3 by up to 60.4%, which reduced cell viability by 33.6%. Additionally, to evaluate antitumor efficiency, siRNA/PEI-loaded MNs were administered to B16F10 melanoma tumor-bearing mice. The results demonstrated a dose-dependent reduction in tumor volume without producing biohazardous sharp waste, which makes it an effective and safe strategy for targeted gene delivery (104).

Among these polymers, PBAE is especially advantageous due to its exceptional properties. Because of the primary amines in its structure, cellular internalization is not only improved but also cell-specific, which is shown in the results of a study comparing PLGA and PEM containing

PBAE for delivering the genetic cargo (79). Furthermore, its buffering capabilities and amphiphilicity can also help with endosomal escape (105). These properties can be further tuned to exhibit greater hydrophobicity and higher molecular weight by adjusting the molar ratio of acrylate and amino monomers, which would ultimately result in higher transfection efficacy.

For instance, plasmid DNA coated onto the surface of cationic NPs, which were prepared with PLGA and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), was evaluated for transcutaneous immunization. PLGA and DOTAP concentrations significantly affected the particle size and zeta potential with both increasing at higher concentrations. In the end, NPs consisting of 1.5 mg/ml of PLGA and 20 mg/ml of DOTAP were prepared by the nanoprecipitation-solvent evaporation method and coated with plasmid DNA (pCMV-β) by gently mixing the suspension with cationic NPs with a solution containing DNA at a concentration of 200 µg/ml. The stability of these complexes was evaluated by treating them with 1 unit of DNAase I for 30 min, which showed some protection compared with naked DNA. The *in vitro* permeation ability was then tested by pretreating the dorsal skin of BALB/c mice with a derma roller containing 192 MNs (1000 µm in length, 80 µm in base diameter). The skin was then placed in a Franz diffusion cell with the dorsal side facing the donor compartment, which was filled with pCMV-β alone or pCMV-β-coated net positive or net negative NPs (50 µg of pCMV-β). The results showed a significant increase in NPs, especially in positively charged complexes. Furthermore, their ability to deliver the genetic cargo to antigen-presenting cells (APCs) was tested by comparing the uptake of fluorescein-labeled β-galactosidase gene-encoding pCMV-β coated on the cationic PLGA NPs with that of the genetic cargo alone by DC2.4 cells. It was significantly higher for the coated NPs. Their transfection efficiency was then tested by incubating dendritic cells (DC2.4) with formulations containing 0.3 µg/well of pCMV-β and measuring β-galactosidase activity using a β-galactosidase assay kit. Despite the higher cellular uptake rate in positively charged NPs, their transfection efficacy was similar. For the *in vivo* immunization test, the derma roller was rolled in 2 perpendicular lines, 10 times each, over the dorsal skin of mice. Naked pDNA and NP coated DNA (20µg/mouse) were then dripped onto the treated area and covered with a self-adhesive patch. This process was repeated twice more, 10-14 days apart. Serum samples were collected 3 weeks post the last immunization and compared with the IM positive control group for antibody response via ELISA. Although a strong induction of IgG1 and IgG2a was detected in both IM and SC groups, the immune response from net positively charged pCMV-β-coated PLGA NPs was stronger compared with the IM administration of pCMV-β alone. However, anti-IgA was detected only in mice treated with MNs, indicating a specific mucosal response. Overall, results from both *in vitro* and *in vivo* experiments indicated that coating plasmid DNA onto the cationic PLGA NPs clearly increased cellular uptake and enhanced immunogenicity (106).

Hybrid NPs

Hybrid NPs consisting of organic and inorganic compounds generate nanomaterials with unique and enhanced physicochemical properties. A polyelectrolyte multilayer (PEM) NP was incorporated in a PLGA MN array, each 250 µm in diameter and 900 µm in height, to deliver plasmid DNA encoding firefly luciferase. The PEM film consisting of degradable poly(- amino ester) (PBAE) and PLGA NPs coated with the zwitterionic lipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and anionic lipid Dioleoylphosphatidylglycerol (DOPG) was then designed via spray LbL multilayer self-assembly. To test the penetration and gene delivery ability of the array, PEM-coated MNs containing Cy3-Labeled pLUC DNA were applied to the dorsal ear skin of C57BL/6 mice for 1 min, 5 min, and 24 hr, and examined by CLSM and whole-animal bioluminescence imaging. The results of the study showed that while MNs without the NP released their cargo over 24 hr, MNs containing the NP released the plasmid DNA upon insertion with higher intensity, which conveys better and enhanced delivery efficiency (79). Another potential use of NPs is for diagnosis or tracking MN penetration and delivery using up-conversion NPs (UCNPs), which emit red and blue signals upon NIR irradiation. This strategy was exploited in a system containing a dissolvable hyaluronic acid (HA) MN and mesoporous silica-coated UCNP (UCNPs@mSiO2). The mesoporous silica (mSiO2) shell was used to protect the loaded siRNA from enzymatic and endosome lysosome degradation, while the UCNP helped to monitor insertion depth of MNs, gene expression, and efficient gene silencing of target mRNA (98).

Hybrid complexes

Hybrid complexes can also be made by combining different carrier molecules or materials to form complexes with improved properties. For example, RALA is a hybrid gene delivery system that combines characteristics of both polyplexes and lipoplexes to create an optimized delivery platform. The RALA system consists of a cationic peptide segment derived from R9, which exhibits cell-penetrating properties, and a hydrophilic polymer backbone synthesized via RAFT polymerization. Like polyplexes, RALA utilizes a cationic component (the R9 peptide) to interact with and condense the genetic material, forming a compact complex. Similarly, RALA also exhibits features of lipoplexes. The hydrophilic polymer backbone serves as a lipid-like component, providing stability and structural integrity to the complex. This lipid-like structure contributes to the ability of RALA to interact with cell membranes, facilitating cellular uptake and endosomal escape, which are important steps in efficient gene delivery (30, 46).

Cole *et al.* incorporated RALA with various N:P ratios (the ratio of the positively charged nitrogen atoms from the peptide to the negatively charged phosphates on the DNA backbone) complexed with plasmid DNA encoding luciferase into dissolvable MNs of PVA and PVP to investigate the improvement in transfection efficiency in NCTC 929 murine fibroblast cells. The results indicated that, with increasing the N:P ratio, the transfection efficiency significantly increased, with the highest being 43.69% ± 4.598% at an N:P ratio of 12. Furthermore, DNA recovery and stability, either alone or complexed with RALA, within PVP formulation, were evaluated by gel electrophoresis. The results illustrated a change in DNA conformation, and therefore a loss of stability and degradation in naked pDNA and at lower N:P ratios. In contrast, RALA/pDNA complexes preserved the pDNA conformation and prevented its degradation, thereby remaining intact. Therefore, to achieve a more stable structure the N:P ratio could be modified (85).

Enhanced payload protection by protective coatings

Payload protection can be achieved through the application of protective coatings on the surface of smart MNs/NNs. These coatings act as physical barriers, shielding the payload from enzymatic degradation, immune recognition, and non-specific interactions, thereby ensuring its stability and improving its bioavailability (85, 93). Coatings can be composed of biocompatible polymers, such as PEG (28, 93), which enhance stability and prevent clearance by the immune system. The choice of protective coating depends on factors such as the desired release profile, target cells or tissues, carrier materials, and the specific requirements of the gene delivery system (107).

Biomimetic coatings

Biomimetic coatings involve the incorporation of biomolecules, such as cell membrane proteins or peptides, onto the surface of gene delivery vehicles. These coatings mimic the natural cell membrane environment and can help evade immune recognition by resembling the surface properties of natural cells, improve cellular uptake by promoting interactions with specific cell-surface receptors, and enhance payload protection (108). Biomimetic coatings can also facilitate targeted delivery by incorporating ligands that enable specific binding and recognition of target cells (38). For instance, a DNA vaccine was designed for malignant melanoma (MM) therapy in which the genetic cargo was complexed with cell-penetrating peptide-low molecular weight PEI copolymer (CPP-PEI1800). Since mannose receptors are overexpressed on dendritic cells (DCs), the nanocomplex was also mannosylated to enhance DC-targeting. Its penetration efficacy was then tested by treating the naked abdominal skin of BALB/c mice with the Man-PEI1800-CPP/QD nanocomplex suspension before and after applying the MN array. The results showed the strongest fluorescence activity in the MN pretreatment group. The tumor prevention efficiency was also evaluated. BALB/c mice (10 per group) were given Man-PEI1800-CPP/QD nanocomplex three times at weekly intervals and compared to the control group treated with bare DNA. One week following the third vaccination, the mice were subcutaneously challenged with B16 melanoma cells. The size of the tumor was used to gauge its progression, and the survival curve was calculated. The results showed a tumor growth inhibition rate of 48%, with prolonged survival time and a stronger immune response compared to the control group (38).

Layer by layer (LbL) coatings

LbL coatings involve the sequential deposition of multiple layers onto gene delivery vehicles. These layers can consist of polymers such as PEM (89), peptides (38), or other materials. Deposition can be achieved through various techniques, including electrostatic interactions (47, 52), covalent bonding (64), and physical adsorption (47, 79). Each layer is deposited one at a time, resulting in a controlled and precise coating thickness (64). LbL coatings provide a versatile approach for tailoring the physicochemical properties of gene delivery vehicles, enabling enhanced stability, controlled release, and improved payload protection (47, 64). When exploiting this method, usually the MN is precoated by bilayers of protamine-sulphate (PS) or Linear poly (ethylene imine) (LPEI) (64) as the cation and poly(4-styrene-sulphonate) (SPS) as the anion is initially deposited through electrostatic interactions to provide a uniform charge density in the base layer thus making it suitable for the adsorption of the following layers (47, 79). This method was exploited by DeMuth *et al.* to develop a light-responsive DNA vaccine against a model HIV antigen, as explained in detail earlier. After the initial 20 bilayers of PS/SPS, an overlying PEM film composed of Cy5-labelled pDNA encoding luciferase and a transfection agent (PBAE) was deposited by iterative adsorption. One great advantage of this strategy is the ability to store the product at room temperature for up to 28 days before application, thereby eliminating the need for a cold chain and providing easier access (47).

Strategies for enhanced tissue penetration and intracellular delivery of genes using MNs/NNs

Tissue penetration is a critical factor in gene delivery systems, as it determines the efficient delivery of genetic material to the desired target cells within tissues. Smart MNs/NNs offer distinct advantages in terms of tissue penetration due to their small size, sharp tips (94), and precise control over their mechanical properties (104, 109). These properties include needle geometry, which allows minimally invasive penetration; mechanical properties such as stiffness and flexibility, and insertion techniques (53, 110). These key parameters can be investigated and optimized using excised human skin explants (42, 94) or neonatal porcine skin as a model for human skin and post-penetration staining (74). Considering the results of different previous research, the needles should be rigid enough to penetrate tissues (approximately 10 to 20 μm) but flexible enough to withstand deformation and minimize tissue damage without rupturing (111). Control over these properties can be achieved through material selection and fabrication techniques (60, 85).

Intracellular delivery involves the successful delivery of genetic material into the cytoplasm or nucleus of target cells. Surface modifications and coatings play a crucial role in enhancing cellular uptake of smart MNs/NNs in gene delivery (112). By modifying the surface properties of the needles, such as charge, hydrophobicity, or the presence of targeting ligands, the interaction with target cells can be optimized, resulting in improved cellular uptake and intracellular delivery of genetic material (49, 93). Furthermore, NNs have the advantage of delivering cargo straight to the nucleus and escaping endosomal degradation (49, 84).

These modifications can be broadly categorized into charge-based and hydrophobic/hydrophilic modifications, which are summarized in Table 5.

Charge-based modifications

The surface charge of smart MNs/NNs can be modified to enhance the cellular uptake of negatively charged genetic materials, such as DNA or siRNA, through improved interactions with negatively charged cell membranes (93). There are two primary charge-based modifications utilized in gene delivery: positive charge enhancement and surface charge neutralization.

Positive charge enhancement involves introducing cationic moieties onto the surface of gene delivery MNs/NNs, which can interact with negatively charged components on cell membranes, such as sulfated proteoglycans and heparan sulfate (93). This interaction

Table 5. Modification strategies for gene delivery carriers in micro/nanoneedles (MNs/NNs) mediated delivery. These examples highlight how tailoring gene cargo properties enhances stability, transfection efficacy, and therapeutic potential

Modification methods	Modified gene delivery carrier	Type of MMs/NNs	Experimental model *In vitro/in vivo*	Results	Ref.
Charge-based modifications	PLGA-PLL EboDNA modified with negatively charged γPGA to induce electrostatic repulsion	PVA-PVP MNs	HeLa cells for *in vitro* test to assess transfection efficacy BALB/c mice divided into 4 groups for *in vivo* experiment	A significantly stronger transfection rate and higher total antigen specific IgG titers were observed in PLGA-PLL/γPGA-EboDNA group compared with naked pDNA Furthermore, a higher stability rate of 94.3% in NPs compared with 35.4% in naked pDNA and a 3.2 folds higher loading capacity per patch were observed	(54)
Hydrophilic modifications	DNA vaccine encoding the viral proteins of SFTSV / pGFP	Nano-patterned stainless steel MNs / MNs etched with NaOH solution / MNs modified by hydrothermal treatment with a chromium (Cr) precursor	NIH/3T3 fibroblasts as the *in vitro* transfection test BALB/c mice as the *in vivo* immunization test	Significantly decreased surface contact angle in Cr MNs and therefore increased hydrophilicity and loading capacity of pGFP were observed. As a result, an enhanced transfection efficacy and immune response were demonstrated after SFTSV vaccination	(70)
	P53 expression plasmid (p53 DNA)-PEI	PCL MNs pretreated with vacuum plasma deposition to increase hydrophilicity	*In vitro* release profile in phosphate buffer saline and insertion profile in porcine cadaver skin *In vivo* anti-tumor efficacy of PEI-p53 complex in tumor bearing BALB/c mice	Pretreating the PCL MNs significantly decreased solvent contact angle and therefore improved its wettability which resulted in uniformly dispersed droplets of PEI-p53 DNA. The anti-tumor efficacy was improved to be 84.7% compared with naked plasmid DNA group which was 59.3%	(80)
Hydrophobic modifications	Cholesterol-modified housekeeping gene (Gapdh) siRNA to improve hydrophobicity	Silicone MN array	*In vivo* delivery to female C57 mice	Increased cellular uptake and therefore reduced Gadph gene expression up to 66% and prolonged blood circulation after administration of cholesterol-modified Gadph siRNA	(58)

PLGA: Poly(d,l-lactic-co-glycolic acid); PLL: Poly-L-lysine; γPGA: Poly-γ-glutamic acid; PCL: Polycaprolactone; PEI: Polyethylenimine

promotes electrostatic attraction and facilitates the binding and uptake of the gene delivery vectors by the target cells. Positive charge enhancement strategies have demonstrated improved cellular uptake and enhanced intracellular delivery of therapeutic genes compared to negatively charged vectors (93). However, it is important to optimize the balance between positive charge and cytotoxicity, as excessive positive charge can lead to increased toxicity, stronger immune responses, and nonspecific interactions with negatively charged biomolecules (93, 106).

Several key methods are used to achieve positive charge enhancement. One of them is incorporating cationic polymers, such as PEI (36, 104), PLL (54), and PLGA (106) or cationic lipids which contain amino groups that confer a positive charge, enabling efficient binding to negatively charged cell membranes and subsequent internalization as was tested in Ebola DNA vaccine loaded on PLGA-PLL/poly-γ-glutamic acid (γPGA) NPs to induce a stronger immune response with increased stability during storage (54).

Initially, the genetic cargo was loaded onto PLGA-PLL NPs prepared by the nanoprecipitation-solvent evaporation method for enhanced cellular uptake (0.24μg of EboDNA per microgram of NPs). However, despite expectations, the release of EboDNA was slow and inefficient, reaching only 17.6% during the first 10 hr. Therefore, to improve the release rate, negatively charged γPGA was also coated to weaken the bond via electrostatic repulsion, thereby increasing the release rate to 68.9% during the first 10 hr. To test the stability of the complex, gel electrophoresis was performed before and after incubation with DNase, which showed complete protection compared with naked DNA. To assess transfection efficacy, GFP-pDNA was loaded into NPs and delivered to HeLa cells *in vitro*, demonstrating strong transfection compared with the negative naked pDNA, which showed essentially no transfection. Next, PVA-PVP MNs were fabricated using a 2-step molding method to load the NPs into the needle tips. The MNs, which were 700 μm in height, consisted of a first layer of PVA/ PLGA-PLL/γPGA-EboDNA and a backing layer of PVA/PVP and were arranged in 10’10 arrays. The loading capacity of the MNs increased 3.2-fold to 44.7 ±1.5 μg of EboDNA per patch when EboDNA was loaded into NPs rather than in its naked form. Furthermore, the results from the stability test of EboDNA showed major damage due to MN fabrication and reconstitution in naked pDNA, with 46.7% of the naked pDNA transforming from supercoiled to nicked circle form. In contrast, 98.3% of EboDNA complexed with PLGA-PLL/γPGA retained their supercoiled form after reconstitution from MNs, which could be explained by electrostatic interactions. Guided by these results, MN patches were stored at 37 °C for 6 weeks and showed 94.3% stability in DNA conformation, compared with naked DNA, which showed only 35.4% stability. For the *in vivo* test, BALB/c mice were divided into four groups and injected intramuscularly or via MN patch with 18 μg of EboDNA vaccine, either naked or in PLGA-PLL/γPGA complex, four times at four-week intervals. Serum samples were evaluated 2 weeks post the final immunization. Results showed significantly lower total antigen-specific IgG titers in mice treated with naked EboDNA via the MN patch, which is correlated with the

damaged DNA resulting from the MN patch formulation. IgG1 subtype responses were also significantly higher in PLGA-PLL/γPGA NPs compared with the control group. Overall, formulation with PLGA-PLL/γPGA NPs increased HeLa cell transfection and induced a stronger immune response in mice (54).

Hydrophobic/hydrophilic modifications

Lipophilic and hydrophilic modifications are two types of surface modifications used to enhance the performance and functionality of smart MNs/NNs in various applications, including gene delivery. These modifications alter the surface properties of the needles to improve their interaction with lipids (lipophilic) or water (hydrophilic), enabling better stability, biocompatibility, and targeted delivery, enhancing interactions with aqueous environments, improving biocompatibility and enabling better solubility (70, 113). MNs/NNs can be coated with hydrophilic polymers, such as PEG (28, 93), PVA (30), or polyvinylpyrrolidone (PVP) (47). These polymer coatings create a hydrophilic layer on the surface, reducing non-specific interactions with proteins and improving biocompatibility. Hydrophilic nano-coatings such as silica NPs (98) or hydrogels (55), can also be applied to the surface of MNs/NNs. These nano-coatings provide a hydrophilic shell, enhancing colloidal stability, reducing aggregation, and improving compatibility with biological systems.

Lipophilic modifications involve introducing hydrophobic or lipid-like components to the surface of MNs/NNs. These modifications are particularly useful when targeting cell membranes, which are primarily composed of lipids. For instance, lipid Anchors are lipophilic groups, such as lipid tails or cholesterol moieties, that can be conjugated to the surface of MNs/NNs. These lipid anchors enhance the affinity of the needles for lipid bilayers, promoting membrane insertion and cellular uptake (58, 67). The choice between lipophilic and hydrophilic modifications depends on the specific application, target cell type, and desired interactions. Often, a combination of lipophilic and hydrophilic modifications is employed to optimize the performance of smart MNs/NNs, achieving both effective cellular uptake and stability in aqueous environments.

In one study, solid silicone MNs were used to deliver cholesterol-modified housekeeping gene (Gapdh) siRNA to the mouse ear skin. To test its efficacy, a silicone MN array patch (pyramidal with a height of 200 μm) was pressed into the skin, which was pretreated with Cy5-labelled cholesterol siRNA. Results from this test demonstrated a significant dose-dependent reduction of Gapdh gene expression up to 66%, which clearly shows facilitated cellular uptake without the need for a transfection reagent. Furthermore, siRNA accumulation was limited to the treated skin and liver, with the fluorescent signal from the skin being much more intense. This data indicates prolonged blood circulation and targeted accumulation of siRNA as a result of conjugated cholesterol (58).

Tang *et al.* also designed an injectable silicone MN system to locally deliver cholesterol-siRNA targeting the HPV16 E6 oncogene in cervical cancer. The injectable system consisted of a micro-syringe connected to a pipetting needle (304 25G stainless steel needle) with a tube and mounted by three hollow MNs 800 μm in height. E6 gene silencing and SiHa cells growth inhibition were evaluated *in vitro* via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and qRT-PCR, which showed a marked reduction of HPV16 E6 gene expression. Furthermore, as an *in vivo* anti-tumor test, xenograft mouse models of cervical cancer were injected with Cy5-Labeled human HPV16 E6 siRNA and evaluated for tumor suppression and distribution of the cargo using biophotonic imaging analysis and siRNA fluorescence histology. The results following administration not only showed significant suppression of tumor growth but also target-site accumulation in the tumor, unlike the heart, lung, spleen, liver, and kidney at both the 1.5 and 6 hour time points, thus making it a potential alternative to the conventional approaches (88).

Hydrophilic modifications involve introducing hydrophilic or water-attracting components to the surface of MNs/NNs via techniques such as vacuum plasma deposition. Li *et al.* used this system to pretreat PCL MNs, decreasing the solvent contact angle and thereby increasing their wettability. Due to this improvement, the next 2 layers, consisting of p53 DNA and PEI, were uniformly dispersed after being ultrasonically sprayed onto the MN patch (80).

Nano-patterning is another strategy that could be exploited for increasing surface hydrophilicity. Using this method, Jung *et al.* designed nano-patterned stainless steel (SS) MNs to deliver a DNA vaccination against the severe fever with thrombocytopenia syndrome virus (SFTSV). To investigate its efficiency, three groups consisting of the original SS MNs, MNs etched with NaOH solution, and MNs modified by hydrothermal treatment with a chromium (Cr) precursor were compared in various tests. The SEM images and X-ray diffraction (XRD) analysis showed that the hydrothermal treatments formed a chromium-rich oxide layer on the SS with an improved Cr/iron (Fe) ratio, thus resulting in a more fortified, compact, and uniform surface texture. Contact angle, which is correlated with the surface hydrophilicity, was also significantly decreased in Cr MNs, indicating an increased hydrophilicity. Based on these results, to test the coating efficiency and loading capacity, MNs were dip-coated with green fluorescence plasmid DNA (pGFP) 1, 3, 6, 9, and 12 times. The results from UV-visible spectroscopy showed an overall significant increase in loaded pGFP in Cr MNs. This could be explained by the adjacent nano grains in Cr MNs, which serve as proper attachment sites for pGFP. Furthermore, its ability transfect NIH/3T3 fibroblasts was also improved. The cellular immune response in female BALB/c mice was evaluated and found to be slightly enhanced following Cr-etched MN-DNA vaccination. These results indicated a controlled, sustained release of the genetic cargo, along with improved biocompatibility due to nano-patterning (70).

These results were demonstrated in a study in which silicone porous NNs were used to deliver VEGF plasmid DNA to muscle tissue, achieving localized neovascularization for healing and scar tissue remodeling, and potentially improving the success of implant grafting. Initially, porous biodegradable silicone NNs were fabricated and tested in HeLa cells for their loading and transfection capacity. NNs were co-loaded with Cy3-labelled siRNA and a GFP-expressing DNA plasmid and evaluated by assessing GFP expression. The results demonstrated transfection efficacy of 90% and 80% silencing of GADPH expression, which indicated the direct co-delivery of two types of nucleic acids into the cell cytoplasm. For the *in vivo* test, NNs loaded with human VEGF165 plasmid DNA (pVEGF165) were injected into mice and compared with direct injection

of naked plasmid DNA as the control. RT-PCR showed higher expression than the control group, along with a sixfold increase in perfusion, which demonstrated tissue neovascularization resulting from modulated VEGF gene expression following confined intracellular delivery of plasmid DNA (62).

AI and predictive modeling in MN/NN systems

AI has emerged as a promising tool for predicting process outcomes by analyzing input data and is therefore helpful in many fields, including disease prediction and cancer diagnosis. Machine learning (ML) is a branch of AI that employs algorithms to discover patterns in data and use them to make predictions (72). ML is categorized into traditional ML, which requires the scientist's understanding of the data's structure, and deep learning (DL), which utilizes deep artificial neural networks to process large, complex datasets automatically (114). Additionally, AI and DL can be exploited to design and fabricate smart MNs/NNs (51). The dimensions of MNs/NNs which include needle length, tip and base diameter, and inter-needle distance, can significantly impact the penetration performance of MN/NN and patient comfort and can be optimized to minimize pain using the LiveLink interface between COMSOL and MATLAB with incorporation of Bayesian (114). ML has also enabled the prediction of 3D-printed MN/NN features, which is a time and cost-efficient manufacturing. Sarabi *et al.* used ML models and pre-trained networks to develop a predictability code using fabrication parameters such as base diameter, height, and drafting angle of MNs/NNs, etching solution concentration, and etching duration. First, ten different geometric shapes of PLA MNs were designed via computer-aided design (CAD) and fabricated via FDM 3D printing. The MNs were then etched with KOH and pictured to create a dataset of defective/non-defective image samples. Three DL models based on CNNs, including ResNet34, MobileNetV2 and ConvNeXt_Base, were trained for anomaly detection. For this purpose, ConvNeXt_Base demonstrated the best performance with an accuracy of 0.96. To make the model more user-friendly, a graphical user interface (GUI) was designed to allow users with no programming background to utilize the model to predict optimized parameters via a classification tree based on design and etching features, which can greatly improve the efficiency and accuracy of the printing process, leading to faster and more precise manufacturing of MNs with reduced material waste (72). Furthermore, to optimize material selection, Chumpu *et al.* used tapered-cone MNs made from 15 different materials to create a dataset for evaluation of random forest (RF), gradient boosting (Gboost), ANN, GNN, GAT, and PointNet models using three-dimensional stress data at the tips. The results indicated that the best model at predicting the von Mises stress of MNs was GAT, with an MSE of 8.3 ´ 10-5 MPa and assessment time of 7 milliseconds (115).

Additionally, machine learning algorithms can also be used to analyze large datasets and identify the most effective gene delivery strategies for specific diseases and conditions (105). ML can also help with predicting transfection efficacy and cytotoxicity when designing polymers for DNA complexation. For instance, PBAE polymers showed a dramatic variability in transfection depending on the end groups used in the polymer structure. Adding cell type-specific transfection into consideration, we have a great range of pattern possibilities that each should be tested, which is time-consuming and expensive. By employing a random forest regression model, high predictive accuracy regarding the optimized polymer properties was achieved, which reduced screening burden in an experimental setting (105). Therefore, by leveraging AI, researchers can optimize the design and performance of smart MNs/NNs, analyze complex biological processes, and improve the safety and efficacy of gene therapies.

Other advances and recent progress

The field of smart MNs/NNs is rapidly evolving, driven by interdisciplinary collaborations and cutting-edge research. Researchers are developing MNs/NNs with multiple functionalities, such as combining gene delivery with imaging or sensing capabilities, which allow real-time monitoring of gene expression and therapeutic responses. For instance, NNs were used as biosensors in patients with esophageal cancer to track intracellular activity, which can ultimately distinguish cathepsin B (CTSB) positive cancer cells (CTSB+ve) from CTSB negative cells (CTSB-ve). To design the sensor, the CFKK peptide, modified with carboxy tetramethyl rhodamine (TAMRA) as the fluorescent label, was conjugated to porous silicone NNs and used as a CTSB-specific sensing probe. The sensing probe was then tested by transfecting HET-1A (CTSB–ve) and OE33 (CTSB+ve) for its ability to map CTSB activity. As expected, CTSB caused a cleavage in the peptide, triggering the release of the fluorescent label into the cytosol, and resulting in an average 15-fold higher fluorescence signal in OE33 cells. To further test the sensor, the NN array was used on excised tissue samples from patients with esophageal adenocarcinoma. The results revealed three regions: a CTSB+ve area with elevated fluorescence signals, a CTSB-ve, visually healthy region with low fluorescence signals, and a third region consisting of CTSB+ve and CTSB-ve cells adjacent to each other. These results demonstrated a single-cell-level mapping within a single tumor resection specimen, making it a potential platform for cancer diagnostics (116).

The same principle could be useful when treating cancers with gene therapy. Another example is UCNP, which allows the tracking of MN depth penetration and cargo delivery via up-conversion luminescence imaging or optical coherence tomography (OCT). Using this principle, dissolvable HA MNs were fabricated and loaded with silica-coated up-conversion NPs (UCNPs@mSiO2), which provides enlarged signal-to-noise ratios upon NIR irradiation that is correlated with the embedding depth of the NPs. To test the ability of UCNPs to be an indicator of insertion depth, HA MNs were inserted into pork skin, which showed an increase in I_{654}/I_{540} with deeper insertion. To further evaluate the loading capacity of the NPs, a GAPDH molecular beacon (MB) was used as a model cargo, which showed an initial burst release of 75% followed by gradual release over three days. Furthermore, MBs targeting GADPH mRNA were co-administered with CTGF mRNA, which is overexpressed in abnormal tissue scar, to normal dermal fibroblast (NDF) and hypertrophic scar fibroblasts (HSF) cultured in 3D agarose gel. Results demonstrated a 1.6-fold higher signal in HSF cells compared to NDF cells, suggesting that CTGF is a potential biomarker to evaluate and track the gene therapy process in abnormal scars. At last, transforming growth factor-beta type I receptor (TGF-βRI) siRNA was used to reduce CTGF expression by up to 50% in HSF cells

and therefore avoid abnormal scarring (98). Additionally, advances in exploring ligands, antibodies, and aptamers as targeting agents have enabled selective and targeted delivery, while stimuli-responsive polymers offer effective protection, prolonged blood circulation, and controlled release of nucleic acid drugs.

Clinical trials have been conducted to evaluate smart MNs/NNs in humans. These trials focus on safety, efficacy, and pharmacokinetics. Early results show promise in cancer therapy, genetic disorders, and regenerative medicine. For instance, in a 2002 trial, patients received plasmid DNA encoding luciferase and hepatitis B surface antigen via silicone MNs fabricated using the isotropic KOH etch technique. The designed MN array, referred to as micro-enhancer arrays (MEA), was first loaded with naked plasmid DNA encoding firefly luciferase and tested for its ability to transfer genes via the skin barrier. Results showed not only similar, and even greater expression levels compared to IM or ID injections, but also gene transfer with only a single lateral pass. Additionally, MEAs loaded with plasmid DNA encoding hepatitis B surface antigen (HBsAg) were administered to mice. A great population of antigen-specific cytotoxic T cells (CTLs) was detected in both lymph nodes and the spleen, resulting in 100% seroconversion following two immunizations, which is significantly higher than 40% with IM and 50% with IV injection. Furthermore, antibody titers were less variable and considerably higher post-second and third doses compared with conventional injections. These data, along with the minimal discomfort of the MEAs, make this method an attractive platform for future vaccine production (71).

Integrating small-scale robotics into MNs/NNs, particularly micro- and nano-robots, has attracted significant attention due to their potential for precise targeting and access to cell-sized sites. While MNs/NNs ensure secure attachment to target cells and controlled release of genetic cargo, and facilitate efficient uptake of the robot, miniature robots offer precise spatial and temporal control, minimizing systemic side effects and further enhancing therapeutic efficiency (117).

Employing robotics can also be advantageous in the manufacturing process. A MN vaccine printer (MVP) was designed to fabricate dissolvable MN patches loaded with lipid NP (LNP)-encapsulated mRNA COVID-19 vaccines that remain stable at room temperature for 6 months. The MVP exploited a vacuum-based technique in which the PVA:PVP solution was dispensed into the molds, followed by applying vacuum from below for the loading process and on top of the sheets to accelerate drying, creating a pressure gradient that drives the polymer downwards without forming bubbles. This process was combined with a robotic arm for programmable application with microliter precision level and enabling the manufacturing of 100 patches in 48h with minimal user interaction. The designed LNPs containing mRNA encoding firefly luciferase (fLuc) were first tested *in vitro* by transfecting HeLa cells successfully without impairing cell viability. Furthermore, MNs were applied to the footpad of mice for ID delivery of fLuc-mRNA, which showed significantly higher expression compared with IM administration of the same amount of cargo. At last, LNPs encapsulating SARS-CoV-2 mRNA were used to transfect HEK cells *in vitro*. To evaluate immunogenicity, vaccines were also administered to the footpad of C57BL/6 mice and compared with IM injection into the hind limb as the control. Results following a booster 28 days post prime application demonstrated slower expression kinetics from MN administration compared to IM injection, which can be explained by a slower dissolution rate from a solid matrix. However, a robust immune response was observed in both groups within 3 weeks of the booster, which shows that MVP can serve as a new platform for the design of mRNA vaccines for diseases of interest (67).

In addition, centrifugation-induced supergravity was also used in research to temporarily deform the cell membrane and thereby facilitating cellular uptake while achieving precise control of the applied force via NNs (84).

The integration of graphene nanosheets has further enhanced the functionality of MNs/NNs due to their unique properties. The sharpness and strength of graphene allow MNs/NNs to penetrate biological barriers with minimal force, reducing tissue damage and improving patient comfort during gene delivery. Most importantly, graphene nanosheets can be engineered to be responsive to both internal and external stimuli and thereby providing needles with dual stimuli-sensitive properties and biosensing capabilities (73). Additionally, due to their large surface area, graphene nanosheets can hold a significant amount of genetic cargo, enabling the delivery of adequate doses without the need for multiple administrations. Graphene can also be modified to be biocompatible and biodegradable without inducing adverse immune responses. However, the stability of the genetic material on the graphene surface and the degradation rate must be carefully considered (73).

Challenges and future perspectives

Smart MNs/NNs hold immense promise in gene delivery; there are challenges that need to be overcome if successful integration in clinical practice is the goal.

Manufacturing

Material selection and geometric structure can greatly influence the efficacy of MNs/NNs (60, 61). One of the primary concerns is the potential for tissue damage and the associated pain, which can lead to patient discomfort and reluctance to undergo treatment. The fabrication of these needles requires precise engineering to ensure they can penetrate cellular barriers without causing undue stress or damage (84, 110, 111). Data from a clinical trial demonstrated an increase in trans-epidermal water loss (TEWL) following the administration of MNs at projection heights of 100 μm or greater and little to no increase with 50-μm MNs, which clearly shows the importance of optimizing the MN array's dimensions and geometry (71). Additionally, geometry can also affect dissolution rate. For instance, in one study, both conical and pyramidal MNs were used, and although both are potentially viable geometries, pyramidal MNs not only outperformed conical MNs in terms of peak force and stiffness but also showed a much higher volume dissolution (67). However, achieving optimal dimensions could be costly as well (60, 67). Furthermore, the integration of smart materials into needle design adds complexity to the manufacturing process, and most fabrication techniques require not only specialized equipment but also trained and professional personnel and are often time-consuming (67, 80, 84). Additionally, when designing MNs/NNs with a matrix for cargo loading, it is important to ensure that a large portion of the cargo is present in the needle tips and the baseplate. However, more often than not, the payload is

lost to the MNs/NNs' sidewalls, indicating a loss of genetic material during manufacturing due to the Marangoni effect (67, 85). This loading capacity limitation can be overcome by concentrating the cargo using centrifugal filtration or a two-step tip loading process. Straeten *et al.* exploited this method by first loading and drying vaccine ink with the minimum amount of polymer followed by a polymer-only layer to form the backing (67). Therefore, developing scalable and cost-effective fabrication methods for MNs/NNs is essential for clinical translation (65).

One challenge in MN/NN design is poor tissue adhesion when long-term gene delivery is required, particularly when thermos-responsive materials are incorporated (91). Conventional manufacturing techniques often yield needles with smooth sides and also have limitations in the design of microfeatures on MNs/NNs. 4D printing, which involves programmed shape deformation after 3D printing, has emerged as a recent trend aimed at further enhancing needle geometry design (66). An MN system with bio-inspired backward-facing curved barbs was designed using this technique, which resulted in tissue adhesion 18 times stronger than that of conventional MNs. 3D MN arrays were fabricated via projection micro-stereolithography (PμSL) and to achieve the backward-facing barbs, the curing process was manipulated. Light initiates polymerization from the top down, creating a density gradient. The top solidifies more than the bottom, leaving some unlinked monomers. These monomers are washed away with ethanol, causing the lower part of the barbs to contract and bend downwards as they dry. A final UV exposure ensures the barbs retain their curved shape (66). The same strategy can be employed in gene delivery systems. Another solution is separable needle tips, which can form a reservoir in the skin for prolonged gene delivery. This strategy can be achieved via integrating thermosensitive material, as was explained earlier (95).

Another point worth mentioning is the advantages of dissolvable MNs/NNs in comparison to coated ones. Although coating solid MNs/NNs can result in a more consistent distribution and a more stable cargo state, it has the limitation of a small DNA loading capacity, which dissolvable MNs/NNs overcome (109). Additionally, coated MNs/NNs can deliver cargo only from the inserted region of the tip, whereas incomplete insertion of dissolvable MNs/NNs is less limiting (59). This is because the dissolution of MNs/NNs creates a transient pore that facilitates the delivery of cargo from the portion of the needle that is un-inserted (85). Furthermore, dissolvable MNs/NNs avoid the generation of biohazardous sharp wastes, which is an advantage both for the human body and the environment (104).

Biocompatibility and safety

When MNs/NNs interact with biological systems, they can trigger immune responses. Ensuring that MNs/NNs do not provoke adverse reactions or cause inflammation is crucial (62, 109). Different tissues have distinct mechanical and chemical properties, and MNs/NNs must be compatible with those properties and should not disrupt their normal function (62, 118). Chronic exposure to MNs/NNs may lead to unforeseen consequences; therefore, assessing the long-term impact of MNs/NNs on tissues and organs is essential (60, 111). In terms of toxicity, MNs/NNs may release materials during degradation or breakdown. Ensuring that these materials are non-toxic is critical (119). Surface modifications, such as coating MNs/NNs with biocompatible materials, can reduce toxicity and improve interactions with biological components, and thereby enhance their safety profile (86). For instance, as mentioned before, Jung *et al.* designed nano-patterned DNA vaccines against severe fever with SFTSV. The nanopatterning not only improved the loading capacity of the MNs by increasing surface hydrophilicity but also enhanced cell viability. This modification made the vaccine more cyto-compatible with fibroblasts and thus improved DNA transfection efficiency (70). Additionally, NNs have the advantage of targeting only a few superficial layers of cells and are therefore less invasive compared to MNs. Rigorous preclinical testing is necessary to evaluate biocompatibility and safety. Since animal models can provide insights into MNs/NN behavior *in vivo*, they are a great candidate for these tests (62). Furthermore, complexing genetic material, especially to lipid particles, can give them a higher chance of toxicity, which should be tempered with the reduction of incubation time (36, 84).

Stability of genetic cargo

The stability of the genetic material is paramount to ensure it remains viable and effective until the point of delivery to the target cells. The interaction between the genetic material and the needle matrix can affect the long-term stability (63, 120). The needle's composition must not react with the genetic cargo in a way that could lead to its degradation or denaturation, as DNA can be degraded following incorporation into PVP since it can form hydrogen bonds with DNA (85). This can be particularly challenging, especially when working with LNPs, since their chemical and colloidal stability can be altered during drying. To prevent destabilization and aggregation of the LNPs, the formulation must contain at least 50% PVA. However, because PVA is both viscous and hygroscopic, with a slow drying rate, it can be modified with PVP at a PVA: PVP 1:1 mass ratio (67).

The conditions under which the needles are stored can also impact the stability of the genetic cargo. Factors such as temperature, humidity, and light exposure need to be carefully controlled to prevent degradation (67, 87). The needle's composition can also be modified to be more resistant to these factors (46, 47, 67). For instance, research shows that adding 1.5% w/v disaccharide can have a stabilizing effect on plasmid DNA by preserving its tertiary physical structure (74). Complexing the DNA cargo itself can also have a positive effect on its durability in storage (85, 121). However, data from a study by Chong *et al.* also indicate that the structure of siRNA lipoplex could potentially be altered during the drying and manufacturing process, which results in a loss of functionality that should be considered. Unlike cells treated with MNs that were coated with siRNA and then complexed with Lipofectamine, cells treated with MNs complexed before coating and recovery showed no reduction in mRNA synthesis (59). Therefore, Accell-modified "self-delivery" siRNA was exploited as a better alternative, which can enter cells passively without the need for being complexed with lipid transfection reagents and is also modified for enhanced stability, resulting in a significant reduction in CD44 mRNA expression in HaCaT cells (59, 81).

Regarding the same matter, as mentioned earlier, naked mRNA's transfection efficacy is the same as when it's encapsulated within NPs. However, the main challenge is its

stability in high concentrations of polymer solutions, such as PVP, when kept at room temperature for an extended period of time. RNase is enriched in high concentrations of polymer and could result in mRNA degradation. Therefore, the concentration of polymer should be carefully adjusted (86). In addition, its solubility in PVP is limited. When mRNA was loaded in PVP at concentrations greater than 5μg/μL, a transparent gel-like phase appeared, and nearly all of the mRNA payload was lost to it due to its thermodynamically favorable properties. The gel phase, however, could not flow into MN molds and resulted in a waste of genetic material (86).

Another issue is the potential for the genetic material to aggregate or agglomerate within the needle when high concentrations are involved (59, 80, 95) or when materials such as CMC, which can affect the delivery efficiency and the dosage accuracy, are used (75). This issue is more noticeable when the genetic material is complexed, especially with PEI, which can aggregate with genes and inhibit their release profile down to as low as 20% (80). The design of the needle and the materials exploited in it play a role in the stability of the genetic cargo. The needle must be engineered to allow for the stable encapsulation of the genetic material and its subsequent release at the target site without loss of function (59, 67).

Clinical translation and regulatory considerations

Recently, clinical trials have begun to assess the translational potential of smart MNs/NNs for gene delivery. For instance, a Phase I clinical trial (NCT04492943) is evaluating the safety, tolerability, and immunogenicity of silicon NN arrays for intradermal delivery of mRNA vaccines in healthy adult volunteers. This study seeks to determine optimal dosing regimens, local skin responses, and preliminary immune activation profiles in comparison to conventional injection methods. Another early-phase study (NCT05853107) examines titanium dioxide nanowire arrays as a platform for targeted retinal gene therapy in patients with inherited retinal degeneration. This trial evaluates the feasibility of minimally invasive subretinal delivery, monitoring outcomes such as gene expression efficiency, preservation of retinal function, and adverse events. While these pioneering trials represent important steps toward clinical validation, most gene delivery applications using smart MN/NN platforms remain in preclinical or early-phase development, highlighting the need for further rigorous clinical investigation (50). The absence of robust, long-term clinical data on efficacy, safety, and patient outcomes hinders regulatory approval and broad adoption. Although short-term biocompatibility and cytotoxicity studies yield encouraging results, data on long-term safety remain limited. Most toxicity assessments are confined to *in vitro* or short-term *in vivo* models, with few investigations addressing chronic exposure, biodegradation, or systemic effects. While the use of biodegradable polymers such as PLA, PLGA, and chitosan is promising, a comprehensive evaluation of degradation products and their potential toxicity is necessary (122).

Conclusion

Smart MN/NN platforms have emerged as transformative tools in gene delivery, providing minimally invasive, targeted, and patient-friendly alternatives to conventional systemic and viral methods (42, 43). These platforms have shown considerable promise in preclinical and early clinical studies for delivering a range of genetic payloads, including DNA, mRNA, siRNA, and CRISPR components (109). The integration of functionalities such as stimuli-responsiveness (pH, near-infrared, and enzyme) (48, 52), hybridization with nanocarriers (92), and AI-driven design has further enhanced the therapeutic potential of these systems (72, 115). However, the progression from laboratory innovation to clinical application continues to encounter significant challenges.

The literature consistently highlights the predominance of proof-of-concept and preclinical studies, with relatively few platforms advancing to human trials. Moreover, manufacturing scalability continues to present a significant challenge. Techniques including photolithography, micro-molding, and three-dimensional printing provide high precision but are frequently constrained by limited throughput, high costs, and variable reproducibility (123). The shift from laboratory-scale prototypes to Good Manufacturing Practice (GMP)-compliant production is further complicated by material selection and process optimization (124).

In summary, smart MN/NN platforms for gene delivery represent a leading advancement in precision medicine, enabling highly targeted, minimally invasive, and patient-centered therapeutic strategies. Despite their transformative potential, widespread clinical adoption is limited by ongoing challenges in clinical translation, comprehensive long-term safety assessment, and scalable manufacturing. Addressing these barriers will require coordinated efforts among scientific, engineering, and regulatory communities, as well as sustained investment and a commitment to equitable, patient-focused innovation. Continued progress in overcoming these limitations will position smart MN/NN gene delivery technologies to play a central role in the advancement of gene therapy and the evolution of personalized medicine.

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Authors' Contributions

M H was responsible for conceptualization, methodology, and supervision of the study. M F performed literature search and wrote the original draft. G K assisted in writing the original draft and designing the tables. Z S and F M conducted critical review and approved the final version. All authors had a role in reviewing, editing, and approving the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

The authors declare that AI tools were used solely for grammatical correction and sentence rephrasing during manuscript preparation. All scientific content, concepts, and interpretations were produced and validated entirely by the authors without AI utilization.

References

1. Qie B, Tuo J, Chen F, Ding H, Lyu L. Gene therapy for genetic diseases: Challenges and future directions. MedComm 2025; 6: e70091.
2. Henderson ML, Zieba JK, Li X, Campbell DB, Williams MR, Vogt DL, *et al.* Gene therapy for genetic syndromes: Understanding the current state to guide future care. BioTech 2024; 13: 1.
3. Shahgordi S, Oroojalian F, Hashemi E, Hashemi M. Recent advances in development of nano-carriers for immunogene therapy in various complex disorders. Iran J Basic Med Sci 2022; 25:134-147.
4. Abas MDM, Asri MFM, Yusafawi NAS, Rosman NAZ, Baharudin NAZ, Taher M, *et al.* Advancements of gene therapy in cancer treatment: A comprehensive review. Pathol Res Pract 2024; 261: 155509.
5. Belete TM. The current status of gene therapy for the treatment of cancer. Biologics 2021: 67-77.
6. Azimifar MA, Hashemi M, Babaei N, Salmasi Z, Doosti A. Interleukin gene delivery for cancer gene therapy: *In vitro* and *in vivo* studies. Iran J Basic Med Sci 2023; 26: 128-138
7. Wu Y, Zhang H. Treatment of infectious diseases by *in vivo* gene editing. Natl Sci Open 2023; 2: 20220061.
8. Danaeifar M. Recent advances in gene therapy: Genetic bullets to the root of the problem. Clin Exp Med 2023; 23: 1107-1121.
9. Singh D, Singh S, Tandon N, Thakur A, Bedi N. Emerging trends in nanomedicine: The role of RNAi-based therapies and onpattro's clinical journey. Iran J Basic Med Sci 2026; 29: 812-822.
10. Zinder ND, Lederberg J. Genetic exchange in Salmonella. J Bacteriol 1952; 64: 679-699.
11. Temin HM. Mixed infection with two types of Rous sarcoma virus. Virol J 1961; 13: 158-163.
12. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, *et al.* T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. Science 1995; 270: 475-480.
13. Wirth T PN, Ylä-Herttuala S. History of gene therapy. Gene 2013;525:162-169.
14. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet 2002; 3: 737-747.
15. Alhasso B, Shareef A, Baldaniya L, Oweis R, Jyothi SR, Singh U, *et al.* CRISPR/Cas9 in colorectal cancer: Revolutionizing precision oncology through genome editing and targeted therapeutics. Iran J Basic Med Sci 2025; 28: 1279-1300.
16. Lotfi M, Morshedi Rad D, Mashhadi SS, Ashouri A, Mojarrad M, Mozaffari-Jovin S, *et al.* Recent advances in CRISPR/Cas9 delivery approaches for therapeutic gene editing of stem cells. Stem Cell Rev Rep 2023; 19: 2576-2596.
17. Khademi Z, Ramezani M, Alibolandi M, Zirak MR, Salmasi Z, Abnous K, *et al.* A novel dual-targeting delivery system for specific delivery of CRISPR/Cas9 using hyaluronic acid, chitosan and AS1411. Carbohydr Polym 2022; 292: 119691.
18. Miller JC, Holmes MC, Wang J, Guschin DY, Lee Y-L, Rupniewski I, *et al.* An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol 2007; 25: 778-785.
19. Chen X. Current and future technological advances in transdermal gene delivery. Adv Drug Deliv Rev 2018; 127: 85-105.
20. Escoffre J-M, Teissié J, Rols M-P. Gene transfer: How can the biological barriers be overcome? J Membr Biol 2010; 236: 61-74.
21. Sabatino DE, Bushman FD, Chandler RJ, Crystal RG, Davidson BL, Dolmetsch R, *et al.* Evaluating the state of the science for adeno-associated virus integration: An integrated perspective. Mol Ther 2022; 30: 2646-2663.
22. Barquinero J, Eixarch H, Perez-Melgosa M. Retroviral vectors: New applications for an old tool. Gene Ther 2004; 11: S3-S9.
23. Cockrell AS, Kafri T. Gene delivery by lentivirus vectors. Mol Biotechnol 2007; 36: 184-204.
24. Robbins PD, Ghivizzani SC. Viral vectors for gene therapy. Pharmacol Ther 1998; 80: 35-47.
25. Yang Y, Su Q, Wilson JM. Role of viral antigens in destructive cellular immune responses to adenovirus vector-transduced cells in mouse lungs. J Virol 1996; 70: 7209-7212.
26. Salmasi Z, Hashemi M, Mahdipour E, Nourani H, Abnous K, Ramezani M. Mesenchymal stem cells engineered by modified polyethylenimine polymer for targeted cancer gene therapy, *in vitro* and *in vivo*. Biotechnol Prog 2020; 36: e3025.
27. Baum C, Kustikova O, Modlich U, Li Z, Fehse B. Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors. Hum Gene Ther 2006; 17: 253-263.
28. Haigh O, Depelsenaire AC, Meliga SC, Yukiko SR, McMillan NA, Frazer IH, *et al.* CXCL1 gene silencing in skin using liposome-encapsulated siRNA delivered by microprojection array. J Control Release 2014; 194: 148-156.
29. Salmasi Z, Mokhtarzadeh A, Hashemi M, Ebrahimian M, Farzad SA, Parhiz H, *et al.* Effective and safe *in vivo* gene delivery based on polyglutamic acid complexes with heterocyclic amine modified-polyethylenimine. Colloids Surf B Biointerfaces 2018; 172: 790-796.
30. Cole G, Ali AA, McCrudden CM, McBride JW, McCaffrey J, Robson T, *et al.* DNA vaccination for cervical cancer: Strategic optimisation of RALA mediated gene delivery from a biodegradable microneedle system. Eur J Pharm Biopharm 2018; 127: 288-297.
31. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy-an overview. J Clin Diagn Res 2015; 9: GE01.
32. Bråve A, Gudmundsdotter L, Sandström E, Haller BK, Hallengärd D, Maltais AK, *et al.* Biodistribution, persistence and lack of integration of a multigene HIV vaccine delivered by needle-free intradermal injection and electroporation. Vaccine 2010; 28: 8203-8209.
33. Wells JM, Li LH, Sen A, Jahreis GP, Hui SW. Electroporation-enhanced gene delivery in mammary tumors. Gene Ther 2000; 7: 541-547.
34. Stein U, Walther W, Stege A, Kaszubiak A, Fichtner I, Lage H. Complete *in vivo* reversal of the multidrug resistance phenotype by jet-injection of anti-MDR1 short hairpin RNA-encoding plasmid DNA. Mol Ther 2008; 16: 178-186.
35. Lin MT, Wang F, Uitto J, Yoon K. Differential expression of tissue-specific promoters by gene gun. Br J Dermatol 2001; 144: 34-39.
36. Pamornpathomkul B, Wongkajornsilp A, Laiwattanapaisal W, Rojanarata T, Opanasopit P, Ngawhirunpat T. A combined approach of hollow microneedles and nanocarriers for skin immunization with plasmid DNA encoding ovalbumin. Int J Nanomed 2017; 12: 885-898.
37. Mehier-Humbert S, Guy RH. Physical methods for gene transfer: Improving the kinetics of gene delivery into cells. Adv. Drug Deliv. Rev. 2005; 57: 733-753.
38. Hu Y, Xu B, Xu J, Shou D, Liu E, Gao J, *et al.* Microneedle-assisted dendritic cell-targeted nanoparticles for transcutaneous DNA immunization. Polym Chem 2015; 6: 373-379.
39. Shin CI, Jeong SD, Rejinold NS, Kim Y-C. Microneedles for vaccine delivery: Challenges and future perspectives. Ther Deliv 2017; 8: 447-460.
40. Phoka T, Thanuthanakhun N, Visitchanakun P, Dueanphen N, Wanichwecharungruang N, Leelahavanichkul A, *et al.* Detachable-dissolvable-microneedle as a potent subunit vaccine delivery device that requires no cold-chain. Vaccine: X 2023; 15: 100398.
41. Saeedi M, Morteza-Semnani K, Akbari J, Hajheydari Z, Goodarzi A, Rostamkalaei SS, *et al.* Green formulation of spironolactone loaded chitosan-coated nano lipid carrier for treatment of acne vulgaris: A randomized double-blind clinical trial. Adv Pharm Bull 2023; 14: 161.
42. Chabri F, Bouris K, Jones T, Barrow D, Hann A, Allender C, *et al.* Microfabricated silicon microneedles for nonviral cutaneous gene delivery. Br J Dermatol 2004; 150: 869-877.
43. Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: A novel approach to transdermal drug delivery. J Pharm Sci 1998; 87: 922-925.
44. Donnelly RF, Moffatt K, Alkilani AZ, Vicente-Pérez EM, Barry J, McCrudden MT, *et al.* Hydrogel-forming microneedle arrays can be effectively inserted in skin by self-application: A pilot study

centred on pharmacist intervention and a patient information leaflet. Pharm Res 2014; 31: 1989-1999.
45. Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, Allen MG, *et al.* Lack of pain associated with microfabricated microneedles. Anesth Analg 2001; 92: 502-504.
46. Ali AA, McCrudden CM, McCaffrey J, McBride JW, Cole G, Dunne NJ, *et al.* DNA vaccination for cervical cancer; a novel technology platform of RALA mediated gene delivery via polymeric microneedles. Nanomed Nanotechnol Biol Med 2017; 13: 921-932.
47. DeMuth PC, Min Y, Huang B, Kramer JA, Miller AD, Barouch DH, *et al.* Polymer multilayer tattooing for enhanced DNA vaccination. Nat Mater 2013; 12: 367-376.
48. Duong HTT, Yin Y, Thambi T, Nguyen TL, Phan VG, Lee MS, *et al.* Smart vaccine delivery based on microneedle arrays decorated with ultra-pH-responsive copolymers for cancer immunotherapy. Biomater 2018; 185: 13-24.
49. Liu B, Yi X, Zheng Y, Yuan Z, Yang J, Yang J, *et al.* A Review of Nano/Micro/Milli Needles Fabrications for Biomedical Engineering. Chin J Mech Eng 2022; 35: 106.
50. Akbuğa FJ, Arpa MD, Şalva E. Nanoneedle-Based Transdermal Gene Delivery: A Minimally Invasive Strategy for Gene Therapy. Int. J. Mol. Sci. 2025; 26: 6235.
51. Bagde A, Dev S, Sriram LMK, Spencer SD, Kalvala A, Nathani A, *et al.* Biphasic burst and sustained transdermal delivery *in vivo* using an AI-optimized 3D-printed MN patch. Int J Pharm 2023: 122647.
52. Duong HTT, Kim NW, Thambi T, Phan VG, Lee MS, Yin Y, *et al.* Microneedle arrays coated with charge reversal pH-sensitive copolymers improve antigen presenting cells-homing DNA vaccine delivery and immune responses. J Control Release 2018; 269: 225-234.
53. Xu Q, Li X, Zhang P, Wang Y. Rapidly dissolving microneedle patch for synergistic gene and photothermal therapy of subcutaneous tumor. J Mater Chem B 2020; 8: 4331-4339.
54. Yang HW, Ye L, Guo XD, Yang C, Compans RW, Prausnitz MR. Ebola vaccination using a DNA vaccine coated on PLGA-PLL/γPGA nanoparticles administered using a microneedle patch. Adv Healthc Mater2017; 6: 1600750.
55. Kim M, Jung B, Park J-H. Hydrogel swelling as a trigger to release biodegradable polymer microneedles in skin. Biomater 2012; 33: 668-678.
56. Xu C, Wu F, Duan Z, Rajbanshi B, Qi Y, Qin J, *et al.* Microneedle-aided nanotherapeutics delivery and nanosensor intervention in advanced tissue regeneration. J Nanobiotechnology 2025; 23: 330.
57. Liu G, Deng Y, Song Y, Sui Y, Cen J, Shao Z, *et al.* Transdermal delivery of adipocyte phospholipase A2 siRNA using microneedles to treat thyroid associated ophthalmopathy-related proptosis. Cell Transplant 2021; 30: 9636897211010633.
58. Deng Y, Chen J, Zhao Y, Yan X, Zhang L, Choy K, *et al.* Transdermal Delivery of siRNA through Microneedle Array. Sci Rep 2016; 6: 21422.
59. Chong RHE, Gonzalez-Gonzalez E, Lara MF, Speaker TJ, Contag CH, Kaspar RL, *et al.* Gene silencing following siRNA delivery to skin via coated steel microneedles: *In vitro* and *in vivo* proof-of-concept. J Control Release 2013; 166: 211-219.
60. Seong H, Higgins SG, Penders J, Armstrong JPK, Crowder SW, Moore AC, *et al.* Size-tunable nanoneedle arrays for influencing stem cell morphology, gene expression, and nuclear membrane curvature. ACS Nano 2020; 14: 5371-5381.
61. He G, Chen H-J, Liu D, Feng Y, Yang C, Hang T, *et al.* Fabrication of various structures of nanostraw arrays and their applications in gene delivery. Adv Mater Interfaces 2018; 5: 1701535.
62. Chiappini C, De Rosa E, Martinez JO, Liu X, Steele J, Stevens MM, *et al.* Biodegradable silicon nanoneedles delivering nucleic acids intracellularly induce localized *in vivo* neovascularization. Nat Mater 2015; 14: 532-539.
63. Luo X, Yang L, Cui Y. Microneedles: Materials, fabrication, and biomedical applications. Biomed Microdevices 2023; 25: 20.
64. Saurer EM, Flessner RM, Sullivan SP, Prausnitz MR, Lynn DM. Layer-by-layer assembly of DNA-and protein-containing films on microneedles for drug delivery to the skin. Biomacromolecules 2010; 11: 3136-3143.
65. Lutton REM, Larrañeta E, Kearney M-C, Boyd P, Woolfson AD, Donnelly RF. A novel scalable manufacturing process for the production of hydrogel-forming microneedle arrays. Int J Pharm 2015; 494: 417-429.
66. Han D, Morde R, Mariani S, La Mattina A, Vignali E, Yang C, *et al.* 4D printing of a bioinspired microneedle array with backward-facing barbs for enhanced tissue adhesion. Adv Funct Mater 2020; 30: 1909197.
67. Vander Straeten A, Sarmadi M, Daristotle JL, Kanelli M, Tostanoski LH, Collins J, *et al.* A microneedle vaccine printer for thermostable COVID-19 mRNA vaccines. Nat Biotechnol 2024; 42: 510-517.
68. Lee K, Kim JD, Lee CY, Her S, Jung H. A high-capacity, hybrid electro-microneedle for *in-situ* cutaneous gene transfer. Biomater 2011; 32: 7705-7710.
69. Li Y, Zhang H, Yang R, Laffitte Y, Schmill U, Hu W, *et al.* Fabrication of sharp silicon hollow microneedles by deep-reactive ion etching towards minimally invasive diagnostics. Microsyst Nanoeng 2019; 5: 41.
70. Jung D, Rejinold NS, Kwak JE, Park SH, Kim YC. Nano-patterning of a stainless steel microneedle surface to improve the dip-coating efficiency of a DNA vaccine and its immune response. Colloids Surf B Biointerfaces 2017; 159: 54-61.
71. Mikszta JA, Alarcon JB, Brittingham JM, Sutter DE, Pettis RJ, Harvey NG. Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. Nat Med 2002; 8: 415-419.
72. Rezapour Sarabi M, Alseed MM, Karagoz AA, Tasoglu S. Machine learning-enabled prediction of 3D-printed microneedle features. Biosensors 2022; 12: 491.
73. Jin Q, Chen HJ, Li X, Huang X, Wu Q, He G, *et al.* Reduced graphene oxide nanohybrid-assembled microneedles as mini-invasive electrodes for real-time transdermal biosensing. Small 2019; 15: e1804298.
74. Pearton M, Saller V, Coulman SA, Gateley C, Anstey AV, Zarnitsyn V, *et al.* Microneedle delivery of plasmid DNA to living human skin: Formulation coating, skin insertion and gene expression. J Control Release 2012; 160: 561-569.
75. Kim Y-C, Song J-M, Lipatov AS, Choi S-O, Lee JW, Donis RO, *et al.* Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch. Eur J Pharm Biopharm 2012; 81: 239-247.
76. Raju L, Hiremath SS. A state-of-the-art review on micro electro-discharge machining. Procedia Technology 2016; 25: 1281-1288.
77. García-López E, Siller HR, Rodríguez CA. Study of the fabrication of AISI 316L microneedle arrays. Procedia Manuf 2018; 26: 117-124.
78. Azizi Machekposhti S, Khanna S, Shukla S, Narayan R. Microneedle fabrication methods and applications. MRS Commun 2023; 13: 212-224.
79. DeMuth PC, Su X, Samuel RE, Hammond PT, Irvine DJ. Nano-layered microneedles for transcutaneous delivery of polymer nanoparticles and plasmid DNA. Adv Mater 2010; 22: 4851-4856.
80. Li X, Xu Q, Wang J, Zhang P, Wang Y, Ji J. A gene-coated microneedle patch based on industrialized ultrasonic spraying technology with a polycation vector to improve antitumor efficacy. J Mater Chem B 2021; 9: 5528-5536.
81. Gonzalez-Gonzalez E, Speaker TJ, Hickerson RP, Spitler R, Flores MA, Leake D, *et al.* Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD). Mol Ther 2010; 18: 1667-1674.
82. Biswas A, Bayer IS, Biris AS, Wang T, Dervishi E, Faupel F. Advances in top–down and bottom–up surface nanofabrication: Techniques, applications & future prospects. Adv Colloid Interface Sci 2012; 170: 2-27.
83. Golshadi M, Wright LK, Dickerson IM, Schrlau MG. High-

Efficiency Gene Transfection of Cells through Carbon Nanotube Arrays. Small 2016; 12: 3014-3020.
84. Wang Y, Yang Y, Yan L, Kwok SY, Li W, Wang Z, *et al.* Poking cells for efficient vector-free intracellular delivery. Nat Commun 2014; 5: 4466.
85. Cole G, McCaffrey J, Ali AA, McBride JW, McCrudden CM, Vincente-Perez EM, *et al.* Dissolving microneedles for DNA vaccination: Improving functionality via polymer characterization and RALA complexation. Hum Vaccin Immunother2017; 13: 50-62.
86. Koh KJ, Liu Y, Lim SH, Loh XJ, Kang L, Lim CY, *et al.* Formulation, characterization and evaluation of mRNA-loaded dissolvable polymeric microneedles (RNApatch). Sci Rep 2018; 8: 11842.
87. Noh I, Lee K, Rhee YS. Microneedle systems for delivering nucleic acid drugs. J Pharm Investig 2022; 52: 273-292.
88. Tang T, Deng Y, Chen J, Zhao Y, Yue R, Choy KW, *et al.* Local administration of siRNA through microneedle: optimization, bio-distribution, tumor suppression and toxicity. Sci Rep 2016; 6: 30430.
89. Li X, Xu Q, Zhang P, Zhao X, Wang Y. Cutaneous microenvironment responsive microneedle patch for rapid gene release to treat subdermal tumor. J Control Release 2019; 314: 72-80.
90. Makvandi P, Jamaledin R, Chen G, Baghbantaraghdari Z, Zare EN, Di Natale C, *et al.* Stimuli-responsive transdermal microneedle patches. Mater Today (Kidlington) 2021; 47: 206-222.
91. Liu X, Song H, Sun T, Wang H. Responsive Microneedles as a New Platform for Precision Immunotherapy. Pharmaceutics 2023; 15:1407.
92. Karimi M, Ghasemi A, Zangabad PS, Rahighi R, Basri SMM, Mirshekari H, *et al.* Smart micro/nanoparticles in stimulus-responsive drug/gene delivery systems. Chem Soc Rev 2016; 45: 1457-1501.
93. van den Berg JH, Oosterhuis K, Hennink WE, Storm G, van der Aa LJ, Engbersen JF, *et al.* Shielding the cationic charge of nanoparticle-formulated dermal DNA vaccines is essential for antigen expression and immunogenicity. J Control Release 2010; 141: 234-240.
94. Pearton M, Allender C, Brain K, Anstey A, Gateley C, Wilke N, *et al.* Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations. Pharm Res 2008; 25: 407-416.
95. Yin Y, Su W, Zhang J, Huang W, Li X, Ma H, *et al.* Separable microneedle patch to protect and deliver DNA nanovaccines against COVID-19. ACS Nano 2021; 15: 14347-14359.
96.Wei Z, Zheng S, Wang R, Bu X, Ma H, Wu Y, *et al.* A flexible microneedle array as low-voltage electroporation electrodes for *in vivo* DNA and siRNA delivery. Lab on a Chip 2014; 14: 4093-4102.
97. Hisazumi J, Kobayashi N, Nishikawa M, Takakura Y. Significant role of liver sinusoidal endothelial cells in hepatic uptake and degradation of naked plasmid DNA after intravenous injection. Pharm Res 2004; 21: 1223-1228.
98. Wang M, Han Y, Yu X, Liang L, Chang H, Yeo DC, *et al.* Upconversion nanoparticle powered microneedle patches for transdermal delivery of siRNA. Adv Healthc Mater 2020; 9: 1900635.
99. Piotrowski-Daspit AS, Kauffman AC, Bracaglia LG, Saltzman WM. Polymeric vehicles for nucleic acid delivery. Adv Drug Deliv Rev 2020; 156: 119-132.
100. Blömer U, Naldini L, Verma IM, Trono D, Gage FH. Applications of gene therapy to the CNS. Hum Mol Genet 1996; 5: 1397-1404.
101. Shi H, Xue T, Yang Y, Jiang C, Huang S, Yang Q, *et al.* Microneedle-mediated gene delivery for the treatment of ischemic myocardial disease. Sci Adv 2020; 6: eaaz3621.
102. Dinh L, Mahon L, Yan B. Nano-encapsulation and conjugation applied in the development of lipid nanoparticles delivering nucleic acid materials to enable gene therapies. Appl Nano 2024; 5: 143-161.
103. Liang X, Zhang J, Ou H, Chen J, Mitragotri S, Chen M. Skin delivery of siRNA using sponge spicules in combination with cationic flexible liposomes. Mol Ther Nucleic Acids 2020; 20: 639-648.
104. Pan J, Ruan W, Qin M, Long Y, Wan T, Yu K, *et al.* Intradermal delivery of STAT3 siRNA to treat melanoma via dissolving microneedles. Sci Rep 2018; 8: 1117.
105. Gong D, Ben-Akiva E, Singh A, Yamagata H, Est-Witte S, Shade JK, *et al.* Machine learning guided structure function predictions enable in silico nanoparticle screening for polymeric gene delivery. Acta Biomater 2022; 154: 349-358.
106. Kumar A, Wonganan P, Sandoval MA, Li X, Zhu S, Cui Z. Microneedle-mediated transcutaneous immunization with plasmid DNA coated on cationic PLGA nanoparticles. J Control Release 2012; 163: 230-239.
107. Ingrole RSJ, Gill HS. Microneedle coating methods: A review with a perspective. J Pharmacol Exp Ther 2019; 370: 555-569.
108. Briffault E, Garcia-Garcia P, Martinez-Borrajo R, Evora C, Delgado A, Diaz-Rodriguez P. Harnessing extracellular vesicle membrane for gene therapy: EVs-biomimetic nanoparticles. Colloids Surf B Biointerfaces 2024; 239: 113951.
109. Chen W, Li H, Shi D, Liu Z, Yuan W. Microneedles as a delivery system for gene therapy. Front Pharmacol 2016; 7: 137.
110. Zhang X, Zhou C, Chen T, Jiang Z, Lu C, Wu C, *et al.* State-of-the-art strategies to enhance the mechanical properties of microneedles. Int J Pharm 2024; 663: 124547.
111. Makvandi P, Kirkby M, Hutton AR, Shabani M, Yiu CK, Baghbantaraghdari Z, *et al.* Engineering microneedle patches for improved penetration: Analysis, skin models and factors affecting needle insertion. Nanomicro Lett 2021; 13: 93.
112. Sun Y, Miao D, Zeng Z, Li X, Pu Y, Liu L, *et al.* Recent advances in micro/nanoneedle arrays mediated intracellular delivery of biomacromolecules *in vitro* and *in vivo*. Adv Funct Mater 2025: 2422234.
113. Han S, Wan H, Lin D, Guo S, Dong H, Zhang J, *et al.* Contribution of hydrophobic/hydrophilic modification on cationic chains of poly (ε-caprolactone)-graft-poly (dimethylamino ethylmethacrylate) amphiphilic co-polymer in gene delivery. Acta Biomater 2014; 10: 670-679.
114. He W, Kong S, Lin R, Xie Y, Zheng S, Yin Z, *et al.* Machine learning assists in the design and application of microneedles. Biomimetics 2024; 9: 469.
115. Chumpu R, Chu C-L, Treeratanaphitak T, Marukatat S, Hsu S-H. Physics-informed graph neural networks accelerating microneedle simulations towards novelty of micro-nano scale materials discovery. Eng Appl Artif Intell 2023; 126: 106894.
116. Chiappini C, Campagnolo P, Almeida CS, Abbassi-Ghadi N, Chow LW, Hanna GB, *et al.* Mapping local cytosolic enzymatic activity in human esophageal mucosa with porous silicon nanoneedles. Adv Mater 2015; 27: 5147-5152.
117. Sun Z, Hou Y. Intelligent micro/nanorobots for improved tumor therapy. BMEMat 2023; 1: e12012.
118. Shah SA, Oakes RS, Kapnick SM, Jewell CM. Mapping the mechanical and immunological profiles of polymeric microneedles to enable vaccine and immunotherapy applications. Front Immunol 2022; 13: 843355.
119. Zhang XP, He YT, Li WX, Chen BZ, Zhang CY, Cui Y, *et al.* An update on biomaterials as microneedle matrixes for biomedical applications. J Mater Chem B 2022; 10: 6059-6077.
120. Choi H-J, Yoo D-G, Bondy BJ, Quan F-S, Compans RW, Kang S-M, *et al.* Stability of influenza vaccine coated onto microneedles. Biomaterials 2012; 33: 3756-3769.
121. McCaffrey J, McCrudden CM, Ali AA, Massey AS, McBride JW, McCrudden MT, *et al.* Transcending epithelial and intracellular biological barriers; a prototype DNA delivery device. J Control Release 2016; 226: 238-247.
122. Cary JH, Li BS, Maibach HI. Dermatotoxicology of microneedles (MNs) in man. Biomed Microdevices 2019; 21: 66.
123. Tarbox TN, Watts AB, Cui Z, Williams III RO. An update on coating/manufacturing techniques of microneedles. Drug Deliv Transl Res 2018; 8: 1828-1843.
124. Moawad F, Pouliot R, Brambilla D. Dissolving microneedles in transdermal drug delivery: A critical analysis of limitations and translation challenges. J Control Release 2025; 383: 113794.