Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

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Cite this: *Biomater. Sci.*, 2023, **11**, 7018

Microneedle systems: cell, exosome, and nucleic acid based strategies

Shufei Zhang,† Lian Yang,† Jianfeng Liu, Hanyue Li, Shasha Hong and Li Hong*

Received 1st July 2023,
Accepted 23rd September 2023

DOI: 10.1039/d3bm01103h

rsc.li/biomaterials-science

Cells, exosomes, and nucleic acids play crucial roles in biomedical engineering, holding substantial clinical potential. However, their utility is often hindered by various drawbacks, including cellular immunogenicity, and instability of exosomes and nucleic acids. In recent years, microneedle (MN) technology has revolutionized drug delivery by offering minimal invasiveness and remarkable versatility. MN has emerged as an ideal platform for the extraction, storage, and delivery of these biological components. This review presents a comprehensive overview of the historical progression and recent advances in the field of MN. Specifically, it highlights the current applications of cell-, exosome-, and nucleic acid-based MN systems, while presenting prevailing research challenges. Additionally, the review provides insights into the prospects of MN in this area, aiming to provide new ideas for researchers and facilitate the clinical translation of MN technology.

1. Introduction

Cells play a crucial role in biomedical engineering, and cellular therapies, such as Chimeric Antigen Receptor (CAR)-T cell therapy and stem cell therapy, have emerged as important therapeutic modalities for various diseases.[1] However, these therapies often face challenges related to immunogenicity. To address this issue, researchers have explored the potential of exosomes, which are nanoscale lipid bilayer-encapsulated vesicles secreted by various cell types, as a means of intercellular communication.[2] Exosomes, with a diameter ranging from 30 to 150 nm, have demonstrated the ability to reduce immunogenicity while retaining some cellular functions. They have been used in diverse fields, including disease diagnosis, treatment, and drug delivery. The effectiveness of exosomes lies in their cargo, which includes proteins and nucleic acids such as DNA, mRNA, and non-coding RNA (Fig. 1). Among these, microRNA (miRNA) has garnered significant interest due to its role in precision medicine. miRNA function by binding to target genes, thereby exerting inhibitory effects and reducing the expression of these target genes.[3,4]

Cell-based therapies, exosomes, and nucleic acids have emerged as important components in biomedical engineering, showing great potential for disease treatment. However, ensuring effective transplantation and action of these seeds remains a key challenge. While the use of biocompatible materials like hydrogels has partially addressed the issue of exogenous delivery, repeated invasive procedures such as hydrogel injections contradict the concept of minimally invasive treatments. Therefore, it is imperative to explore approaches that can ensure therapeutic effectiveness while minimizing invasiveness. Microneedle (MN) systems present a promising solution.

MN systems are novel drug delivery systems consisting of micron-sized needles. By forming an array of MNs, these systems enable minimally invasive transdermal drug delivery with virtually no pain, bridging the gap between non-invasive and invasive therapies.[5] MNs have been extensively studied in the fields of drug delivery, vaccination, and disease monitoring.[6–8] Based on different fabrication methods and structures, MNs can be classified into solid, hollow, coated, soluble, and hydrogel types and these MNs have found widespread applications in transdermal drug delivery and other areas (Fig. 2).[9] This review aims to provide an overview of the historical development and recent advancements of MN applications in the context of cell-based therapies, exosomes, and nucleic acids. It also discusses the current challenges and prospects, aiming to inspire new ideas and provide references for researchers in the field.

2. Research progress of various types of MN

2.1 Research progress on various types of MN materials and manufacturing processes

In the early stages of MN development, teams from Georgia Tech fabricated solid MNs and hollow MNs using etching or

Department of Obstetrics and Gynecology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, People's Republic of China.
E-mail: dr_hongli@whu.edu.cn
†These authors contributed equally to this work and share first authorship.

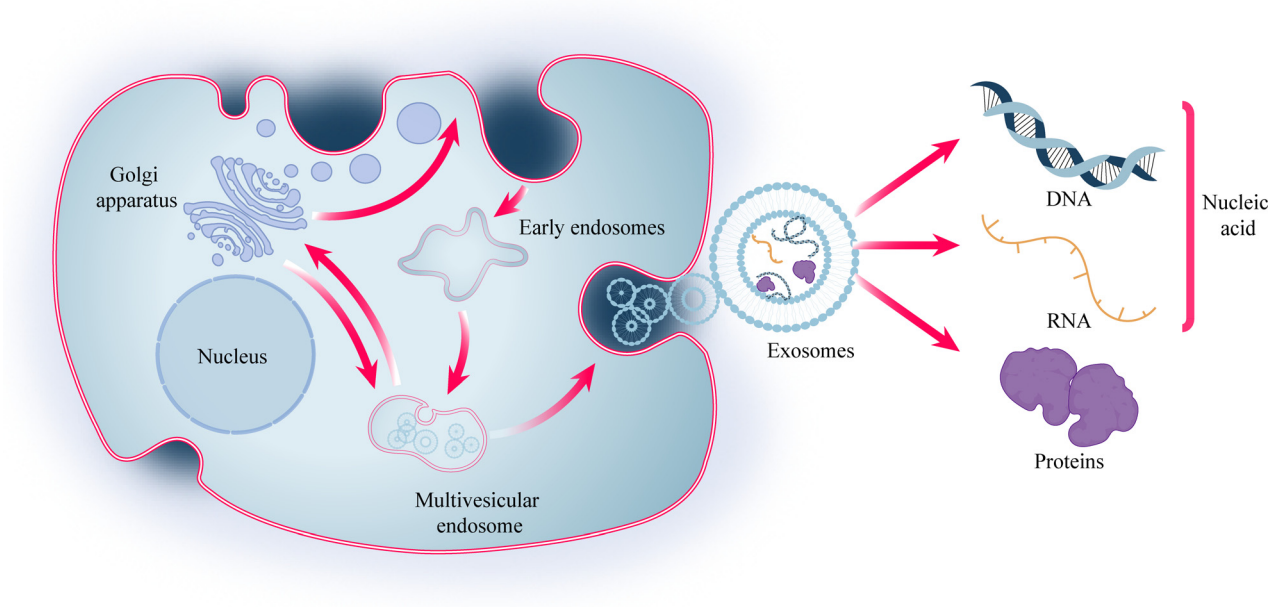

Fig. 1 Cell-secreting exosomes and the basic substances contained in exosomes.

stencil-filling methods, which served as the basis for solid, hollow, and hydrogel MN fabrication processes and dominated the field for a long time.[10] However, achieving all aspects of the MN manufacturing process has been challenging. The introduction of technologies such as stereolithography laser cutting and ablation, as well as Xenon Difluoride Dry Etching, has optimized the MN manufacturing process and enabled microfabrication and mass production of MNs. However, there are still limitations such as high cost, complex processes, and potential chemical contamination.[11,12]

Silicon, metal, and glass are common MN raw materials with excellent mechanical properties. However, their potential for fracturing after insertion into tissues can lead to complications, limiting the application of MN. As a result, sugar glass MNs and biodegradable polymers such as polylactic acid and polycaprolactone have emerged as new options for MN materials. The choice of biocompatible and biodegradable MN materials aligns more closely with the original design intention of minimally invasive procedures.[13–17]

Coated MNs are composite structures consisting of a solid MN core and a coating layer. The quality of the coating, process reproducibility, and delivery efficiency are crucial for their application. Unlike MN fabrication techniques such as etching, the dip coating method is the simplest way to fabricate coated MNs. In this method, the solid MN is immersed in a solution and the coated MN is obtained by drying and molding. However, this construction method faces challenges in precisely controlling the dose and is time-consuming. It is also difficult to achieve a uniform coating on the inner tip surface due to surface tension. Ideally, the coating should only cover the MN tip uniformly without unnecessarily wasting coating material.[18] Several spraying-based coating methods, such as Gas Jet Drying, Spray Coating, electrohydrodynamic atomization, and Piezoelectric Inkjet Printing, have been developed to achieve uniform coating by replacing liquid immersion with droplet spraying (Fig. 3).[19,20] The use of microforming further ensures precise dose control of the coating. When combined with industrialization tools like ultrasonic spraying technology, it facilitates the industrialization and clinical translation of coated MNs.[21,22]

2.2 Research progress of various MN application methods

The traditional method of MN application is finger pressure. Although simple and easy to use, this method lacks control over the magnitude and angle of applied pressure, leading to uneven force distribution and difficulty in handling larger MN patches or shorter MN needles. As a result, finger pressure is gradually being replaced by devices such as electronic applicators and impact-insertion applicators. Digitally controlled MN applicators allow precise control of applied pressure and impact speed, ensuring repeatability in MN applications. The impact force provided by these applicators also enables shorter micro-needle tips (<300 μm) to overcome skin elasticity, enhancing the penetration ability of MNs. However, the MN application method has been neglected for a long time, with only a few studies focusing on its improvement. Further research is needed to address issues such as differences in puncture performance and uneven force distribution in MN patches.[23] As MN treatments become more popular, MN administration may need to evolve towards an intelligent and personalized patient self-management model.

2.3 Advantageous applications of various types of MN

2.3.1 Solid MN. Solid MNs are widely used in clinical treatments, primarily as physical penetration enhancers for drugs. They are often combined with other drug-carrying materials or

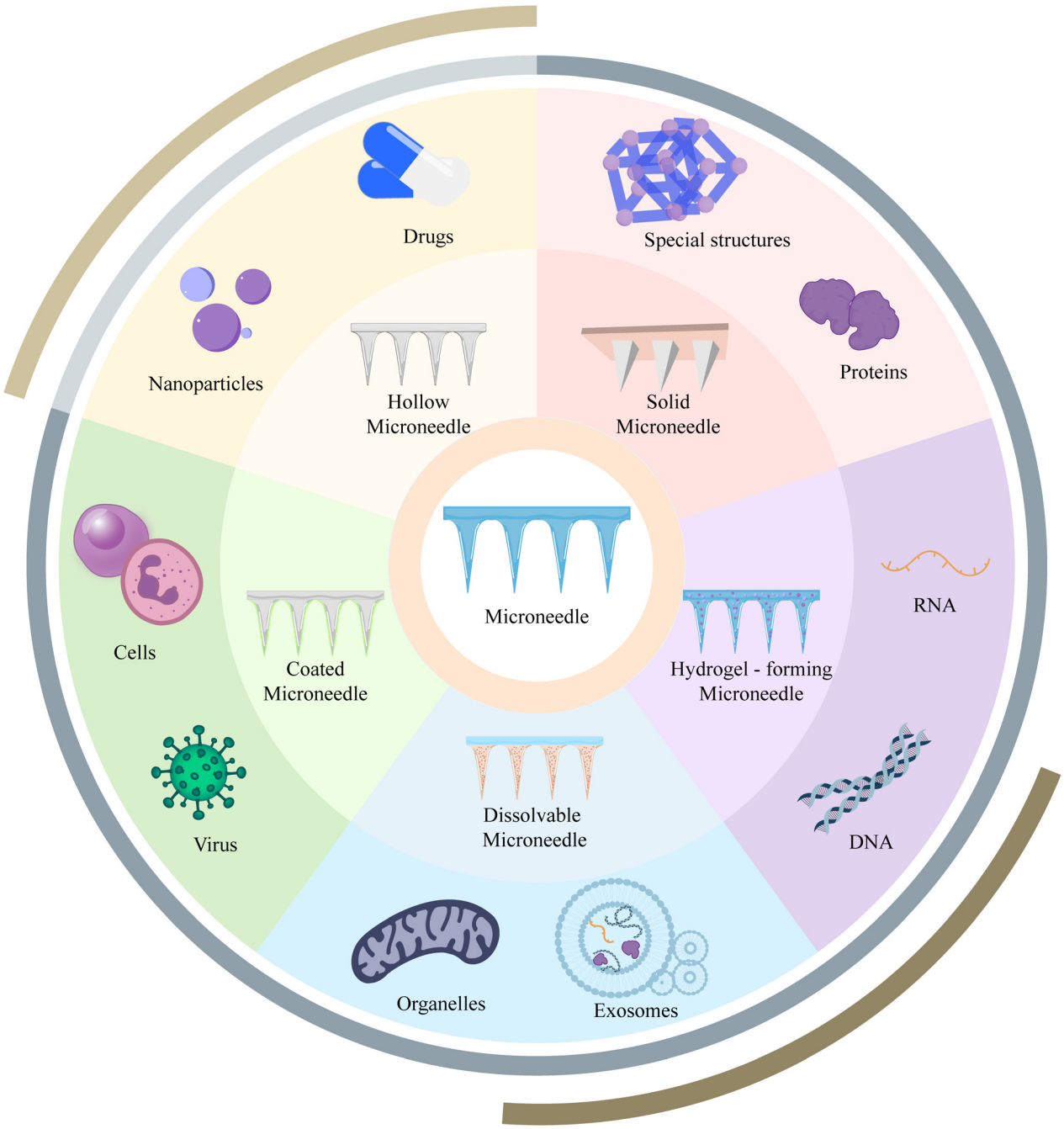

Fig. 2 The five basic types of MNs and their delivery substances.

transdermal patches to promote drug penetration and absorption by pretreating tissues. Solid MNs, with their excellent mechanical properties, are not limited to the stratum corneum of the skin. Their mechanical advantages are better demonstrated when dealing with tougher tissues such as vaginal walls, scar tissue, or sclerotic lesions.[24,25]

2.3.2 Hollow MN. Hollow MNs create channels between tissues and the external environment, making them suitable for precise treatments in various microsites, such as the suprachoroidal space or damaged arteries. They play a crucial role in targeted drug delivery to specific sites.[26,27] Additionally, their unique mode of action has made them valuable in disease diagnosis and tissue fluid collection. By utilizing prevacuum negative pressure in combination with hollow MNs, one-stop blood collection can be achieved.[28] Furthermore, when combined with personalized tests, hollow MNs enable rapid diagnostic tests, such as the use of colloid gold-based lateral flow immunoassay technology.[29] Combining hollow biodegradable MNs with biosensing devices and electroosmotic pumps allows for the comprehensive management of dia-

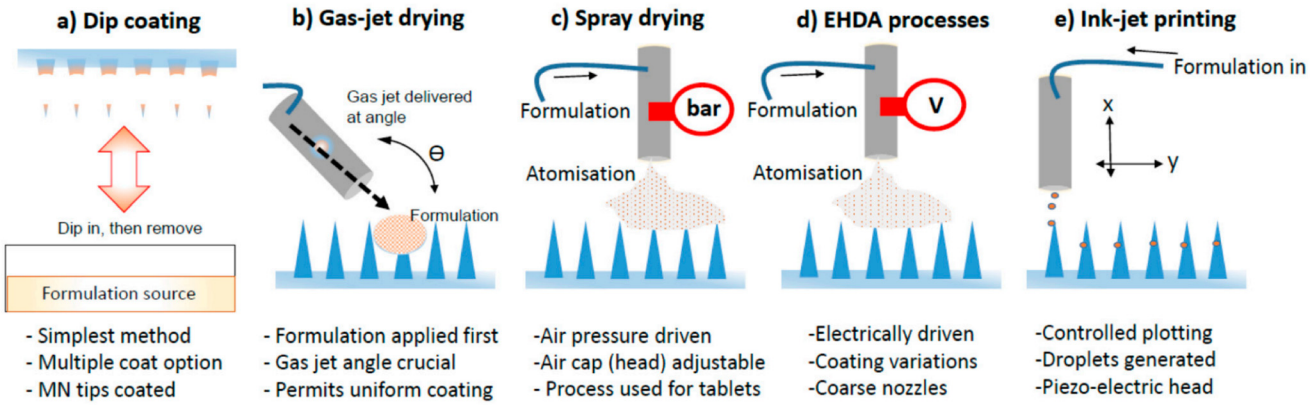

Fig. 3 Illustrated examples of techniques used to coat MNs. (a) Dip coating; (b) gas-jet drying; (c) spray drying; (d) EHDA processes; (e) ink-jet printing. Reprinted with permission from MDPI,[20] Copyright 2015.

betes.[30] By leveraging the unique characteristics of hollow channels and integrating physical sensors and other technologies for localized microfluid management, minimally invasive techniques have the potential to replace surgery and fill gaps in conservative treatment options.

2.3.3 Coated MN. Subcutaneous allergen immunotherapy is a classical treatment for allergies. However, long-term repetitive injections can be lengthy and painful, leading to poor patient compliance. To address this issue, researchers have used medium-length (250 μm) coated MNs to treat airway allergies in mice (Fig. 4). The MNs are cleverly inserted at an acute angle to achieve localized delivery within the epidermis (20–100 μm below the skin surface). This approach reduces systemic uptake, avoids the pain associated with multiple subcutaneous injections, and improves patient compliance.[31–33]

2.3.4 Soluble MN. Soluble MNs are composed of materials that can dissolve or degrade in the body after insertion. One of the earliest compositions was dextrin and polypropylene for insulin delivery. Current research focuses on the local delivery of photosensitizers in photodynamic therapy to minimize systemic uptake and enhance the treatment of infected wounds, tumors, and hyperplastic scarring.[34,35] An important aspect of

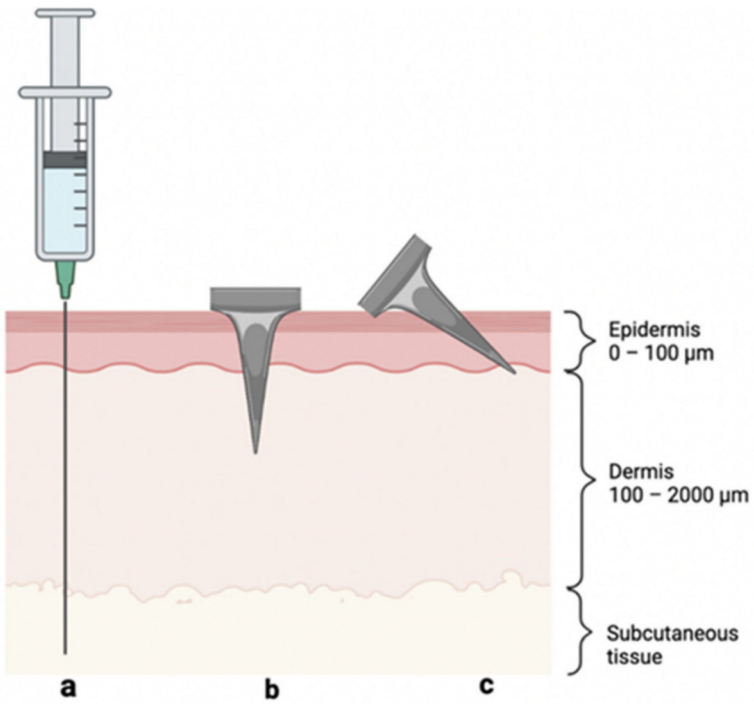

Fig. 4 Visual representation of skin layers and three methods of antigen delivery: (a) hypodermic needle injection to the subcutaneous space, (b) perpendicular (*i.e.*, 90°) MN insertion crossing epidermis and into the dermis, and (c) angled MN insertion targeting the epidermis. Reprinted with permission from MDPI,[32] Copyright 2022.

soluble MNs is the use of materials with different dissolution rates. This allows for sequential therapy, where fast-dissolving components provide rapid symptomatic relief while slow-dissolving components target specific disease conditions.[36] The varying dissolution rates of these materials can also enable the rapid separation of the MN needle from the substrate.[37] Researchers have explored the use of hyaluronic acid (HA) and sericin to construct a rapid separation MN patch (Fig. 5). In this design, sericin acts as a slow-release component, while the HA substrate supports the MN. Once the MN is inserted into the skin, the soluble HA quickly dissolves in the tissue fluid, separating the needle tip from the sericin matrix. As the sericin matrix swells and gradually degrades, the loaded drug is released at a constant rate, providing sustained therapeutic effects.[38]

2.3.5 Hydrogel MN. Hydrogel materials have gained significant attention in biomedical engineering due to their hydrophilicity, high biocompatibility, and ability to protect the skin barrier. These properties make them ideal for drug delivery applications.[39,40] Hydrogel MNs, in contrast to hollow MNs that rely on negative pressure vacuum, have superior hydrophilicity, allowing them to rapidly extract interstitial fluid upon insertion into the skin. This makes them particularly suitable for disease monitoring. Hydrogel MNs can be used not only for blood glucose detection but also for the detection of proteins, exosomes, and other substances when combined with probes, enabling *in situ* detection of biological indicators.[41,42] The wide variety of hydrogel materials available allows for the development of MNs with a high potential for expansion and innovation. For example, chitosan, a hydrogel material, exhibits inherent antibacterial and hemostatic properties, which can enhance the functionality of MNs.[43] By incorporating responsive properties such as near-infrared light, temperature, or glucose response, hydrogel MNs can achieve controlled drug release.[44–46] Additionally, the manufacturing method can be modified to create hydrogel ice MNs, which effectively deliver active substances.[47]

3. MN system: cell, exosome, and nucleic acid-based treatment strategy

3.1 Extraction and detection

Whether it is cell therapy or exosome delivery, the extraction of isolated seed cells is crucial for subsequent treatment and detection. Stem cells play a key role in cell therapy, but their clinical application is hindered by the challenges of purification and low viability during *in vitro* transplantation.[48]

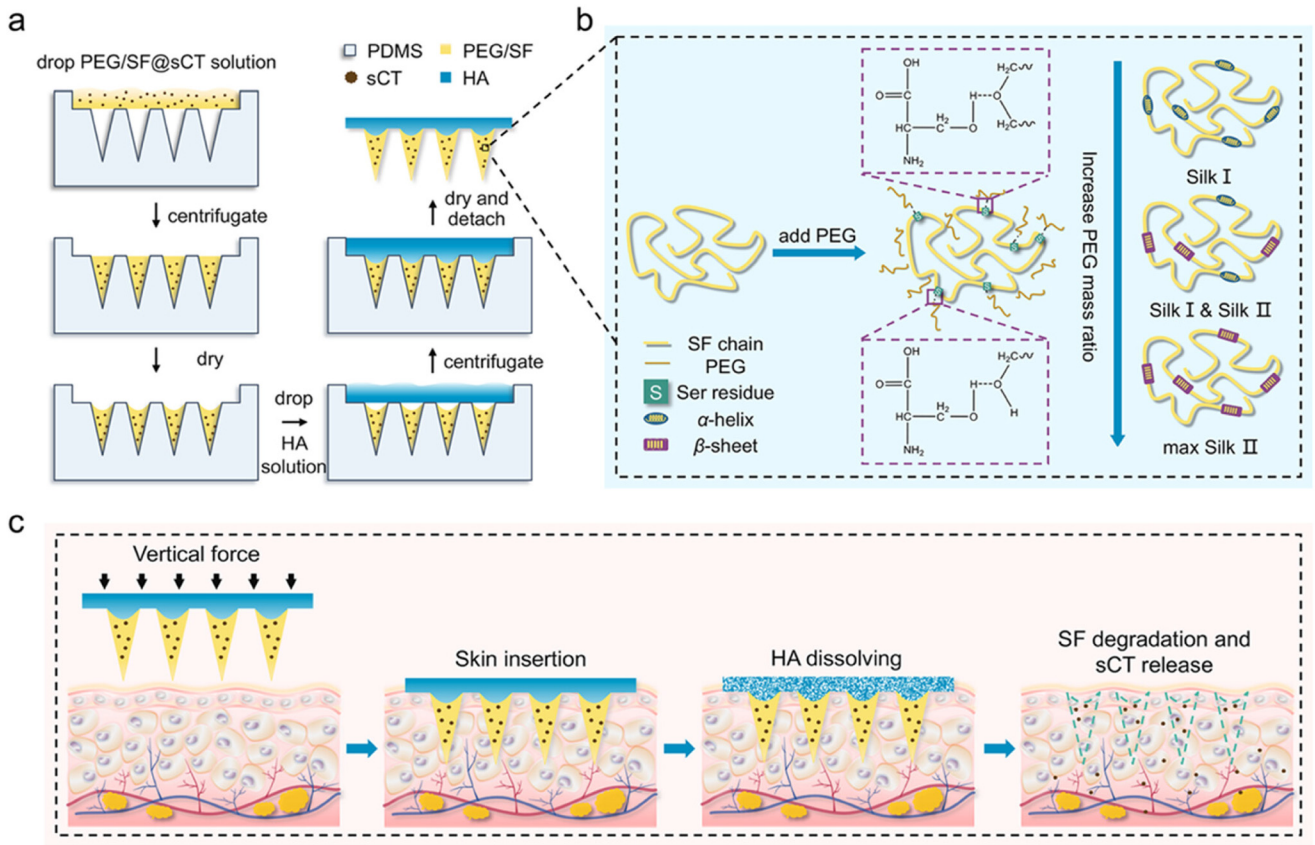

Fig. 5 Schematic illustrations of the fabrication process of HA-PEG/SF@sCT MNs (a), PEG-induced conformational transition of SF (b), and transmucosal delivery of sCT using the HA-PEG/SF@sCT MNs (c). Silk fibroin (SF); poly(ethylene glycol) (PEG); salmon calcitonin (sCT). Reprinted with permission from American Chemical Society,[38] Copyright 2023.

Traditional methods, such as flow cytometry, are often used to purify heterogeneous cells after isolation, but the mechanical pressure exerted on cells during high-speed flow can negatively affect their state, compromising further research or therapeutic use. To address this, Yuli Wang *et al.* explored an alternative strategy for isolating stem cells using PLGA degradable microrafts and a magnetic needle system, which could be effective for small sample sizes or fragile and sensitive transplanted cells.[49] However, this method is currently only applicable to adherent cells and involves a complex procedure, limiting its translational application. Therefore, the next research direction is to achieve targeted binding of microrafts to suspension cells and develop high-throughput screening methods.

Similarly, the first step in exosome research is often the extraction, isolation, and identification of exosomes. Common methods for exosome extraction, such as ultracentrifugation and ultrafiltration, are time-consuming and yield low concentrations of exosomes[50] (Fig. 6A). In contrast, some researchers have successfully used hollow MNs to extract trace amounts of dermal interstitial fluid exosomes. This extraction process is simple, minimally invasive, and provides a higher concentration of exosomes suitable for proteomic sequencing and detecting circulating biomarkers for disease diagnosis (Fig. 6B). However, these extracted exosomes are still limited in quantity and cannot be directly used for disease treatment. Moreover, although the simplification of the extraction process is beneficial, it does not address the issue of time-consuming exosome extraction or enable the direct extraction of MN-derived exosomes. Future research may focus on combining exosome-specific targets with hollow MNs to achieve direct extraction and arranging individual MNs into matrices for high-throughput collection of exosomes, advancing disease treatment strategies.

Interesting findings have been made regarding the application of MN in nucleic acid extraction. Initially, these applications were focused on the plant, animal, and food sectors. Polyvinyl alcohol (PVA) MN patches were utilized to extract DNA without relying on tissue and cell lysis, resulting in a significant reduction in plant DNA extraction time from 3 to 4 hours to approximately 1 minute. This approach also reduced extraction costs, making it a transformative strategy for the rapid detection of plant diseases.[51,52]

In the realm of human nucleic acid extraction, there have been notable studies. For instance, Yuchun Qiao *et al.* developed MN patches composed of methacrylamide gelatin (GelMA) and graphene oxide (GO) for the enrichment and detection of multiple miRNA biomarkers in skin interstitial fluid. These patches exhibited good mechanical strength, rapid sampling ability, and a high swelling rate to enrich miRNA fragments in interstitial fluid. The team also achieved simultaneous fluorescence detection of three psoriasis-specific

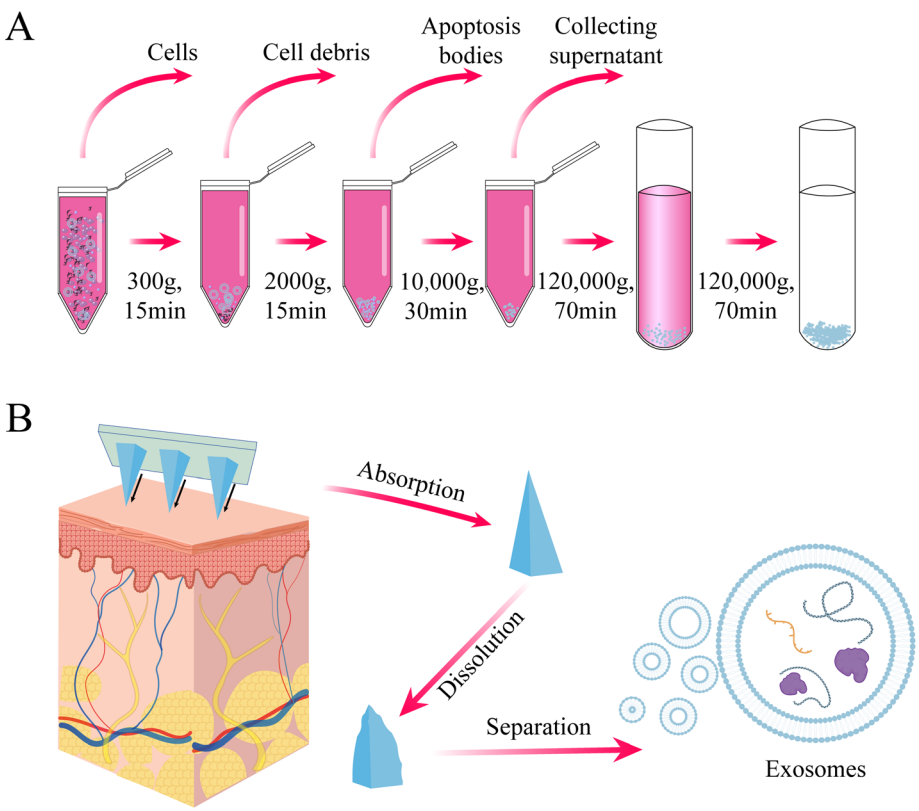

Fig. 6 (A) Conventional procedure for the extraction of exosomes by ultracentrifugation (B) extraction of skin tissue fluid for exosome isolation using hydrogel MNs.

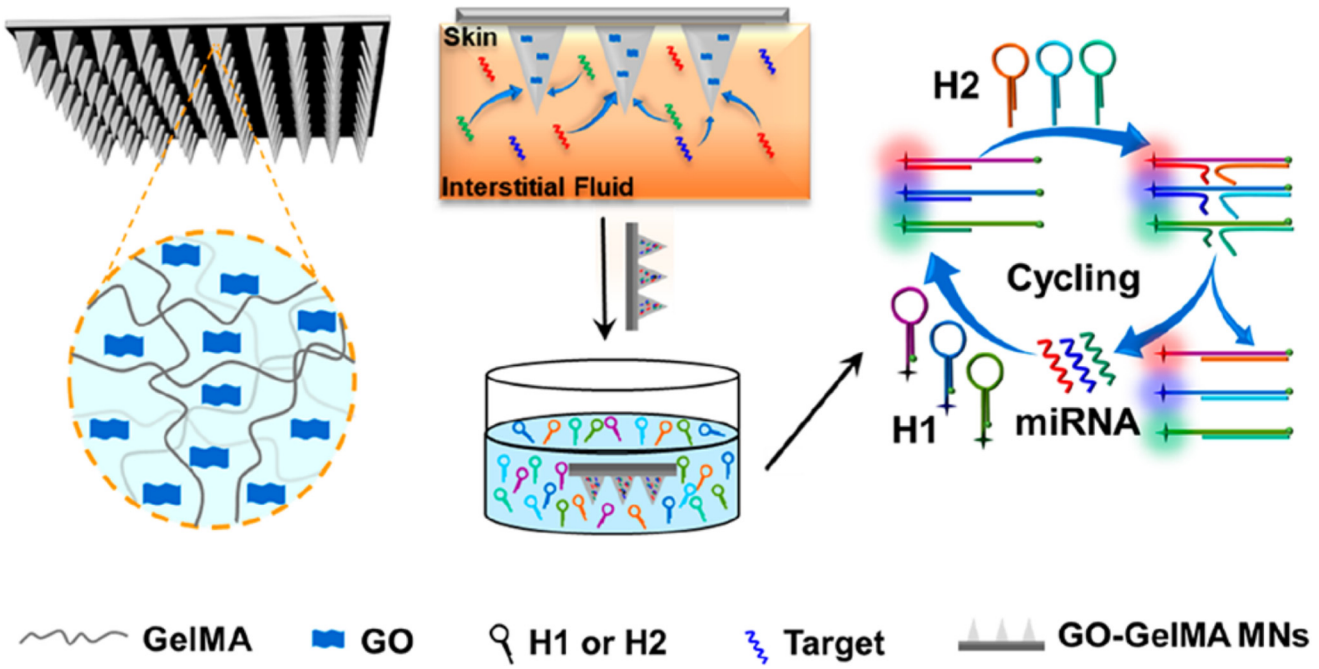

Fig. 7 Schematic illustration of GO-GelMA MNs used for interstitial fluid extraction and the detection of miRNA. Reprinted with permission from American Chemical Society,[53] Copyright 2023.

miRNAs by combining the isothermal catalytic hairpin assembly signal amplification technique (Fig. 7).[53] Furthermore, Bin *et al.* took a step further and constructed an online CRISPR-Cas9-activated wearable MN patch. This innovative patch leverages the synergy between CRISPR-Cas9 and graphene biointerfaces, as well as a conductive MN patch with reverse ion electroosmosis therapy. It enables efficient extraction and real-time monitoring of different DNA targets in a minimally invasive manner, exhibiting excellent electrochemical properties and up to 10 days of *in vivo* stability. This wearable MN patch holds great potential for early screening and treatment of diseases.[54]

In general, MNs have been extensively utilized in the field of cell, exosome, and nucleic acid extraction and detection. However, direct extraction of cells and exosomes remains a challenge. Although MNs can enrich higher concentrations of exosomes, their limited size restricts the total amount available, making it difficult to apply them in subsequent disease treatments. To overcome this limitation, the development of high-throughput continuous sampling MNs is crucial for expanding their application in the future.

Surprisingly, most studies have focused on using hydrogel MNs rather than hollow MNs for nucleic acid extraction. Nowadays, the use of MNs extends beyond nucleic acid extraction to include enrichment, extraction, and *in situ* detection of nucleic acids in combination with probes, fluorescence techniques, gene editing, and other technologies. Hydrogels offer good biocompatibility and editing properties, making them well-suited for these applications. However, one challenge is that while MNs are minimally invasive, they have limitations in terms of volume and scalability. This may necessitate the use of additional devices, such as fluorescence detection devices, to achieve all the expected functions, thereby adding redundancy to the detection process. Simplifying the steps and transforming nucleic acid extraction tests into individual test kits holds great potential in the field of disease diagnosis, treatment, and early prevention. Moreover, the development of wearable devices for real-time long-term disease monitoring places higher demands on the structure of MNs. Wearable devices are exposed to harsh conditions that can affect hydrogel properties and bioactivity over an extended period. A possible solution strategy could involve adding a layer of isolation colloid at the contact interface to mitigate these effects. In Table 1, MNs for extraction and detection of cells, exosomes, and nucleic acids are presented.

3.2 Seed activity retention

Studies have shown the potential of MN to facilitate the delivery of cellular and bioactive substances. However, it is critical to maintain the activity of cells, exosomes, and other components throughout the manufacturing, storage, and transport processes. To protect the activity of cells during MN manufacturing, KangJu Lee *et al.* proposed the design of a detachable hybrid MN receptacle. The mechanical strength of the reservoir was ensured by using a PLGA MN shell, and a biocompatible and biodegradable GelMA pre-mixed with cell culture medium was used to deliver bone marrow MSCs. This approach effectively reduces cell loss during MN manufacturing and provides valuable cells, offering a promising strategy for cell therapy applications involving challenging cells.

Table 1 Microneedles for extraction and detection of cells, exosomes, and nucleic acids

		MN design				
Seeds	Type	Materials	Height/ diameter	Application	Advantages	Drawbacks
Stem cell[49]	Solid MN	Anodized steel	NA/150 μm	Isolation of microrafts	Allows sorting and purification of small samples of walled cells under delicate conditions and maintains cell viability	Only walled cells can be sorted; the process is complex
Exosome[55]	Hollow MN	Glass	1500 μm/ 2.8 mm	Extraction of dermal interstitial fluid for exosome isolation and identification	Simple, minimally invasive, and high enough concentration of exosomes for transcriptomic or proteomic analysis	Low volume for therapeutic use in a single extraction; requires exosome extraction process
Plant DNA[51]	Hydrogel MN	PVA	800 μm/ 150 μm	Instant extraction of plant DNA for rapid pest detection	Fast, economical, minimally invasive, no lysis, no purification	Relatively low early detection rate of pathogen infection
Food DNA[52]	Hydrogel MN	PVA	840 μm/ 280 μm	Instant extraction of food DNA, rapid detection of food allergens	Fast, economical, simple	Difficult to handle complex food products
miRNA[53]	Hydrogel MN	Gelma/GO	600 μm/ 370 μm	Detection of psoriasis-related miRNA	Sensitive, automated	Longer detection time compared to other MN patches
DNA[54]	Hydrogel MN	Synthesis of polymethyl vinyl ether-*alt*-maleic acid (PMVE/MA) hydrogel/ chitosan/graphene	600 ± 50 μm/300 ± 10 μm	Long-term capture and real-time monitoring of free DNA	Wearable, efficient extraction, real-time monitoring	Interface receptor immobilization and sensitivity to be improved

However, the activity of MSCs tends to decline rapidly after 24 hours, posing a challenge for the long-term storage of these cells.[56]

Despite the beneficial biological functions of exosomes, they cannot be stored for long periods, even at −80 °C, resulting in the loss of bioactive material. In contrast, MN not only facilitates the delivery of exosomes but also serves as a long-term storage method for exosomes. For example, delivery of exosomes has been successfully achieved using HA MN, which can be stored at 4 °C for 6 months.[57]

In addition, Hao Chang *et al.* developed Cryomicroneedles utilizing a cryogenic medium consisting of 2.5% DMSO and 100 mM sucrose solution with gradient cooling in the mold to incorporate cells into Cryomicroneedles.[58] Furthermore, by modifying the formulation, Cryomicroneedles can enable the delivery of various bioactive substances, such as live bacteria and nucleic acids, thus expanding its applicability in delivering various bioactive substances (Fig. 8).[59–61] Cryomicroneedles not only have excellent mechanical properties but also dissolve within tissues to facilitate the rapid and efficient delivery of bioactive substances. Importantly, they possess long-term preservation capabilities not available with normothermic MNs, representing an important milestone in advancing the clinical translation of MN technology.

The issue of bioactive component deactivation during MN manufacturing and storage has been partially addressed through the use of Cryomicroneedles. However, there are still some common problems associated with these Cryomicroneedles. Firstly, MN loses its mechanical properties shortly after being removed from the cryogenic environment, making it challenging to achieve transdermal delivery. Secondly, deep cryogenic MNs may cause sensory discomfort. One potential solution is to construct the PLGA shell in the template first and then freeze-form the soluble active ingredients. This approach ensures the activity of the contents during MN fabrication and storage while maintaining the mechanical strength of the MN. It also extends the time window between Cryomicroneedle removal from deep cryogenic temperatures and application, reducing the possibility of discomfort. Although this solution may not be optimal, with the rapid development of technologies like 3D printing, finding new manufacturing processes that can maintain the activity of cells, exosomes, and nucleic acids under more general conditions is a future direction worth exploring.[62] In Table 2, MNs for maintaining the biological activity of cells, exosomes, and nucleic acids are presented.

3.3 Local delivery

After solving the problem of cell, exosome, and nucleic acid extraction and preservation, local delivery of seeds is the most important step for clinical therapeutic translation. To summarize, MNs that can achieve topical delivery have the following basic characteristics: (1) good mechanical strength to penetrate the stratum corneum or tissues; (2) the ability to release the contained components effectively and exert therapeutic effects; and (3) no adverse reactions caused by the MN components.

In 2018, B. Gualeni *et al.* successfully delivered melanocytes targeted to the skin for vitiligo treatment using hollow MN. This approach allows flexibility in treating different areas while minimizing pain and discomfort. It enables direct entry

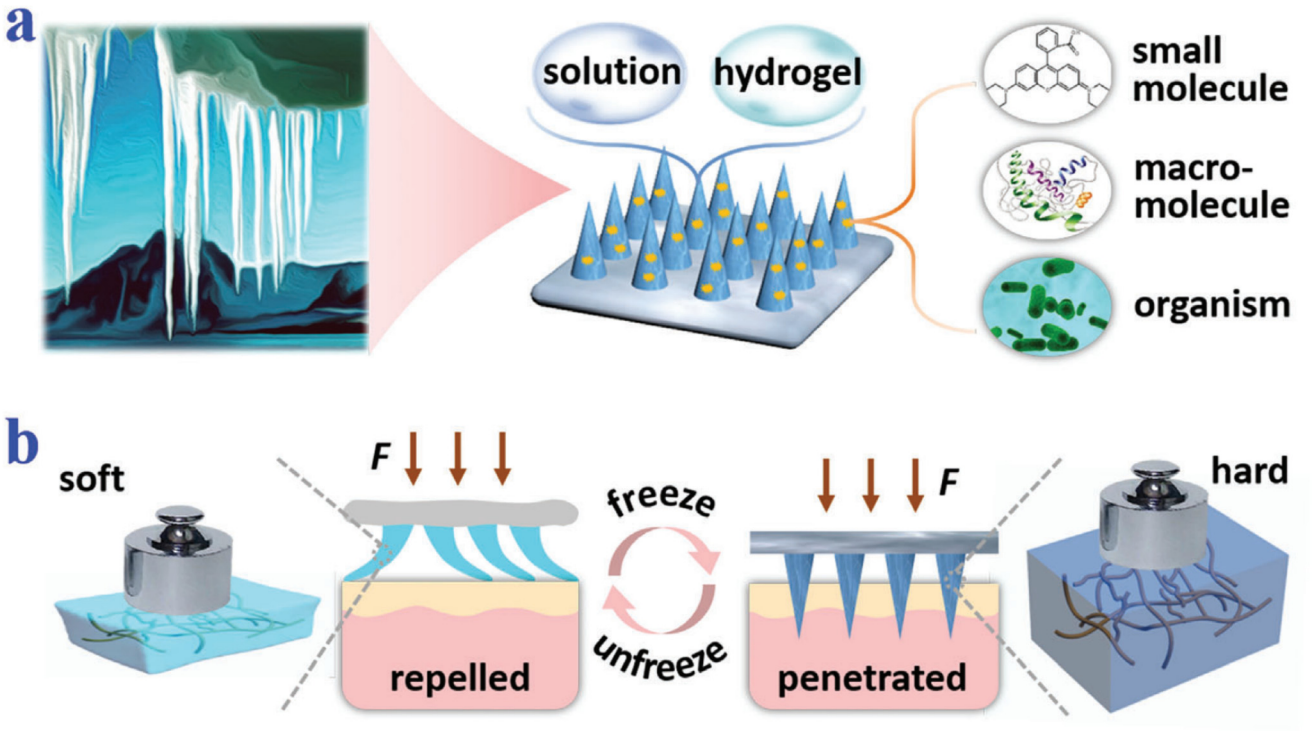

Fig. 8 Schematic illustrations of compositions and properties of ice MNs. (a) Ice–formation–inspired ice MNs can be made from soft materials such as solutions and hydrogels, and can carry diverse actives including small molecules, macromolecules, and living organisms. (b) Scheme of transformation from softness to hardness realized by freezing processes. Reprinted with permission from American Chemical Society Advanced Science published by Wiley-VCH GmbH,[61] Copyright 2021.

Table 2 Microneedles for maintaining the biological activity of cells, exosomes, and nucleic acids

		MN design				
Seeds	Type	Materials	Height/ diameter	Application	Advantages	Drawbacks
MSC[56]	Hydrogel MN	Gelma/ PLGA	700 μm/ NA	Local delivery of MSCs	Removable, avoiding the effects of the drying process on cellular activity in traditional MN manufacturing processes	Cellular activity declines precipitously after more than 24 hours of storage
Exosome[57]	Soluble MN	HA	600 μm	Long-term storage and transdermal delivery of extracellular vesicles	Can maintain the biological activity of extracellular vesicles for more than 4 months under mild storage conditions	Does not solve the problem of short duration of action after extracellular vesicle administration
Cell[58]	Soluble MN	DMSO/ succrose	1200 μm/ 400 μm	Delivers bioactive and maintains bioactivity	Enables long-term storage and transport of actives	Loses mechanical properties quickly after removal from the cryogenic environment; slight discomfort may occur at low temperatures
Bacteria[59]	Soluble MN	PBS// glycerol	600 μm/ 250 μm	Delivers live bacteria	Enables long-term storage and transport of actives	Loses mechanical properties quickly after removal from the cryogenic environment; slight discomfort may occur at low temperatures
mRNA[60]	Soluble MN	HA	900 μm/ 350 μm	Intradermal delivery of mRNA	Enables long-term storage and transport of active material	Loses mechanical properties quickly after removal from the cryogenic environment; may be slightly uncomfortable at low temperatures; no means to facilitate gene transfection

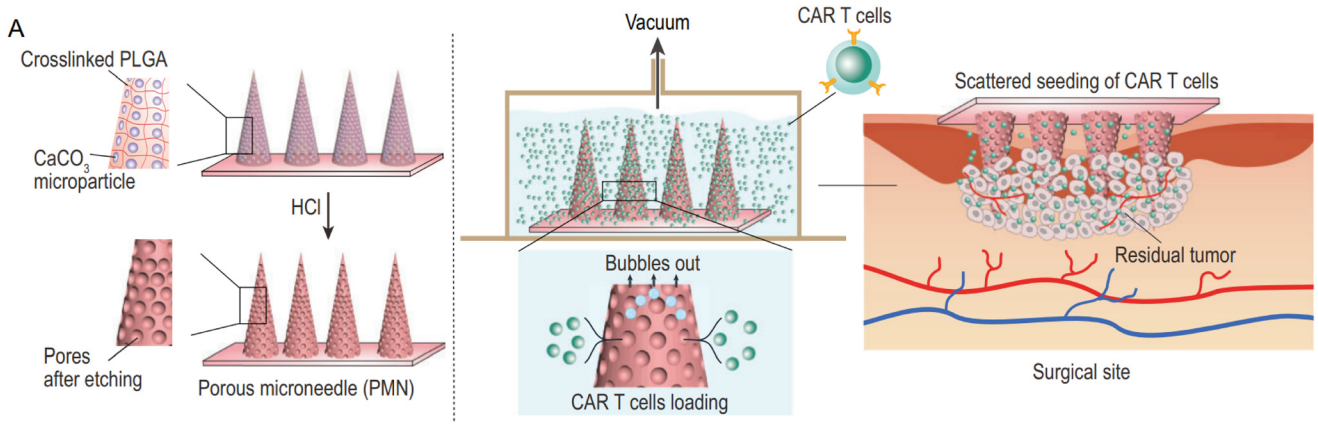

Fig. 9 Characterization of porous MN (PMN) patch for CAR T cell loading and delivery. (A) Schematic of PMN fabrication, CAR T cell loading, and implant of CAR-T-cell-loaded PMN (PMN@CAR T) within the tumor bed after surgery. Reprinted with permission from National science review,[64] Copyright 2021.

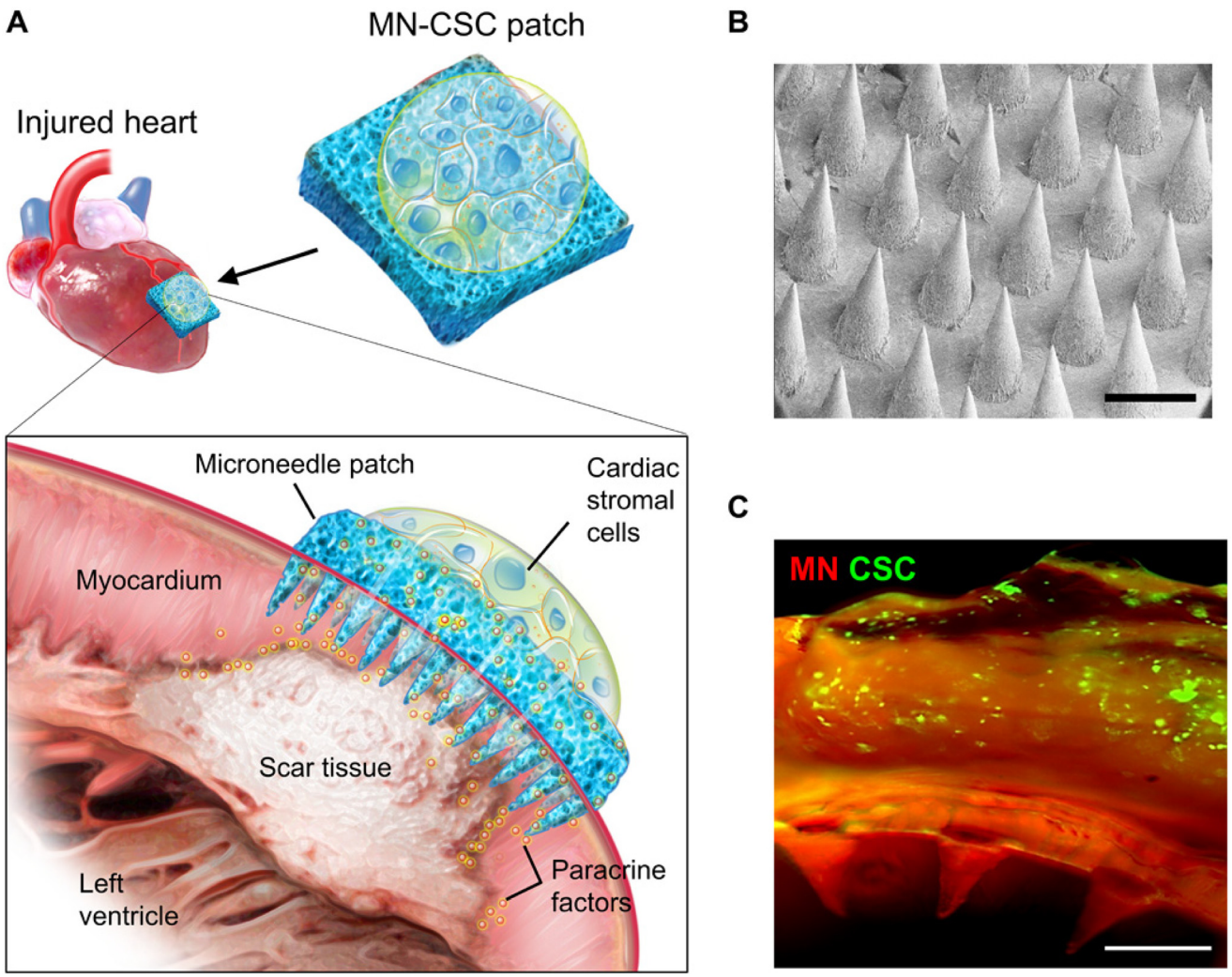

Fig. 10 Characterization of MN-CSCs. (A) Schematic showing the overall design used to test the therapeutic benefits of MN-CSCs on the infarcted heart. (B) SEM image of MN. Scale bar, 500 μm. (C) Representative fluorescent image indicating that DiO-labeled CSCs (green) were encapsulated in fibrin gel and then integrated onto the top surface of MN array (red). Scale bar, 500 μm. Reprinted with permission from Science Advances,[67] Copyright 2018.

of cells into the skin without significant cell loss, providing a model for cellular delivery using MNs.[63] Different application scenarios and MN contents pose additional demands on MN technology.

In the case of CAR-T cell therapy, for example, the abnormal vascular system, dense extracellular matrix, and tumor microenvironment cytokines in solid tumors hinder its effectiveness. To overcome these challenges, Hongjun *et al.* designed porous MNs that utilize an acid–base chemical reaction to etch the MN exterior and create pores for loading CAR-T cells (Fig. 9). This approach provides a multi-point, dispersed delivery vehicle for CAR-T cells, enhancing their infiltration within the tumor. The porous MNs can be applied to the resection cavity to prevent local tumor recurrence and potential metastatic dissemination.[64]

The application of MN for cell delivery extends beyond the skin and includes important areas such as the heart.[65] However, delivering cells to the myocardium poses specific challenges. The limited injected dose and the potential leakage of injected fluid and gel due to the beating of the heart put additional demands on MN-based cell delivery systems. There are two approaches for myocardial MN delivery. (1) Cell-loaded MN: this approach involves rapidly inserting the needle tip and inducing rapid substrate lysis to allow cell growth into the myocardium. Shiqi Hu *et al.* used a soluble HA substrate to achieve rapid separation of the MN needle from the substrate and implant cells into the myocardium.[66] (2) Cell-free approach: Tang *et al.* used PVA MN to establish a channel between cardiac stromal cells (CSC) and host cells, allowing therapeutic effects while providing nutrients for implanted seed cells through an ingenious design (Fig. 10).[67] Both approaches require precise administration using specialized instruments and often involve open-heart surgery to expose the heart, making clinical application challenging. Therefore, it is necessary to explore minimally invasive methods for cell delivery to the heart with the assistance of MNs. Mark R. Prausnitz *et al.* proposed a solution using a luminal unfolding MN injector to deliver MN-loaded capsules to the heart through natural lumens such as large blood vessels. The capsules release MNs for insertion into the myocardium. However, controlling the site and precision of MN release may be challenging.[68]

As previously mentioned, certain cells produce exosomes with therapeutic effects, and MSC exosomes are considered as a promising cell-free therapeutic strategy for tissue repair and regeneration.[69] In a brilliant study conducted by Min Han *et al.*

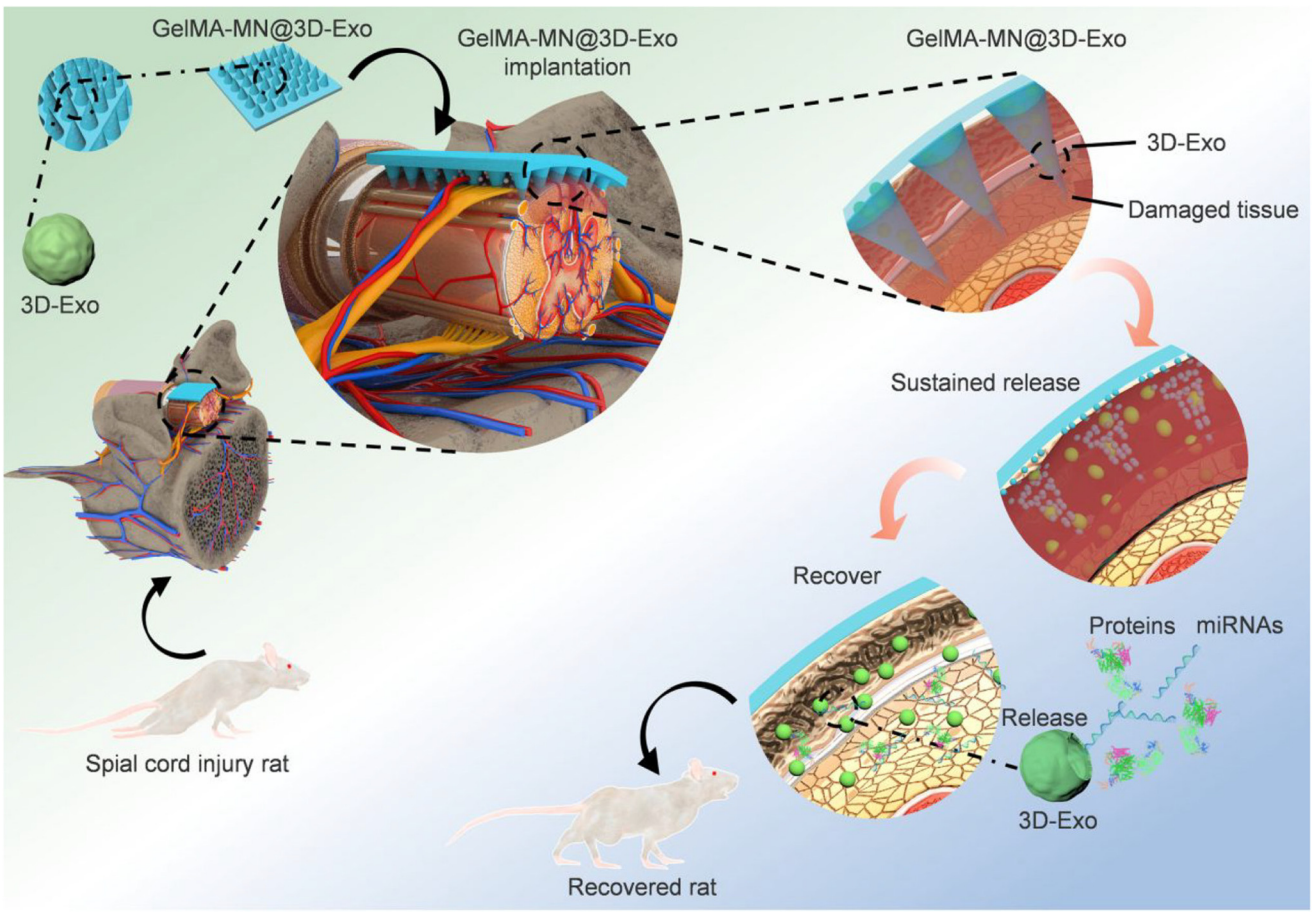

Fig. 11 Flow diagram of the overall study design of GelMA-MN@3D-Exo for the treatment of SCI in rats. Reprinted with permission from American Chemical Society,[70] Copyright 2022.

they utilized MN as a delivery vehicle and employed 3D culture to generate high-quality exosomes (Fig. 11). This approach facilitated *in situ* production and release of exosomes, eliminating the need for exosome extraction. In addition, exosomes from cells cultured in a 3D environment exhibited higher therapeutic efficiency. However, it may be wiser to consider the use of soluble material as a substrate for MN.[70]

In addition to their cellular functions, exosomes possess several advantages including low immunogenicity, low cytotoxicity, high circulating stability, and the ability to address issues related to hydrophobicity and low cellular uptake of drugs due to their phospholipid bilayer structure. As a result, exosomes can serve as carriers and form a dual carrier system with MN systems to facilitate efficient drug delivery and enhance cellular uptake. Yerneni *et al.* demonstrated improved drug uptake and skin-targeted delivery by utilizing MN-carrying exosomes containing curcumin in a dual-carrier system.[71] Yuan *et al.* successfully prevented cardiac fibrosis after myocardial infarction by delivering microRNA-29b-loaded exosomes *via* gelatin MN patches.[72]

To further enhance the effectiveness of exosome-mediated delivery, researchers have combined nanomotors with MN systems to drive the movement of exosomes using self-propelled forces generated through chemical reactions with the surrounding environment (Fig. 12). For instance, the conversion of L-arginine to nitric oxide by NOS or ROS can serve as the driving force for the nanomotors. This shift from a passive to an active mode of transport makes exosomes more effective in their therapeutic effects. However, it should be noted that the efficacy of this nanomotor-based approach may depend on the specific components of the environment and may not be highly compatible for application in different diseases.[73]

The initial gene delivery methods, such as microinjection, were inefficient due to the limitations of microscopic manipulation.[74,75] In contrast, the use of MN for exogenous nucleic acid delivery ensures effective uptake and utilization while meeting basic requirements. Physical techniques like electroporation, iontophoresis, and ultrasound have been shown to enhance nucleic acid uptake and utilization.[76] Previous studies utilized hollow conductive MN combined with electrical pulses for DNA electroporation. However, these early studies encountered challenges such as electric field conditions and high viscosity of DNA solutions, resulting in suboptimal delivery capacity and efficiency.[77] Therefore, subsequent studies focused on optimizing DNA delivery flux and electrode design for MN. The combination of soluble MNs and electrodes, known as hybrid electro-MN, increased the delivery flux by acting as a gene pool and facilitated *in situ* transfection through electrical stimulation. Additionally, the integration of metal transfer microforming technology improved the suitability of MN electrode arrays for electroporation, significantly enhancing nucleic acid delivery and utilization efficiency.[78,79]

Nevertheless, crossing the barrier of nucleic acids is just the first step, and escaping from the endosome-lysosome degradation pathway is crucial for their effectiveness. Coating nucleic acids with SiO_2 shells or using amphiphilic peptide

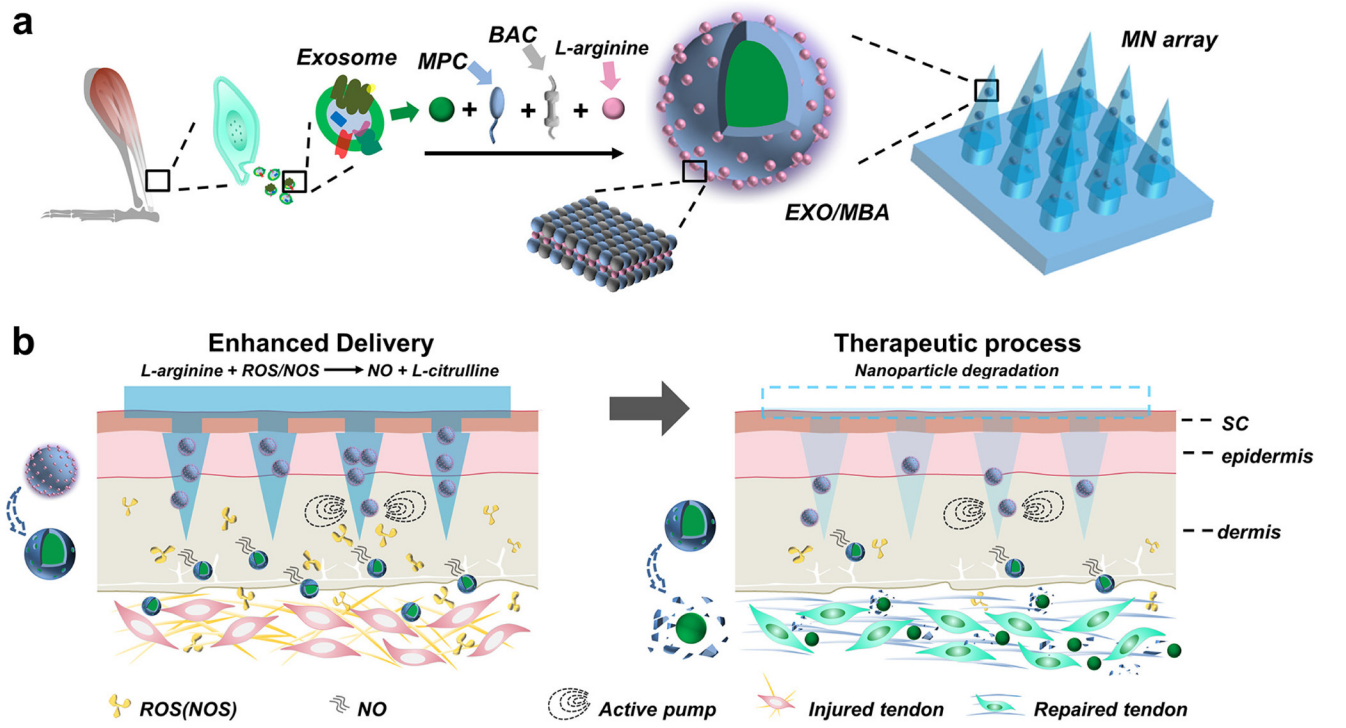

Fig. 12 Nitric oxide nanomotor driving exosomes-loaded microneedles schematic illustrations. (a) The manufacturing procedure of EXO/MBA-loaded MN array. (b) The healing process of Achilles tendinopathy after application of EXO/MBA-loaded MN array. Reprinted with permission from American Chemical Society,[73] Copyright 2021.

RALA has been commonly employed to facilitate intracellular transmembrane delivery and endosomal escape, thereby improving transfection efficiency.[80–82]

DNA vaccines are cost-effective, stimulate effective cellular and humoral immune responses, and are considered an ideal alternative to traditional vaccines. MNs enable the efficient

Table 3 Microneedles for cells, exosomes, and nucleic acids delivery

		MN design				
Seeds	Type	Materials	Height/ diameter	Application	Advantages	Drawbacks
Melanocyte[63]	Hollow MN	Silicon	400–700 μm/ 75–150 μm	Delivery of melanocytes to the skin for vitiligo	Can achieve precise delivery of cells and precise control of cell volume	A high concentration of cells may produce blockage; silicon MN has the risk of fracture
Mesenchymal stromal cell[66]	Hydrogel MN	Elastin-like polypeptide gel/HA	600 μm/ 300 μm	Delivery of mesenchymal stromal cell for myocardial infarction	Basal lysis can be achieved without suturing and disassembly	Requires open-heart surgery and other instruments for delivery, difficult to translate clinically
Cardiac stromal cells[67]	Hydrogel MN	PVA	600 μm/ 300 μm	Delivery of cardiac stromal cells for myocardial infarction	MN generates channels that allow the exchange of material between cells and the host myocardium	Requires open-heart surgery, difficult to translate clinically
CAR-T cells[64]	Solid MN	PLGA	1500 μm/ 500 μm	CAR-T treatment	Etching increases CAT-T cell infiltration	Cellular action is limited by the rate of PLGA degradation
MSC[70]	Hydrogel MN	Gelma	300 μm/ 300 μm	Spinal cord injury repair	*In situ*, the production and release of exosomes can be achieved	The substrate and tip are the same material, substrate separation is not achieved and the needle can be easily dislodged due to external influence
Exosome[86]	Hydrogel MN	Methacrylamide HA	860 μm/ 360 μm	Promotes diabetic wound healing	The ability of MSC NVs to express therapeutic cytokines can be significantly enhanced when treated with iron nanoparticles	The synthesis step is more complicated
Exosome[71]	Soluble MN	Carboxymethylcellulose/ alginate	700 μm/ 210 μm	Enables skin-targeted delivery of curcumin	Addresses the low solubility, bioavailability, and *in vivo* stability of curcumin	Passive uptake of exosomes efficiency needs to be improved
Exosome[72]	Hydrogel MN	Gelatin	500 μm/ 270 μm	Prevents cardiac fibrosis after myocardial infarction	Addresses poor miRNA stability	Passive uptake of exosomes efficiency needs to be improved
Exosome[73]	Hydrogel MN	Gelma/HA/PVA	800 μm/ 250 μm	Promotes Achilles tendinopathy healing	Enables the active release of exosomes	Complex synthesis; environment-dependent efficiency of action
SiRNA[81]	Hydrogel MN	HA	800 μm/ 300 μm	Delivery of siRNA	Can resist partial nucleic acid degradation	Uncontrollable release rate, too fast in the initial phase
DNA[82]	Soluble MN	Poly(vinylpyrrolidone) (PVP)	600 μm/ 300 μm	Delivery of DNA	Helps nucleic acids to undergo endosomal escape	Cannot accurately determine the amount of DNA delivered from the MN patch to the skin
DNA[84]	Soluble MN	HA	750 μm/ 200 μm	Delivery of DNA influenza vaccine	Superior to conventional intramuscular injection in terms of induction of conjugated antibodies, antibody-secreted recall responses, and interferon-secreted T cells	The relatively weak immunogenicity of the DNA vaccine is also not addressed

delivery of DNA vaccines and offer a simple method that enhances patient compliance, particularly during pandemics.[83] Interestingly, studies have shown that DNA vaccine delivery to the skin *via* MNs outperforms traditional intramuscular injection in terms of inducing conjugated antibodies and recalling antibody secretion responses. This superior outcome may be attributed to the direct delivery of vaccine DNA to numerous antigen-presenting cells in the skin through MNs, resulting in improved protection against lethal infections.[84,85] However, the immunogenicity of DNA vaccines is relatively weak compared to inactivated vaccines, necessitating further improvements in MN technology to recruit immune cells, such as antigen-presenting cells, and enhance the potency of DNA vaccines. In Table 3, MNs for delivering cells, exosomes, and nucleic acids are presented.

4. Conclusions

Cells, exosomes, and nucleic acids are essential elements in biomedical engineering with significant clinical potential. However, their application is hindered by challenges such as cellular immunogenicity, instability of exosomes and nucleic acids, and difficulties in extraction and culture. Extensive studies have demonstrated that MNs provide an ideal solution for addressing seed extraction, identification, activity maintenance, and delivery issues. Each type of MN has its advantages and disadvantages, and constructing suitable MNs according to specific usage scenarios is a critical aspect of MN research.

However, there are still many problems in the application and clinical translation of MNs. (1) MN is limited by its volume, and the amount of cells, exosomes, or nucleic acids obtained is small, which makes it difficult to be applied to clinical treatment. (2) Although Cryomicroneedles largely solve the problem of activity retention, they lose their proper mechanical properties in a few tens of seconds after being removed from the cryogenic environment and may cause some sensory discomfort. (3) The administration of MN deviates from the minimally invasive concept, for example, open-heart surgery is required to expose the heart and achieve myocardial delivery of MN. (4) Most MNs of the contained substance are passively released, and efficiency is difficult to ensure. (5) It is difficult to precisely quantify the amount of seeds delivered. There may be many similar problems, and our work gives some ideas, but more research is needed to demonstrate its feasibility.

In the future, the development of MN in biomedical engineering should primarily serve clinical diagnosis and treatment. This can be achieved by: (1) developing integrated disease indicator detection kits, which are highly valuable for early prevention and treatment of diseases. (2) Vigorous development of wearable and smart MN devices for real-time monitoring and integrated disease management. (3) Develop high-throughput MN extraction methods to apply seed components to clinical disease treatment. (4) Promote local delivery of MN to achieve intelligent, on-demand, responsive release, combined with biobatteries to actively release the contained components. (5) Precisely control the dose of MN delivery to facilitate standardized clinical translation. (6) Maintain the concept of minimally invasive surgery and utilize new structures, such as capsule MNs, to achieve minimally invasive clinical applications of MNs. It is foreseeable that MNs will bridge the gap between conservative treatment and surgery and become the backbone of minimally invasive therapies in clinical practice.

Author contributions

Shufei Zhang: Conceptualization, writing – original draft, visualization. Lian Yang: Resources, writing – review & editing. Jianfeng Liu: Resources, supervision. Hanyue Li: Investigation. Shasha Hong: Investigation. Li Hong: Writing-review & editing, funding acquisition.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank all the researchers who have contributed to the relevant studies covered in this paper. This work was financially supported by Hubei provincial Key Research and Development Program (2022BCA045); the National Key Research and Development Program of China (2021YFC2701300); the National Natural Science Foundation of China (81971364; 82001527), Second level fund of the second medical leading talents project of Hubei province (No. [2019]47) and Natural Science Foundation of Hubei Province (2022CFB124, 2021CFB113).

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