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REVIEW

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A Review of Nano/Micro/Milli Needles Fabrications for Biomedical Engineering

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Abstract

Needles, as some of the most widely used medical devices, have been effectively applied in human disease prevention, diagnosis, treatment, and rehabilitation. Thin 1D needle can easily penetrate cells/organs by generating highly localized stress with their sharp tips to achieve bioliquid sampling, biosensing, drug delivery, surgery, and other such applications. In this review, we provide an overview of multiscale needle fabrication techniques and their biomedical applications. Needles are classified as nanoneedles, microneedles and millineedles based on the needle diameter, and their fabrication techniques are highlighted. Nanoneedles bridge the inside and outside of cells, achieving intracellular electrical recording, biochemical sensing, and drug delivery. Microneedles penetrate the stratum corneum layer to detect biomarkers/bioelectricity in interstitial fluid and deliver drugs through the skin into the human circulatory system. Millineedles, including puncture, syringe, acupuncture and suture needles, are presented. Finally, conclusions and future perspectives for next-generation nano/micro/milli needles are discussed.

Keywords: Nanoneedles, Microneedles, Millineedles, Fabrication methods, Biomedical Engineering

1 Introduction

Needles, some of the most widely used medical devices, can easily penetrate cells, skin, and organs by generating highly localized stresses with their sharp tips for sampling, biosensing, drug delivery and surgery. Biomedical needles have a long developmental history in fighting diseases (Figure 1). In 279 BC, the Chinese first developed stone acupuncture needles for bloodletting and apocenosis [1]. Later, metal acupuncture needles, specifically Ag needles, which have good antibacterial properties, were invented and improved with the development of metallurgical technology. In 500 AD, Suxruta, an Indian saint and physician, explored suture needles for stitching wounds [2]. In 1809, Francois Magendie, a French physiologist, reported the earliest prototype of syringe needles for mimicking the lethal effects of Javanese arrow poison. A wooden barb with poison on the end was pierced into the buttocks of a dog, and the poison slowly dissolved and spread in the body [3]. In 1855, Alexander Wood developed modern syringes to treat neuralgia by subcutaneous injection [4]. Similar to the syringe needle, the puncture needle has been exploited to extract biological specimens such as blood, secretions, and tissue samples [5]. The diameter of the acupuncture needle, suture needle, syringe needle and puncture needle are usually on the millimeter scale; therefore, we refer to them "millineedles" in this review. Although millineedles have been widely used in medical fields, they can easily puncture the dermis and reach nerve endings and blood vessels, causing pain and bleeding. Therefore, painless and minimally invasive techniques for these biomedical applications are highly desired.

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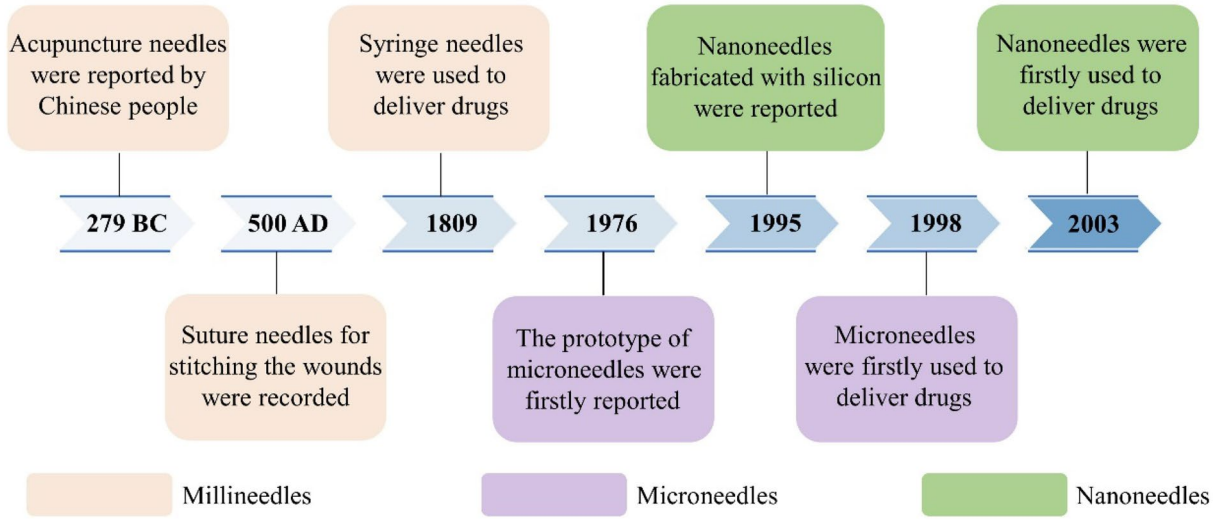


Figure 1 The typical development history of nano/micro/milli needles

Microneedles at the microscale are superior to traditional millineedles in transdermal sensing and drug delivery because of their unique advantages; they are minimally invasive, painless, and can be used for self-administration [6]. In 1976, the microneedle was first proposed and applied for transdermal delivery [7]. In 1998, Henry's team developed Si microneedles to deliver drugs with 4 orders of magnitude of increasing drug permeability [8]. Since then, microneedle fabrication and application have made great progress due to the rapid development of high-precision microfabrication technologies. Emerging microneedle technology has been applied in biomedical applications, including transdermal drug delivery, immunobiological administration, disease diagnosis, and cosmetic applications.

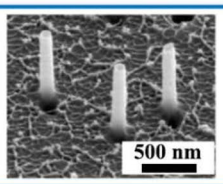
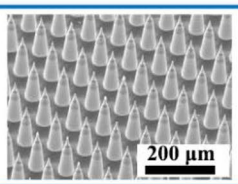
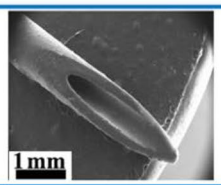
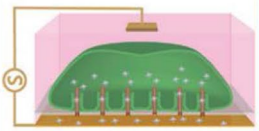
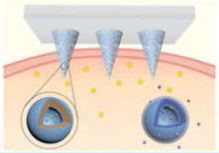
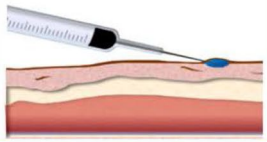
Nanoneedle is a kind of needle with nanoscale diameter, which can penetrate the cytomembrane to probe and manipulate biological processes in living cells [9]. Nanoneedle technology is expected to play an important role in studying biological systems [10]. In 1995, nanoneedles were first described by Heike et al. [11], where silicon nanoneedles were used for direct imaging with scanning tunneling microscope apex tips. In 2003, McKnight et al. [12] demonstrated the use of vertically aligned carbon nanofibers as nanoneedles to deliver plasmid DNA to viable cells for biochemical manipulation. Since then, nanoneedles have been widely studied as a powerful tool in intracellular cell sensing and drug delivery.

Depending on their diameter, needles can be classified into nanoneedles, microneedles and millineedles. Owing to the scale effects of nano/micro/milli needles, different fabrication strategies and biomedical applications have been developed, as shown in Table 1. In this review, we provide an overview of multiscale needle fabrication techniques and their applications in biomedical engineering, as shown in Figure 2. The fabrication strategies for nanoneedles, including bottom-up and top-down methods, are summarized, and representative applications in cell sensing and drug delivery are also highlighted. The fabrication methods of microneedles are divided into subtractive, formative and additive manufacturing, and their applications in transdermal sensing and drug delivery are also described. Typical fabrication of millineedles, grinding process, is introduced. And the applications of millineedles in puncture, springe, acupuncture and suture are presented. Finally, conclusions and future perspectives on the development of next-generation nano/micro/milli needles of use in the biomedical field are proposed.

2 Nanoneedles

Nanoneedles, as thin 1D nanostructures, can penetrate the cell membrane by generating highly localized stress with their sharp nano features, which have diameters of 1–100 nm; these needles can be used for cell sensing and drug delivery [13–15]. Based on their 1D nanostructures, nanoneedles can be divided into solid nanoneedles and hollow nanoneedles. Because of the evolution of advanced fabrication technologies in the 20th century, high aspect ratio nanoneedles with tunable dimensions, compositions, structures, and properties have been fabricated from semiconductors, metals, and metal oxides. According to fundamental fabrication principles, the nanoneedle fabrication process can be classified into a bottom-up strategy and a top-down strategy. In this section, we will briefly focus

Table 1 Illustration of nano/micro/milli needles applied in biomedical engineering

	Cell (nm)	Skin (μm)	Organs (mm)
Needle types	**Nanoneedles**	**Microneedles**	**Millineedles**
Typical needle images			
Schematic illustration			
Typical applications	● Cell sensing ● Cell drug delivery	● Transdermal sensing ● Transdermal drug delivery	● Puncture ● Syringe ● Acupuncture ● Suture

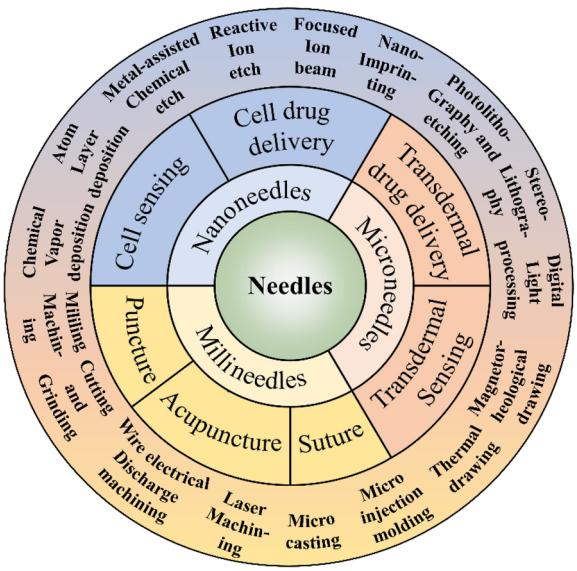


Figure 2 Summary of nano/micro/milli needles fabrication techniques

on the typical fabrication techniques of nanoneedles and their applications in cell sensing and drug delivery.

2.1 Fabrication of Nanoneedles

2.1.1 Bottom-Up Strategy

The bottom-up fabrication strategy of nanoneedles involves stacking atoms or molecules along a certain direction [13]. Hence, the layer-by-layer construction of a structure with a high aspect ratio on a substance is fundamental. Deposition is a fundamental bottom-up fabrication process. According to atom or molecule deposition methods, typical bottom-up fabrication strategies can be classified into physical vapor deposition (PVD), chemical vapor deposition (CVD), and atomic layer deposition (ALD). PVD is rarely reported for building nanoneedle structures; thus, this section will mainly focus on CVD and ALD techniques for nanoneedles.

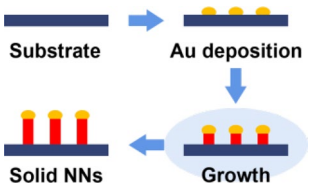
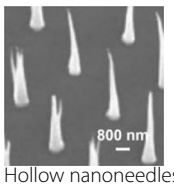
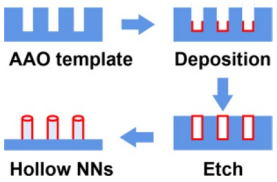
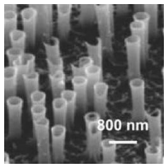
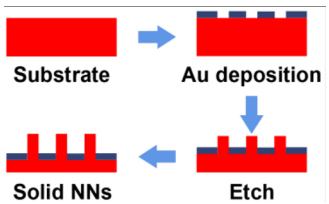
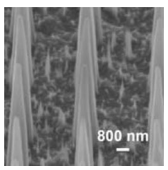
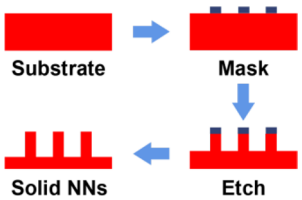
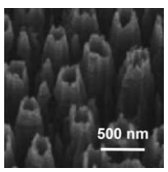
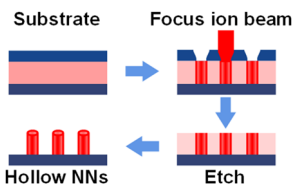
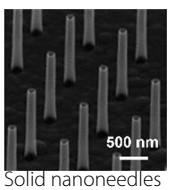
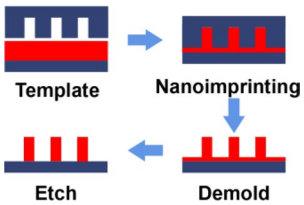
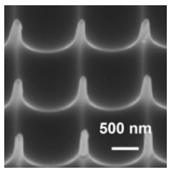
(1) Chemical Vapor Deposition

Chemical vapor deposition is a deposition method in which the substrate is exposed to one or more volatile vacuum precursors, which react and/or decompose on the substrate surface to produce the desired nanoneedles [16]. The main fabrication procedures could be divided into three steps. First, the material to be deposited is gasified under high temperature and high pressure. Second, the gases are fed into the reaction cavity. Third, chemical reactions occur between the gas and the substrate, and a film is deposited on the substrate. This fabrication method is also known as the vapor-liquid-solid (VLS) process. CVD is often applied to the construction of SiO_2 and Si_3N_4 films on silicon wafers, and metals can also be fabricated.

Typical CVD growth of nanoneedles usually depends on metal nanoparticle catalysts (e.g., Au, Pt, etc.) to catalyze the reaction and determine the diameter and density of nanoneedles [17]. McKnight et al. [12] constructed vertically aligned carbon nanoneedle structures via the plasma-enhanced CVD (PECVD) method, as shown in Table 2. The

nanoneedle structures were synthesized on a silicon wafer with the help of 500 nm nickel catalyst dots. A mixture of C_2H_2 and NH_3 was introduced and reacted on the silicon wafer with the help of plasma, and carbonaceous material was deposited on the wafer. Finally, the deposited solid film grew to form nanoneedle structures with nickel particles on their tips. The typical dimensions of the nanoneedle structures were 6–10 μm in length, 20–50 nm in tip diameter and approximately 1 μm in base diameter. The reaction time and the growth time have great influence on the length of the nanoneedles.

Table 2 Fabrication techniques of nanoneedles

Methods	Fabrication illustrations	SEM images	Materials	Advantages	Typical limitations	Typical applications
Bottom-up						
Chemical vapor deposition		Solid nanoneedles 	Silicon, Carbon, GaN	High efficiency, high repeatability	Expensive equipment, high production cost	Cell sensing, cell drug delivery
Atom layer deposition		Hollow nanoneedles 	Ni Al_2O_3	High machining accuracy, high aspect ratio, fit for hollow nanoneedle	Expensive equipment, multiple steps, low efficiency	Gene delivery, protein extraction
Top-down						
Metal-assisted chemical etch		Porous nanoneedles 	Silicon	High efficiency, simple process, low cost, fit for mass production	Random distribution, wide dimension	Cell drug delivery, cell sensing
Reactive ion etch		Hollow nanoneedles 	Silicon SiO_2/Si	High machining accuracy, highly ordered, better controllability	Expensive equipment, high resolution mask, low flexible	Cell drug delivery
Focused ion beam		Hollow nanoneedles 	Silicon	Good flexibility, high aspect ratio, fine controllability, fit for hollow nanoneedle	Expensive equipment, low efficiency	Cell drug delivery, cell sensing
Nanoimprinting		Solid nanoneedles 	Aluminum	High resolution, low cost, high consistency, fit for mass production	Multiple steps, low flexible, high precision mold	Biosensing

Furthermore, hollow nanoneedles could be fabricated by CVD [18, 19]. Park et al. [18] fabricated hollow nanoneedles with highly ordered porous anodized aluminum oxide (AAO templates) by CVD. The carbon layer was grown within the pore structure of the AAO templates to construct the hollow structure. A C_2H_2 and NH_3 gas mixture was injected into the CVD chamber. During the deposition process, argon was introduced and C_2H_2 was pyrolyzed, and carbon was deposited on the surface of the AAO templates. Finally, the AAO template was etched, and hollow nanoneedles was obtained. Golshadi et al. [19, 20] reported a similar fabrication process for constructing hollow nanoneedles.

Another CVD technique, metal organic vapor phase epitaxy (MOVPE), also known as MOCVD, has been proposed for the fabrication of nanoneedles. The growth of nanoneedles was achieved through the surface reaction of metal-organic precursor complexes containing the target chemical elements. Unlike CVD, MOVPE does not require a noble metal catalyst, and the reaction temperature is relatively lower, which makes it satisfactory for continuous and mass production. Persson et al. [21] fabricated solid gallium phosphide (GaP) nanoneedles via the MOVPE method.

The CVD technique for nanoneedle fabrication is highly efficient, has the possibility of mass production and is suitable for numerous materials ranging from semiconductors to metal materials. CVD could fabricate solid and hollow nanoneedles. The diameter, density and height of the nanoneedles could be easily tuned by varying the chemical gas composition, catalyst size, heating temperature and reaction time. However, the CVD process has some limitations, such as poor surface morphology due to layer-by-layer deposition and a high deposition temperature. In addition, the distribution of metal nanoparticle catalysts at desirable locations is difficult, and metal nanoparticles may contaminate nanoneedles.

(2) Atomic Layer Deposition

Atomic layer deposition (ALD) is a thin film deposition technique based on the sequential use of a gas-phase chemical process. The majority of ALD reactions use two precursors that react with the surface of a substrate sequentially and in a self-limiting manner. Thin nanoneedles could be slowly deposited by repeated exposure to separate precursors.

ALD is a low-temperature and uniform deposition technology, and it has been used to produce hollow nanoneedles. He et al. [22] fabricated hollow nanoneedles using ALD combined with etching technique, as shown in Table 2. A track-etched polycarbonate membrane, which had uniform nanopores of 145 ± 2 nm diameter, was chosen as the template. First, the track-etched polycarbonate membrane was coated with ≈ 40 nm Al_2O_3 on all surfaces via ALD. Then, the Al_2O_3 layer on the top surface was fully etched via reactive ion etching (RIE) using BCl_3/Cl_2 as the etchant. Finally, the polycarbonate substrate was partially etched using O_2 RIE to expose the Al_2O_3 hollow nanoneedles.

Compared with CVD technique, ALD is a monatomic layer-by-layer growth process, while CVD is a core-forming growth process. ALD requires repeated exposure to separate precursors to slowly deposit nanoneedles, while CVD simultaneously introduces multiple precursors to form nanoneedles [23]. ALD has higher processing accuracy, more uniform film formation, and lower reaction temperature. However, the fabrication process is more complex, the growth rate is lower and the fabrication process is time consuming. ALD requires expensive equipment and harsh experiment condition, which limits the feasibility of hollow nanoneedle fabrication under conventional laboratory conditions [24].

2.1.2 Top-Down Approach

Compared with the bottom-up strategy, the top-down strategy mainly involves etching substrates into 1D nanoneedles using advanced micro/nano manufacturing technologies. Physical and chemical etchings are key processes in the top-down strategy. Depending on the etchant, the etching process can be divided into dry etching and wet etching. High-energy beams, such as laser beams, electron beams, and ion beams, can also be employed for nanoneedle etching. Nanoimprinting has also been introduced for nanoneedle fabrication.

(1) Metal-assisted Chemical Etching.

Metal-assisted chemical etching (MACE) is a typical wet etching process that utilizes gold or other noble metal particles as catalysts to induce the etching process and thereby construct nanoneedles [25, 26]. MACE has been widely used to fabricate silicon nanoneedles [27, 28]. The fabrication process can be described as follows. A layer of noble metal particles, such as Au, Pt, and Ag, is first deposited on the silicon substrate, and the substrate is then submerged in a mixed etching solution, such as HF and H_2O_2. Noble metal particles act as catalysts, which can locally accelerate the etching process. As the etching process proceeds, the noble metal might sink into the silicon substrate, and silicon nanoneedles are finally obtained.

Chiappini et al. [29–31] fabricated porous silicon nanoneedles using MACE for intracellular nucleic acid delivery, as shown in Table 2. A layer of silicon nitride (160 nm) was deposited on the silicon wafer. The wafer was patterned to a desired pitch using photolithography and etching. Then the substrate was cleaned with 10% HF for 60 s and deposited with Ag particles. After washing and drying, the wafer was etched with 10% HF and 122 mM H_2O_2 for 8.5 min to form porous silicon pillars. Finally, the pillars were sharpened by reactive ion etching to form porous nanoneedles. Experimental results showed that dimensions of nanoneedles could be tuned by adjusting various processing parameters, such as reaction time, temperature, and concentration of the HF.

Compared with the bottom-up strategy, MACE has unique advantages, such as a simple and low-cost fabrication process, highly efficient and suitable for mass production [27]. However, it still has some limitations. It is difficult to control the distribution and dimension of the metal particles, leading to random distribution of nanoneedles and a wide range of nanoneedle dimensions [14, 32]. Furthermore, the etchant is not environmentally friendly.

(2) Reactive Ion Etching

Reactive ion etching (RIE) is a typical dry etching method that involves bombarding the substrate with high-energy gas ions to construct nanoneedles [33, 34]. RIE is usually combined with photolithography or a deposition process to create a mask on the substrate and define the density, dimension, and spacing of the nanoneedles. During the fabrication process, etchant gases are discharged with a high-frequency electric field to produce high-energy ion groups. These ion groups bombard the substrate accompanied by a series of physical and chemical reactions, leading to the removal of the undesired substrate and the remaining parts covered by the mask, finally forming nanoneedles.

Wang et al. [35] used plasma deposition and RIE to develop solid nanoneedles for in situ probing and analyzing intracellular signaling. First, the silicon wafer was ultrasonically polished and deposited with 7 μm thick nanodiamond films using MPCVD. After deposition, the RIE process was performed to construct the nanoneedles with an input microwave power of 800 W for 3 h. The reactive gas was a mixture of Ar and H_2 (volume ratio 45%: 55%). The processed nanoneedles were conical in shape with a height of ~5 μm and a bottom diameter of ~300 nm. Furthermore, not only solid nanoneedles but also hollow nanoneedles can be fabricated using the RIE technique. He et al. [36] fabricated silicon hollow nanoneedles from a single crystal silicon wafer using RIE, as shown in Table 2.

RIE can be used to fabricate highly ordered, highly precise nanoneedles with high aspect ratios. The patterning and etching steps can be precisely controlled by tuning the processing parameters, and the geometries of nanoneedles can also be adjusted intentionally. However, RIE has some typical limitations; gas ions might induce side defects in nanoneedles. The microfabrication equipment for RIE is costly, and nanoscale masks, which are usually fabricated by electron beam lithography, may increase the preparation cost [13].

(3) Focused Ion Beam Fabrication

The focused ion beam (FIB) fabrication method utilizes ion beams generated and accelerated by an ion source to focus and scan the substrate and remove undesired material. High-energy FIB has been employed to fabricate nanoneedles with high aspect ratios.

Angelis et al. [37–40] fabricated hollow nanoneedles using FIB to delivery nanoparticles and molecules, as shown in Table 2. An S1813 photoresist was spin-coated on a Si_3N_4 membrane and soft-baked. A 7 nm-thick titanium layer and a 20 nm-thick gold layer were sputter-coated on the back of the Si_3N_4 membrane. Subsequently, FIB technique was applied to drill hole arrays in the back of the Ti/Au-coated Si_3N_4 sample with a voltage of 30 keV and a current varying from 0.23 to 2.5 nA. After that, the sample was washed with oxygen plasma to smooth the photoresist. Finally, hollow nanoneedles were obtained by dissolving the photoresist.

FIB is a noncontact and high-energy fabrication process, which has good flexibility, and almost all materials can be processed. What's more, FIB is suitable to construct hollow nanoneedles with high aspect ratios and tunable sizes, shapes, layouts and vertical sidewalls [41]. However, FIB is low frequency and the equipment is expensive, which tremendously limits the mass production possibility of hollow nanoneedles using FIB.

(4) Nanoimprinting

Nanoimprinting can fabricate nano-scale patterns by the deformation of imprint resists with the help of external mechanical forces. A negative template is often used to construct nanoneedles. In general, a thin imprint resist layer is first spin coated on the substrate as a transfer medium. Subsequently, patterns are created by mechanical deformation of the

imprint resist when the negative template is compressed toward the substrate. Then, the imprint resist is cured by heat or UV light during imprinting. The residual layer is etched following the imprint process. The nanoneedles are fabricated upon the removal of the template.

Li et al. [42] fabricated aluminum nanoneedles through nanoimprinting combined with electrochemical etching, as shown in Table 2. First, an aluminum sheet was electrochemically polished in a perchloric acid and ethanol mixture (volume ratio 1:3). Under a pressure of 2×10^4 N/cm^2, the pattern was transferred from the silicon template to the aluminum sheet. The dented substrates were then anodized in an etchant composed of citric acid (2 wt%), ethylene glycol (2 wt%) and H_3PO_4 (0.01%) at 10 °C and a voltage of 400 V. The aluminum nanoneedle substrate was finally obtained by etching away the anodization layer. This aluminum nanoneedle was employed for rapid detection of carbohydrate antigen 199.

Nanoimprinting is a simple nanolithography process with low cost, high throughput and high resolution. The key concerns for nanoimprinting are overlay, defects, template patterning and template wear. Specifically, the lingering barrier to nanoscale patterning is the current reliance on other lithography techniques to fabricate the template. Above all, nanoimprinting is a promising fabrication method that can replace traditional photolithography technology.

2.2 Intracellular Applications of Nanoneedles

Cells are the basic structural and functional units of organisms. In-depth exploration of cells is ungently significant in biology. However, the cell membrane, with an approximate thickness of 6 nm, possesses a layered hydrophilic/hydropho-bic structure, a barrier that makes it difficult for most artificial probes and drugs to access the cytoplasm. How to penetrate cell membrane is a fundamental topic of cell research. Traditional cell membrane penetration methods, including microinjection, patch clamping, and electroporation, have attained modest successes and high universality. However, the first two methods have difficulty achieving high-throughput operation for large numbers of cells, and electroporation requires different fine-tuned operations for each cell type. In contrast, nanoneedles can readily penetrate cell membranes by generating highly localized stress with their sharp structures and high aspect ratios. Furthermore, nanoneedles can connect the inside and outside of cells, allowing for various intracellular biomedical applications, including electrical recording, biochemical sensing, and drug delivery. In this section, we will introduce the intracellular applications of nanoneedles.

2.2.1 Cell Sensing

(1) Electrical Recording

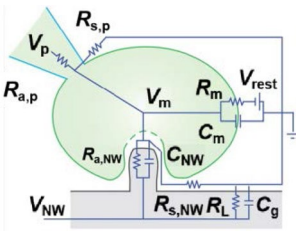
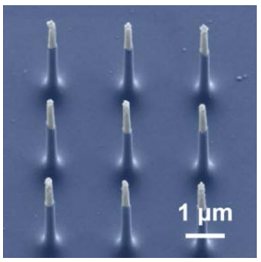
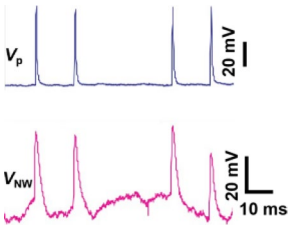
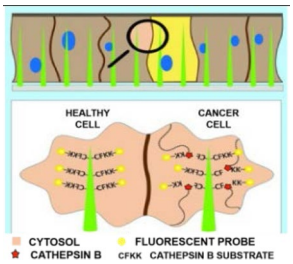
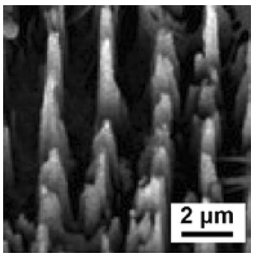
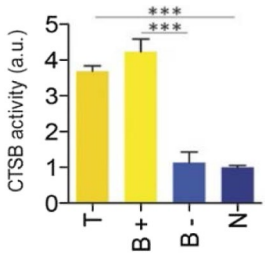
Cellular electrical signals originate from the ions flowing across the membrane and chemical reactions inside cells. Direct measurement of cell electrophysiological states is of great significance for gaining in-depth knowledge of network-forming activity and complicated communications [24]. Extracellular recording suffers from significantly reduced signal strength and quality. The cell membrane is the main barrier to intracellular signal measurement. Nanoneedles can penetrate the cell membrane, not only forming tight junctions with the cell membrane but also decreasing impedance, thus achieving long-term and minimally invasive intracellular signal recordings [43].

Nanoneedle-based intracellular recording also provides a meaningful advantage in obtaining high signal-to-noise ratio signals for cell research. Robinson et al. [44] fabricated a vertical nanoneedle electrode array via a standard top-down nanofabrication process, as shown in Table 3. Each nanoneedle was constructed with a Si core, an insulating SiO_2 shell layer, and a sputter-coated Ti/Au tip, which was utilized as the active electrode for sensing the action potential and recording the intracellular signals. The silicon core and metal tip provided electrical access to the interior of the cell, and the SiO_2 shell both prevented current leakage through the nanoneedle sidewalls and formed a tight seal with the cell membrane. Once the nanoneedle had access to the interior of the cell, the nanoneedle electrode array could measure and control the cell membrane potential (V_m) by taking advantage of the electrochemistry at the nanoneedle tips. Moreover, the electroporation-generated pores could seal within several minutes, indicating that the invasiveness of the nanoneedles was lower than that of a traditional patch clamp [43]. In summary, nanoneedles can measure intracellular electrical signals in the long term with high sensitivity and minimal invasiveness, making them suitable for many potential applications, such as distinguishing cells and cell mapping.

(2) Biochemical Sensing

Profiling intracellular signaling cascades and networks is a core topic in modern biology. Traditional detection of intracellular species requires cell lysis and the extraction of cell contents. In these cases,

Table 3 Cell sensing applications of nanoneedles

Sensing methods	Schematic illustration	SEM images	Performance
Electrical signals			
Electrochemilu-minescence			

cell lysis-based methods are one-off tests, making longitudinal sampling within the same set of cells impossible, thus surrendering the capability of capturing dynamic information within cells. Nanoneedles are an attractive intracellular sensing element due to their unique minimally invasive capacity to penetrate the cell membrane. Moreover, hollow nanoneedles can be used to extract intracellular contents without disrupting cell activity, making them suitable for repeated intracellular sampling and multiplexing detection [30, 45].

Chiappini et al. [30] developed a nanoneedle-based biosensor for mapping intracellular activity and discriminating the difference in cathepsin B (CTSB) activity throughout tissue extracted from patients with esophageal cancer, as shown in Table 3. The sensor consisted of a fluorescently labeled CTSB cleavable peptide covalently conjugated to a nanoneedle. Upon penetrating the cell membrane, nanoneedles interfaced with the intracellular environment, the peptide was cleaved, and the fluorescent label was released to the cytosol to sense the intracellular activity. The nanoneedle-based biosensor could discern CTSB-positive cancer cells from CTSB-negative cells in a mixed culture, highlighting CTSB-positive and CTSB-negative regions within a single tumor resection specimen. The nanoneedles could also sense the difference in CTSB activity in tissue resected from patients with esophageal cancer. These findings highlight the potential for nanoneedles as a minimally invasive exploratory tool for single-cell mapping of intracellular activity.

2.2.2 Cell Drug Delivery

Efficient delivery of molecules into living cells is vital to both the fundamental study of cell biology and novel therapeutic development [33]. Many strategies have been developed to facilitate cross-membrane transport by overcoming the barrier of the cell membrane. Traditional delivery systems usually depend on transfection reagents that initiate endocytosis processes that suffer from cargo degradation, low efficiency, and high toxicity to cells. Nanoneedles have been reported for cell drug delivery, which allows various types of cargo ranging from small molecules to macromolecules to enter the cytosol with nondestructively or locally disrupting the cell membranes. Compared with the traditional carrier-mediated delivery system, nanoneedle techniques can prevent drug degradation due to endosomal entrapment and decrease the potential toxicity of carriers accumulated within the cells [29]. Nanoneedles for cell drug delivery can be divided into solid nanoneedles and hollow nanoneedles depending on their structure.

Solid nanoneedles used in cell drug delivery mainly act as penetrating tools. Matsumoto et al. [46] used solid nanoneedles to create transient pores in the plasma membrane to allow the access of desired macromolecules into live cells, as shown in Table 4. Another common strategy for molecular delivery is to functionalize

the nanoneedle surface with chemically active motifs to promote the electrostatic or covalent conjunction of delivered cargo. Chiappini et al. [47] developed silicon nanoneedles capable of interfacing with cells for drug delivery. The weak binding linkage can be designed to dissociate when it penetrates the cell membrane, allowing the surface-bound biomolecules to be released into the cell. Solid nanoneedle could penetrate the cell membrane for drug delivery; however, it has some limitations, such as low drug load, low drug utilization rate and uncontrollable drug release.

Hollow nanoneedles are tube-shaped structures that are open at both ends, and they can be used to deliver drugs continuously to cells. Once they penetrate the cell membrane, the inner fluidic channel acts as a conduit and can directly access the cytosol, allowing fluidic transfer between the extracellular and intracellular spaces. Wen et al. [24] developed a hollow Pt nanoneedle with a 400 nm diameter integrated with a low-voltage nano-electroporation system to achieve intracellular drug delivery, as shown in Table 4. Moreover, by taking advantage of the nanoscale size of the hollow nanoneedles, negligible damage to the cell is introduced when the nanotubes penetrate the cell membrane, and the viability of the cells can be improved. Hollow nanoneedles enable temporal and dose control of delivered cargo. Specifically, by using hollow nanoneedles as drug containers, more accurate and tiny amounts of drugs could be delivered and administered to the target.

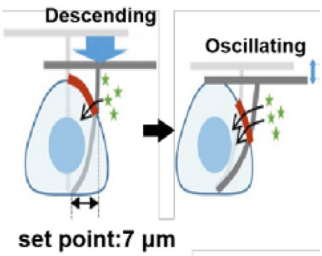
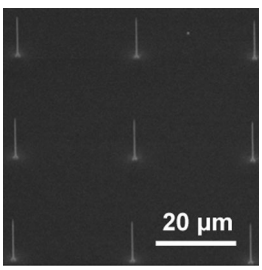
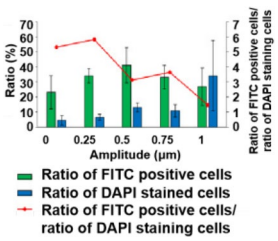
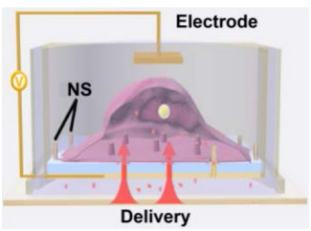
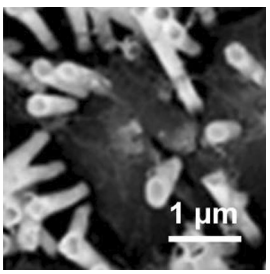
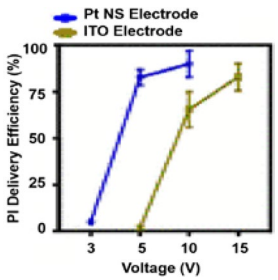
3 Microneedles

Microneedles are a type of miniaturized medical device that contains an array of micro sized needles with heights no more than 1 mm and sharp tips [48, 49]. According to the needle structure, microneedles can usually be divided into solid microneedles, hollow microneedles, and porous microneedles. According to manufacturing materials, microneedles can be fabricated from a variety of materials, including silicon, metals, and polymers. Fabrication of microneedles in a desired structure in a cost-effective manner is vital for the biomedical application of microneedles. In this section, we will briefly introduce the typical fabrication methods and biomedical applications of microneedles.

3.1 Fabrication of Microneedles

According to the manufacturing strategies, the fabrication of microneedles can be classified into subtractive manufacturing, formative manufacturing, and additive manufacturing. Typically, microneedles can be fabricated by subtractive processes. Three-dimensional microneedles are selectively carved out of a two-dimensional substrate through microfabrication processes such as photolithography with wet or dry etching and micromachining. Additive manufacturing, such as typical techniques of 3D printing and drawing lithography, can form three-dimensional polymer microneedles from droplets or two-dimensional surfaces. The formative

Table 4 Cell drug delivery applications of nanoneedles

Nanoneedles	Schematic illustration	SEM images	Delivery performance
Solid nanoneedle			
Hollow nanoneedle			

manufacturing techniques of microcasting and microinjection molding are near-net shape forming processes for the fabrication of microneedles.

3.1.1 Subtractive Manufacturing

The subtractive manufacturing process utilizes various methods to remove the excised part of a block material to obtain the desired microneedles. According to the material removal methods, subtractive manufacturing of microneedles can be classified into photolithography and etching methods and microfabrication methods.

(1) Photolithography and Etching

Photolithography with etching is one of the most widely used techniques in microelectromechanical systems. Photolithography utilizes light to transfer a pattern from a mask to a light-sensitive chemical photoresist on a substrate surface. Subsequently, the substrate is etched to remove the unwanted material, and the microneedle structure is fabricated [50–52]. Etching can be divided into wet etching and dry etching based on the etchant [53, 54].

Ma et al. [54] fabricated tungsten microneedles using photolithography with etching. The SEM image of fabricated microneedles is shown in Table 5. A 4-inch bulk wafer was sputter-coated with an aluminum mask by photolithography to define the dimension, location, and density of the microneedles. The SF6-based DRIE process was conducted to form a tungsten micropost array on the tungsten wafer. Finally, the aluminum mask layer was removed, and the tungsten micropost was sharpened to form tungsten microneedles.

Photolithography with etching is suitable for the highly precise mass production of silicon microneedles. The geometric characteristics of microneedles, such as diameter, height, and density, could be elaborately controlled by adjusting the fabrication parameters. However, there are still some limitations of photolithography with etching, including the requirement of complicated fabrication steps and sophisticated equipment. Furthermore, silicon microneedles with a high aspect ratio may break off in the skin during skin penetration and result in skin infection since silicon is a brittle material [55].

(2) Micro Machining

Numerous micromachining methods, such as laser machining, micromilling, and wire electric discharge machining, have been introduced to fabricate microneedles. The typical micromachining methods for microneedles are presented in this section.

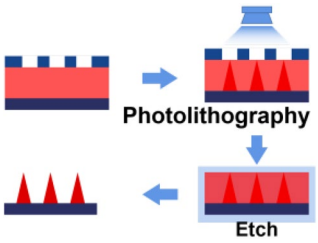
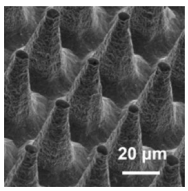
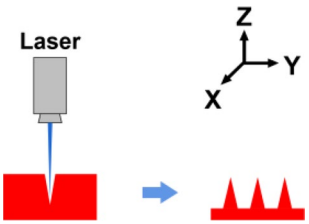
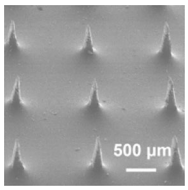
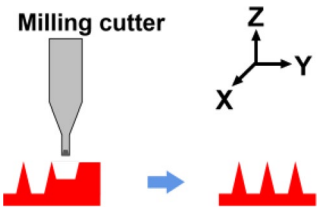
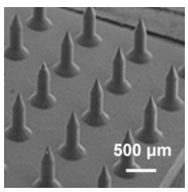
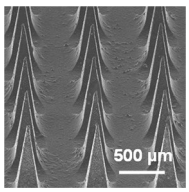
1. Laser Machining

Laser machining utilizes a concentrated laser beam to directly irradiate and ablate the substrate surface to fabricate 2D or 3D microneedles. Laser machining can fabricate in-plane or out-of-plane microneedles. Depending on the machining mechanism, laser machining of microneedles can be classified into laser cutting [56] and laser ablation [57].

Laser cutting uses a laser beam to cut through a plate along a designed route to construct microneedles. This method is suitable for fabricating in-plane metal microneedles. Xie et al. [58, 59] constructed in-plane microneedles from a 100 μm thick stainless-steel plate using laser cutting. Each microneedle patch contained 10 microneedles, whose length and width were 800 μm and 225 μm, respectively. Sun et al. [60] fabricated composite metal microneedles via laser cutting, as shown in Table 5. Subsequently, microneedles were manually bent 90° out of the plane to form 3D microneedle. The 3D microneedles were then coated with SU-8 and a gold layer to construct a composite microneedle electrode for body temperature and biosignal monitoring. No bending or fracture happened on the metal microneedles after 100 times porcine skin penetration, indicated its excellent mechanical property. Laser cutting is highly flexible and can cut almost arbitrary shapes in plane. For example, a bioinspired microneedle with backward micro barbs along its spine [61] was fabricated using laser cutting which was difficult for other microfabrication methods.

During the laser ablation process, the laser beam irradiates and scans the metal surface in a certain sequence layer by layer. The scanned metal gradually melts and evaporates under the high energy of the laser beam to form 3D microneedles. Zhou et al. [62] constructed copper microneedles with microtextures on the surface using laser ablation. Under continuous irradiation of a laser beam, the copper gradually melted, and a groove structure was formed on the surface. Additionally, the molten metal particles splashed and were recast during the process. Some of the recast metal was deposited and integrated on the unscanned area, forming cone-shaped microneedles. Experimental results showed that process spacing has important

Table 5 Subtractive manufacturing of microneedles

Fabrication methods	Fabrication illustrations	SEM images	Materials	Advantages	Typical limitations	Typical applications
Photolithography and etching		Solid microneedles 	Silicon, Tungsten	High precision, high efficiency, fit for mass production	Expensive equipment, harsh environment, multiple steps	Bio-sensoring, drug delivery
Micro machining						
Laser machining		Solid microneedles 	Copper, Titanium, Stainless steel	High efficiency, maskless, high flexibility, non-contact machining	Rough surface quality, lower machining accuracy, arced needle tip	Bio-electrode, bio-sensoring, drug delivery, bioinspired microneedles
Mililing machining		Solid microneedles 	Aluminum alloy, Stainless steel 316L	High precision, good surface quality	Low efficiency, high cost	Microneedle template
Wire electrical discharge machining		Solid microneedles 	Stainless steel	High precision, good aspect ratio, reproducibility	Time consuming, limited material	Bio-electrode

influence on the forming of microneedles; better microneedles were obtained when the process spacing was 0.1 mm.

Laser machining can be used to fabricate microneedles in a maskless, highly flexible and non-contact manner, especially suitable to fabricate metal microneedles. Metal microneedles, with excellent electrical conductivity and mechanical properties, are more suitable for biosignal monitoring [63]. However, high heat generation is inevitable during the laser machining process, leading to poor surface quality, lowering the machining accuracy and creating arced needle tips, which may significantly hinder skin penetration. Some advanced laser equipment, including picosecond lasers [64] and femtosecond lasers [65, 66], has been investigated to reduce these side effects.

2. Milling Machining

Milling machining is a typical subtractive manufacturing method that utilizes the relative movement between the high-speed rotating milling cutter and the workpiece to cut undesired material and obtain microneedles, as shown in Table 5 [67]. Bediz et al. [68] fabricated a rectangular microneedle using micromilling method. A set of tailor-made single-crystal diamond microend mill (straight and tapered diamond microend mills) tools with a 300 μm cutting diameter was designed and fabricated to make the microneedle. Erika et al. [69] manufactured a conical 316L stainless steel microneedle with 1 mm in base diameter and 1 mm in height.

Micromilling enables the fabrication of rectangular or conical microneedles with high precision.

However, the cutting tool should be on micron scale to obtain the microneedle. The feed rate and cutting depth should be sufficiently small, and the spindle speeds should be ultrahigh to reduce the cutting force and breakage risk of the cutting tool. Therefore, more effort should be made to enhance the mechanical properties of the cutting tools, optimize the cutting parameters, and improve the stability of the micromilling machine.

3. Wire Electrical Discharge Machine
Wire electrical discharge machining utilizes electrical discharge to remove conductive material from the substrate and fabricate the desired microneedles. Pigeon et al. [70] fabricated 316L stainless steel microneedles with high aspect ratios that were 40–50 μm in width and 1.5 mm in height by two-pass wire electrical discharge machining. However, the arching between the wire electrode and the workpiece produced a thin layer of thermally degraded metal; thus, electropolishing was needed to polish the surface. Recently, Liu et al. [71] fabricated 316L stainless steel microneedles using low-speed wire electric discharge machining where 0.15 mm copper wire worked as the electrode, as shown in Table 5. The working voltage, cutting speed, and workpiece-wire distance were 35 V, 6.3 mm/min, and 0.08 mm, respectively. Low-speed wire electric discharge machining could fabricate high-quality metal microneedles with excellent mechanical properties and a small tip radius of less than 10 μm, which is beneficial for skin penetration.
In summary, wire electric discharge machining could non-contact fabricate high aspect ratio, and high-precision microneedles, and is suitable for the fabrication of hard and electric conductive materials. However, low fabrication efficiency and potential ablation damage are the main limitations.

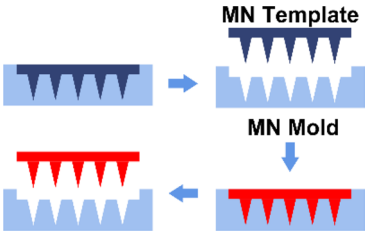
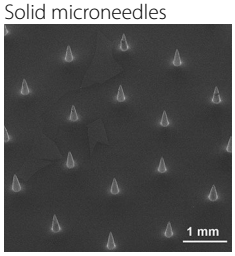
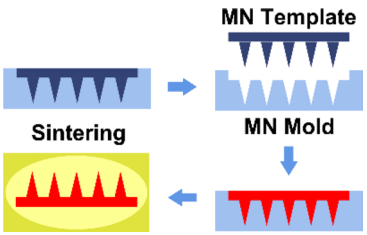
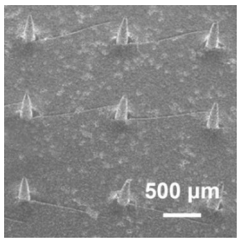
3.1.2 Formative Manufacturing

Formative manufacturing usually utilizes a negative mold to replicate microneedles. According to the fabrication mechanism, formative manufacturing can be divided into two categories, microcasting and microinjection molding.

(1) Microcasting
The preparation of microneedles using microcasting involves 4 steps: master template preparation, mold fabrication, polymer filling, and peeling. A desired microneedle template is first prepared and applied to reproduce the microneedle mold. Subsequently, molten polymers are poured into the microneedle mold via vacuum or centrifugation. After solidification, the filling materials are removed from the mold to obtain a microneedle structure.
Yang et al. [72] fabricated solid polymethyl methacrylate (PMMA) microneedles by the microcasting method for transdermal insulin delivery, as shown in Table 6. A commercial microneedle stamp consisting of 42 conical microneedles was used as a master template to produce the polydimethylsiloxane (PDMS)-microneedle mold. Subsequently, the prepolymerized PMMA was cast into the PDMS mold under a vacuum of 2 kPa. After solidification, the PMMA microneedles were peeled away from the PDMS mold. Microcasting is suitable for producing drug-loaded dissolving microneedles or porous microneedles for transdermal drug delivery. Wang et al. [73] fabricated a dissolvable layered microneedle with a three-step microcasting method. The drug-encapsulated hyaluronic acid solution was cast on top of the dissolvable layered microneedle to form a shell, and the polyvinyl alcohol solution was cast to obtain the core and base of the microneedles. When microneedles were inserted into the skin, the drug was released immediately once the hyaluronic acid layer was dissolved. Microcasting provides a simple manufacturing technique for fabricating polymer microneedles. As a simple and highly efficient fabrication technique, microcasting has potential to be used for the mass production of microneedles. Numerous materials, such as PMMA [72], silk fibroin [74], HA gel solution [75], and hydrogels [76], can be used to fabricate microneedles in cone shapes [77, 78], pyramids [79], double-layered structures [80], core-shell structures [81], tip-hollow [82], and star shapes [83] by the microcasting method.

(2) Microinjection Molding
Microinjection molding has been introduced for the fabrication of porous metal microneedles [84]. Metal and polymer powders (binder, diffusing agent, etc.) were mixed and injected into the micromold using an injection machine to obtain the raw 3D microneedle architecture. After high-temperature sintering, metallurgical bonding occurred between the metal powders, forming the desired metal microneedles.
Li et al. [77] fabricated titanium porous microneedles using microinjection molding, as shown in Table 6. The titanium slurry consisted of titanium (46 wt%), ethanol (46 wt%), poly (vinyl butyral) (1 wt%), butyl benzyl phthalate (6.4 wt%), and

Table 6 Formative manufacturing of microneedles

Methods	Fabrication illustrations	SEM images	Materials	Advantages	Typical limitations	Typical applications
Micro casting		Solid microneedles 	Polymer, Hydrogel	High efficiency, low cost, easy operation, fit for mass production	High resolution mold, low flexibility, multiple steps	Bio-electrode, transdermal drug delivery, triboelectric nanogenerator
Micro injection molding		Porous microneedles 	Titanium, Stainless steel 316L, Aluminum	Fit for porous microneedle, controllable porosity	Low efficiency, low flexible, multiple steps	Transdermal drug delivery, bio-sensing

0.6 wt% Solsperse 20000 as the dispersant. The slurry was injected into a microneedle mold and dried at room temperature for 48 h to obtain the raw microneedle body. The raw body was sintered at 1250 °C for 2 h under an Ar atmosphere to form a porous microneedle. Gholami et al. [85] reported a similar work in which porous microneedles were fabricated from alumina suspensions. Benefitting from the high porosity and interconnected pore structures, porous metal microneedles are suitable for liquid drug storage and *in vivo* biofluid sampling. Microinjection molding is suitable for the fabrication of porous metal microneedles, and the porosity and pore dimensions can be tuned by adjusting the mixed powder composition and sintering parameters. There are many advantages of microcasting and microinjection molding, including high efficiency and low cost, while the fabrication of a high-resolution micromold is required. Numerous microfabrication methods, such as 3D printing [86], re-engraving [72], laser machining [87], micromilling [88], and microdrilling [89], have been applied to produce micromolds. However, fabricated micromolds have relatively low aspect ratios that limit the application of microneedles.

3.1.3 Additive Manufacturing

Additive manufacturing can be used to construct 3D microneedles with metal powders, molten polymers, and even ceramics layer by layer, which makes it suitable for fabricating complex 3D microneedles such as porous, gradient, fractal microneedle structures, which are difficult to be fabricated by traditional subtractive manufacturing or formative manufacturing processes [90–92].

(1) Typical 3D Printing

Typical 3D printing enables the fabrication of 3D microneedles by stacking the materials layer by layer. A 3D numerical model is first designed with CAD software and sliced by slicing software. Then the sliced model is printed into microneedles layer by layer. Numerous 3D printing techniques, such as stereolithography (SLA) [91], digital light processing (DLP) [93], fused deposition modeling (FDM) [94], selected laser melting (SLM) [95], selected laser sintering (SLS) [96], and two-photon polymerization (TPP) [97], have been introduced to produce microneedles. SLS and SLM are powder-based methods suitable for metal microneedle fabrication, but the printing resolution and surface quality of the printed objects are poor. The FDM process uses a nozzle to extrude the fused polymers. It is a simple and cost-effective fabrication process, but the surface quality is poor, especially the layer-by-layer finish. In this section, we will mainly focus on the SLA and DLP techniques of microneedle fabrication.

SLA is a vat polymerization technique and is the most common method of fabricating microneedles due to its high fabrication efficiency and printing accuracy. The laser irradiates the liquid photopolymer resin and causes the polymerization reaction, curing the resin into the designed microneedles. Douroumis et al. [91, 92] fabricated rectangular pyramid microneedles with the SLA process using a Form 2 SLA printer for transdermal drug delivery, as shown in Table 7. Biocompatible Class I resin was applied to fabricate the microneedles. The height and width of the microneedle was 1 mm, and each patch of microneedles was arranged in a 6×8 grid. After printing, the microneedle patch was washed with isopropyl alcohol to remove the unpolymerized resin residues and cured for 60 min at 40 °C under UV radiation.

DLP utilizes a mirror array and a light bulb to cure the photopolymer and shape the desired microneedles. The SLA technique is a point-by-point curing process, while the DLP technique can cure each layer at once; therefore, it has a relatively higher fabrication efficiency than SLA [96]. Lu et al. [93] developed drug-loaded microneedles through a DLP apparatus. A skin anticancer drug was incorporated into the photosensitive polymer blend before photopolymerization. Then, a patterned light was irradiated onto the surface of the liquid material, initiating crosslinking and forming microneedles layer-by-layer. Han et al. [98] fabricated microneedles with bioinspired backward-facing curved barbs using the DLP method, as shown in Table 7. The 3D bioinspired backward-facing barbed microneedle had micro barbs around the surface of the microneedle body, which is difficult to fabricate with traditional methods. The bioinspired microneedle had excellent tissue adhesion performance, which was 18 times stronger than that of barbless microneedles.

3D printing is a high efficiency and high flexible microneedles fabrication method, could fabricate complex 3D microneedle structures which are difficult to fabricate via traditional subtractive or formative manufacturing. However, due to the equipment accuracy, the manufactured microneedles have a relatively rough surface and large tip radius, which substantially affects the mechanical properties of microneedles for skin penetration. The available materials suitable for SLA and DLP are relatively limited, and the drug activity may be reduced or lost during polymerization. Furthermore, several other factors, such as biocompatibility, thermal properties, and chemical stability, should be considered when applying such methods to the biomedical field.

(2) Drawing Method

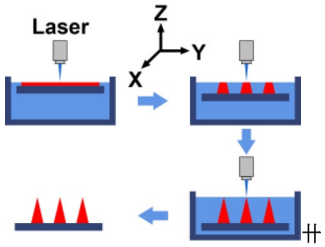
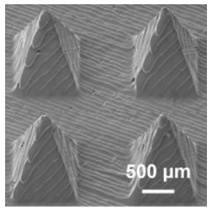
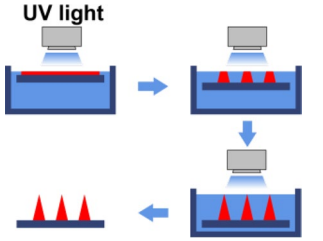
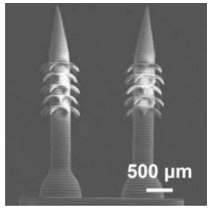
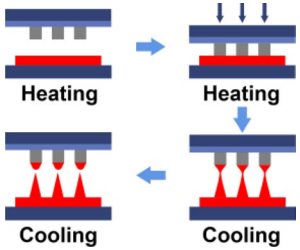
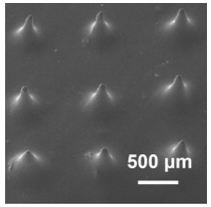
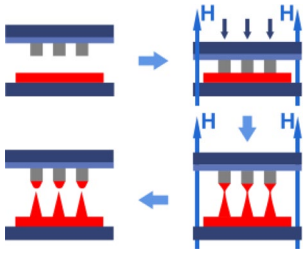
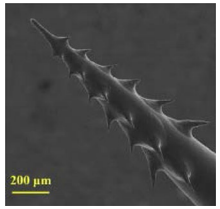
Drawing is a novel additive manufacturing method of fabricating polymer microneedles. Different from the traditional 3D printing method of stacking materials layer by layer to build 3D microneedles, drawing techniques construct microneedles by pulling or stretching 2D viscous polymer materials to form 3D structures under the coupled action of the material properties, gravity and the external field, such as centrifugal force [99], thermal field [100], magnetic field [101], and electric field [102]. Depending on the external field, the drawing method can be divided into thermal drawing, magnetorheological drawing, electric drawing, and centrifugal lithography. However, both the electric drawing method and centrifugal lithography method require complicated procedures and the process parameters are difficult to control. Thus, in this section, we will mainly introduce thermal drawing and magnetorheological drawing for microneedle fabrication.

1. Thermal Drawing

Thermal drawing can be used to directly construct conical 3D microneedle structures from 2D slurry polymers without the use of a mold [103]. Thermal drawing technology utilizes the unique glass transition character of thermosetting polymers to fabricate microneedles. The fabrication process can be divided into three main steps. First, the thermosetting polymer is spin coated and heated above the glass transition temperature (T_g) to a high elastic state on a heating plate. Subsequently, the heated micropillars slowly approach and make contact with the slurry polymer, and finally, the pillars are drawn upward, and the polymer is lifted simultaneously, forming a 3D microneedle array.

Lee et al. [100] first reported the thermal drawing method to process a high-aspect-ratio microneedle of 3600 μm. An SU-8 2050 photoresist was spin-coated on a flat glass panel at 1000 r/min, and the panel was placed on a hot plate at 120 °C for 5 min and then cooled to room temperature. A 3×3 pillar array made contact with the photoresist and was drawn upward at 60 °C for 6 min at a speed of 10 μm/s. Then, the photoresist was cured at room temperature for 30 min. After that, the frame was separated to obtain the microneedle. Ren et al. [103] also fabricated PLGA microneedles with thermal drawing method, as

Table 7 Additive manufacturing of microneedles

Methods	Fabrication illustrations	SEM images	Materials	Advantages	Typical limitations	Typical applications
Typical 3D printing						
Stereo-lithography		Solid microneedles 	Photo curable polymer	High efficiency, high repeatability, high flexibility	Poor surface quality, rough surface, large tip radius	Transdermal drug delivery
Digital light processing		Bioinspired microneedles 	Photo curable polymer	Complex 3D structure, high flexibility, non-contact machining	Rough surface, limited materials,	Transdermal drug delivery, fog harvest
Drawing method						
Thermal drawing		Solid microneedles 	SU-8, PLGA, Maltose	High aspect ratio, high efficiency, maskless	Low repeatability, limited materials, high temperature	Bio-electrode, brain-computer interface
Magnetorheological drawing		Bioinspired microneedles 	Curable magnetorheological fluid	One-step fabrication, maskless, high flexibility	Low biocompatibility, limited material	Transdermal drug delivery, bio-electrode, bio-sensing

shown in Table 7. The tip diameter of the solid microneedle increased as the thickness of the coated SU-8 increased [104]. While the length of the microneedle can be determined by the diameter of the drawing pillars [105].

Thermal drawing is a versatile rapid prototyping method. Thermal drawing can freely form polymer microneedles with ultrahigh aspect ratios and sharp tips without relying on any templates. However, it is still challenging to repeatedly produce microneedles with identical shapes due to the multiple fabrication parameters of the processing conditions, such as the drawing temperature, drawing speed, drawing heights, and parallelism in the drawing setup. In addition, thermal drawing is only possible with thermosetting materials, and the high temperatures applied during the fabrication process may damage the drug activity of drug loaded microneedles.

2. Magnetorheological Drawing

Magnetorheological drawing could draw a droplet of curable magnetorheological fluid to form microneedles under an external magnetic field. Chen et al. proposed this fabrication method and established an automated customized platform [101].

A novel curable magnetorheological fluid was prepared with curable liquid (epoxy/novolac resin), curing agent (modified aliphatic amine), and magnetic particles (iron powder) at a mass ratio of 3:1:3. Copper pins as the drawing pillars were dipped in the magnetorheological fluid pool, leading to firm contact between the magnetorheological fluid droplet and the substrate under an external magnetic field. Subsequently, the copper pins were drawn back, and the magnetorheological fluid droplet was drawn from a hemispherical state to a conical shape, forming a liquid microneedle that could stand on the substrate under the multiple-field action of surface tension, gravity and magnetic force. The liquid microneedle array was prebaked by hot air blowing and then solidified in a vacuum oven to achieve solid microneedles. Theoretical and experimental results showed that the tip radius of the microneedle was mainly influenced by the intensity of the external field, and the height of the microneedle increased with the external field intensity and droplet volume.

In addition to vertical microneedles, tilted microneedles and even multiscale bioinspired microneedles can be fabricated using this technique. A novel honeybee stinger-inspired microneedle with many micro barbs was fabricated via the magnetorheological drawing method, as shown in Table 7 [101, 106]. Microbarbs were directly drawn from the main needle. The barbs on the main needle could significantly decrease the insertion force and increase the force of removal [107], which is helpful for dynamic biosignal measurement and transdermal drug delivery.

Magnetorheological drawing is a simple and inexpensive manufacturing technology that can produce magnetic microneedles with high aspect ratios on almost any surface. In contrast to the thermal drawing method, the fabrication process is conducted at room temperature. However, the biocompatibility of the magnetic materials of microneedles needs further investigation.

3.2 Biomedical Applications of Microneedles

Skin is the outermost layer of the human body, and it can not only prevent external substances from entering the body but also reduce the outflow of internal biological fluids. Microneedles can penetrate the skin barrier, extract health-related information, and deliver bioactive drug molecules in a simple, effective, and minimally invasive manner. Thus, the use of microneedles is feasible in sampling, sensing and drug delivery. Microneedles have been employed for transdermal sampling, biosensing [108–110] and drug delivery [79, 111].

3.2.1 Transdermal Biosensing

Microneedles penetrate the skin and make direct contact with the biological fluid. Biomarkers in biological fluid, such as interstitial fluid (ISF) and blood, can be used as indicators that reflect local physiological states. Microneedles mainly possess two primary functions: absorbing or extracting biological fluid samples from skin and directly detecting biomarkers in the biological fluid. Based on the detection signals, microneedle-based biosensors can be divided into physical signal electrodes and chemical signal sensors.

(1) Microneedle-based Electrode for Bio-electric Signals Monitoring

Bio-electric signals, generated by the electrochemical activity of active cells, are fundamental features of life activities. Bioelectrodes could convert the ionic current signals inner body to electron current for further healthy information analysis [112, 113]. In clinical applications, standard Ag/AgCl electrodes are commonly applied to measure bioelectric signals, including electrode-skin interface impedance (EII), electrocardiography (ECG), electromyography (EMG), and electroencephalogram

(EEG). As a wet electrode, conductive gel and skin preparation are necessary before measurement, as shown in Figure 3(a). However, high electric impedance of the stratum corneum layer and drying of conductive gel may result in signal distortion and motion artifacts and thereby are not suitable for long-term monitoring. The measured bioelectrical impedance signal with Ag/AgCl electrode contains the standard electrode impedance value, conductive gel resistance value (R_{gel}), and skin stratum corneum impedance value (R_{sc} and C_{sc}). Microneedles electrodes are alternative dry electrodes that can directly measure bio-electric signals without skin preparation and conductive gel. Microneedles can penetrate the stratum corneum layer, avoiding high impedance of the stratum corneum and improving the signal quality. As a result, the equivalent circuit model of microneedles electrode could avoid the impedance signal of the stratum corneum (R_{sc} and C_{sc}), resistance value of conductive gel (R_{gel}), and the electric potential difference (E_{sc}) compared with the standard Ag/AgCl electrode, as shown in Figure 3(b). Measurement performance of the ECG, EMG and EEG with Ag/AgCl electrode and microneedles electrode are introduced in the following discussion.

ECG is a kind of weak electric signal generated by the activation of the myocardium when the heart is beating. It is widely used to reflect heart conditions for clinical diagnosis, such as heart attacks and rhythm disorders. ECG signals measured by the microneedles electrode and standard Ag/AgCl electrode were shown in Figure 3(c). The ECG signals, including the P-wave, QRS-complex, and T-wave signals of typical cardiac signatures acquired by the microneedles electrode, were consistent with those of the Ag/AgCl electrode [71]. This result indicated that the microneedles electrode had a good ability to detect ECG signals. EMG is generated during muscle contraction and relaxation. The biceps brachii muscle EMG signals recorded by the microneedles electrode and Ag/AgCl electrode were shown in Figure 3(d). When muscle contracts, muscle cells were activated, and the electrical potential rap-

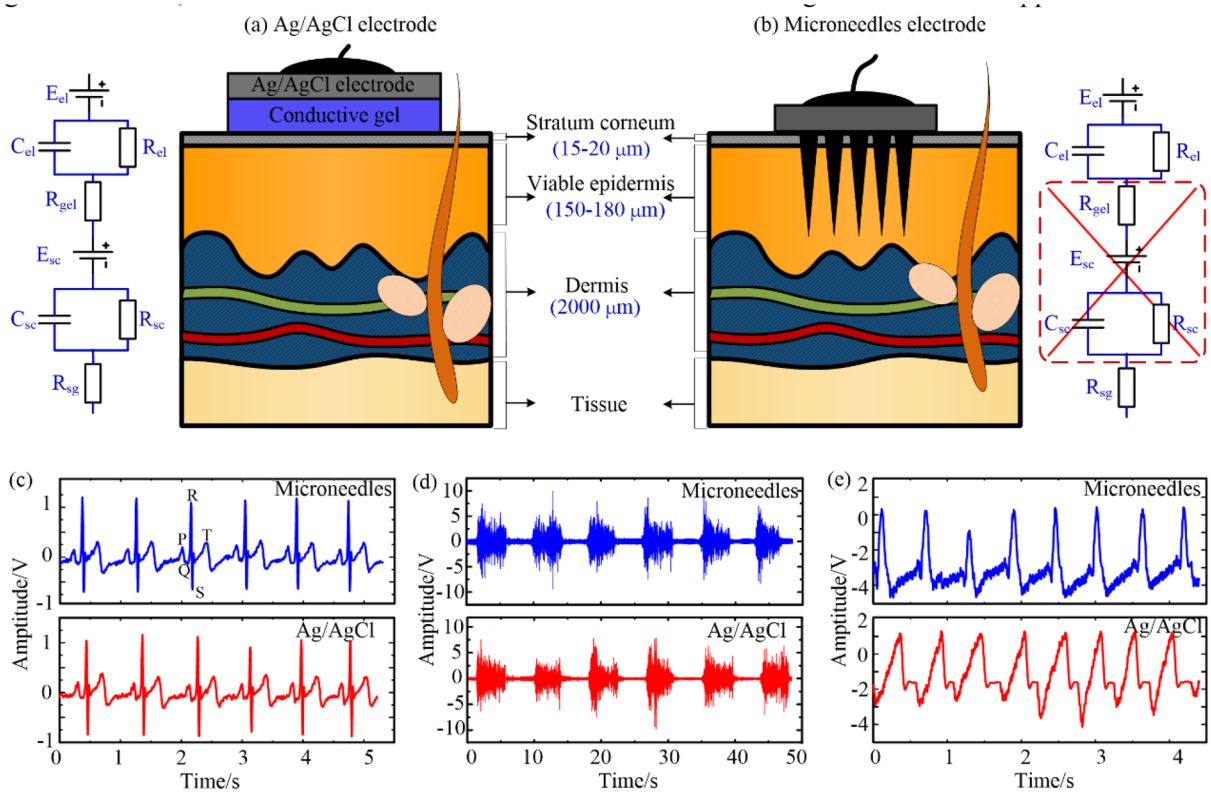


Figure 3 Typical bio-signals measured by Ag/AgCl electrode and microneedles electrode: **a** measure principle and equivalent circuit model of Ag/AgCl electrode, **b** measure principle and equivalent circuit model of microneedles electrode, **c** ECG, **d** EMG, **e** EEG

idly increased to a higher amplitude. Conversely, the muscle relaxed, and the voltages decreased to a low level [71]. Signals recorded by microneedles and Ag/AgCl electrodes were similar. Moreover, microneedles electrode can measure dynamic EMG signals [113]. Thus, microneedles electrode was a good choice for EMG recording. EEG is an electrical signal generated by brain nerve activity. EEG signals of eye blinking detected by microneedles electrode and an Ag/AgCl electrode were shown in Figure 3(e). The signals were almost the same in amplitude and frequency. The signal amplitude recorded by the microneedles electrode was higher and more stable than that recorded by the Ag/AgCl electrode [71].

In summary, microneedles electrode is an alternate method of measuring bio-electric signals without skin preparation and conductive gels. However, the mechanical and conductive properties of the microneedle material should be further investigated for clinical application.

(2) Microneedle-based Sensors for Disease Diagnosis

Biofluids such as ISF and blood contain abundant biomarkers, which can provide comprehensive health information for humans. Clinically, the collection and analysis of biomarkers in biofluids require expensive instruments and professional technicians in hospitals, which are not convenient for home monitoring. Microneedles penetrate the skin, making it easier to collect and detect biomarkers in biofluids. According to the sensing mechanism, microneedle-based sensing systems can be classified as transdermal sampling [114–118] and online detection [52, 58, 119].

In transdermal sampling, biofluid is extracted from the skin using microneedles [120, 121]. Much effort has been made to accomplish biomarker sampling in skin biofluids via microneedles. As shown in Table 8, Zhu et al. [120] manufactured a hydrogel microneedle patch for ISF sampling. The hydrogel microneedle fabricated with gelatin methacryloyl (GelMA) has an excellent swelling ratio ranging from 293% to 423%. After penetrating the rat skin, ISF was absorbed by the hydrogel microneedle patch and ISF in the microneedles were then transferred to a centrifuge machine for further extraction. The test results demonstrated that ISF uptake increased over time until it plateaued at 10 min. Li et al. [121] introduced a novel negative pressure blood extraction system, as shown in Table 8. The system consisted of a hollow microneedle and a prevacuum PDMS actuator. When microneedles were inserted into the dermis, blood was absorbed into the PDMS chamber due to the negative pressure. A sufficient volume of blood (31.3 ± 2.0 μL) was successfully extracted from a rabbit for evaluation using the microneedle system.

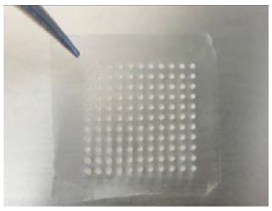
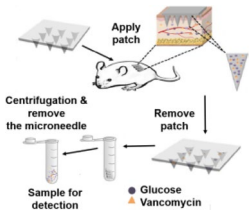
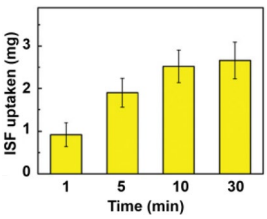
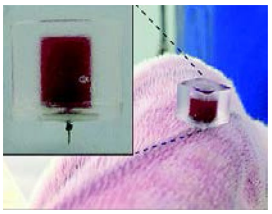
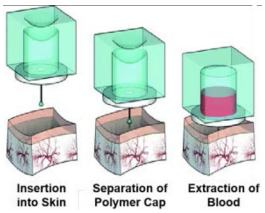
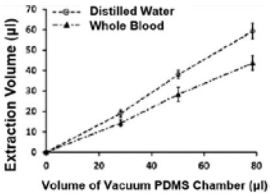
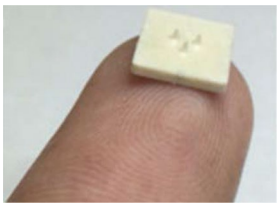
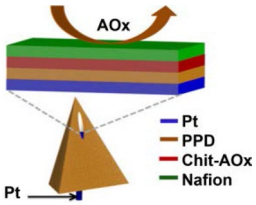
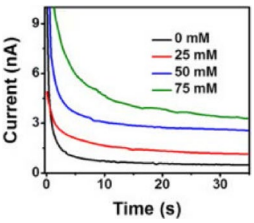
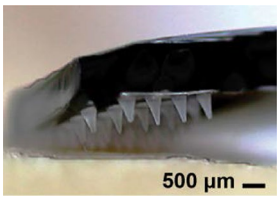
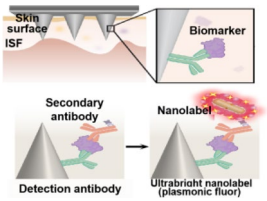
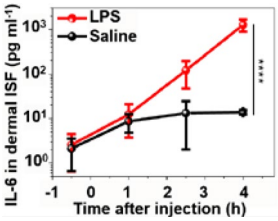
To simplify the detection procedures, microneedles, key sensing components for ISF biomarker detection, have been proposed and investigated. Microneedles with a specially designed morphology or modified with sensitive material to detect the analytes directly have been proposed. According to the sensing mechanism, microneedle-based diagnostic methods can mainly be divided into electrochemical methods [122–124] and photochemical methods [125, 126]. Mohan et al. [123] constructed an alcohol sensor based on the electrochemical method, as shown in Table 8. Alcohol oxidase (AOx) enzyme was immobilized and bound to the surface of the microneedles. AOx could catalyze ethanol to produce hydrogen peroxide (H_2O_2), which could be detected at the Pt working electrode. The oxidation current of the product was correlated to the target ethanol concentration, which could be recorded on an electrochemical workstation. Wang et al. [126] fabricated polystyrene microneedles for sampling and on-needle fluorescence detection of biomarkers in ISF, as shown in Table 8. Microneedles were coated with capture antibodies to specific binding of the target biomarkers. Furthermore, the conventional immunoassay procedure, including calibration process were implemented on the double-layer microneedle directly for on-needle quantification of target protein biomarkers.

In conclusion, microneedles for transdermal diagnostics are promising due to their unique characteristics of being painless, minimally invasive, easy to operate, and suitable for long-term monitoring of chronic diseases and home-based monitoring. Although microneedle-based biosensors have significant advantages, they also have limitations. In particular, the validity, stability, and reliability of microneedle-based devices should be further investigated before applied in clinical settings.

3.2.2 Transdermal Drug Delivery

Drug therapeutics is essential for clinical treatment. Although existing drug delivery methods (oral administration and injection) have met a variety of therapeutic delivery requirements, there are still some limitations, such as low drug absorption rate, the first pass effect associated with oral delivery, unpleasant experiences, professional skill requirements, and infection risk of hypodermic injection. Microneedles create numerous channels as the drug release route and thereby enhance

Table 8 Disease diagnosis of microneedles

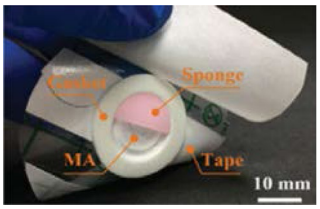
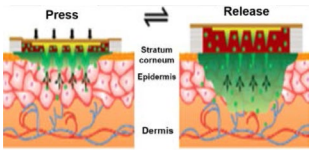
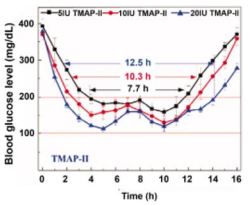
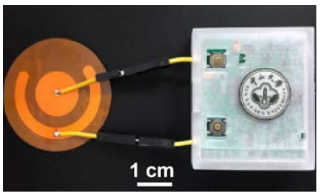
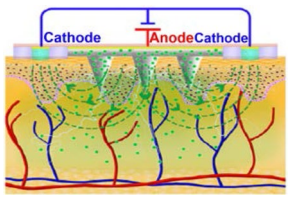
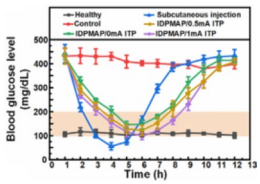
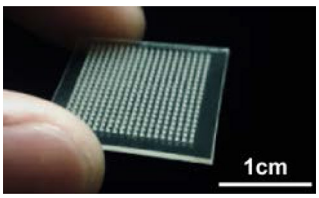
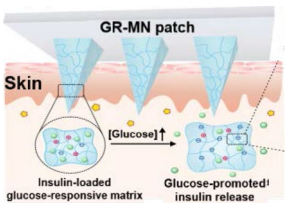
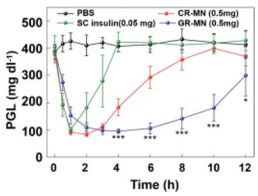
Strategies	Microneedle images	Schematic illustration	Test results
Sampling			
Interstitial fluid			
Blood			
Detecting			
Electrochemistry			
Photochemical sensor			

drug permeability and delivery efficiency. Microneedles have been regarded as a promising tool for transdermal drug delivery. According to the drug release strategies, microneedle-mediated transdermal drug delivery can be divided into passive release, active release, and responsive release delivery methods.

Passive drug delivery is a typical transdermal drug delivery method in which drugs are spontaneously released across the skin, driven by a drug concentration gradient. In this strategy, microneedles are mainly used as penetration tools to create microchannels in the skin. After penetration, drugs coated on or encapsulated in the microneedles [82, 127, 128] or stored within a drug patch [72] are released into the skin and subcutaneous tissue through created microchannels. Yang et al. [72] constructed a passive touch-actuated microneedle array patch for insulin drug delivery, as shown in Table 9. The microneedle patch was made up of solid PMMA microneedles, a sponge for drug storage, medical tape, and a flexible gasket to prevent drug leakage. After pressing, the microneedles soaked with the sponge penetrated the sponge and the stratum corneum layer to create microchannels in the skin. After removal of the compression, microneedles were retracted into the sponge from the skin due to the elastic energy of the sponge and gasket. Simultaneously, drugs stored in the sponge could be released into the body through microchannels formed via passive diffusion of the liquid drug.

To achieve controllable drug release, microneedle-based active drug release systems have been developed. Active drug release systems, such as iontophoresis-driven [129], thermal-driven [130], vibration-assisted [131], and light-controllable [132] microneedle drug delivery systems, have been exploited to control and accelerate drug diffusion and absorption. Li et al. [133] established an iontophoresis-driven porous microneedle patch

Table 9 Transdermal drug delivery of microneedles

Delivery strategies	Device images	Schematic illustration	Delivery performance
Passive release			
Active release			
Responsive release			

for active drug release, as shown in Table 9. A porous microneedle was fabricated and sealed on a flexible printed circuit board surrounded by a specially designed circular electrode. A microportable iontophoresis-driven device was developed. The anode was connected to the porous microneedles, while the cathode was connected to the circular electrode. By loading a constant and safe electric current between two electrodes, drug-loaded charged nanovesicles were released from the porous microneedles under the effect of ion flow.

Responsive drug release is another drug delivery strategy that relies on real-time internal substance stimuli or external environmental stimuli to trigger drug release. In this strategy, the microneedle serves not only as a sensor but also as an actuator. Yu et al. [109] constructed a glucose-responsive microneedle patch to achieve closed-loop blood glucose control, as shown in Table 9. Phenylboronic acid served as the glucose-responsive component. Under hyperglycemic conditions, phenylboronic acid can reversibly form glucose-boronate complexes, promoting the rapid release of insulin. Under normoglycemic conditions, the inhibited variation and the electrostatic interaction can slow the insulin release rates.

In summary, microneedles could be used for transdermal drug delivery. In passive drug delivery, microneedles work as a puncture tool that creates microchannels on the skin surface, and the drug passively diffuses by concentration gradients. In active drug delivery strategy, the drug release could be facilitated with external environment such as electric field, temperature field, magnetic field, vibration, etc. Responsive drug delivery can be activated through in vivo microenvironment or external trigger to achieve drug delivery on demand.

4 Millineedles

Millineedle is an essential medical device in clinic, and it could be used for drug delivery, tissue samples, biopsy aspiration, acupuncture-therapy, skin suture, etc. According to the structure of needles, millineedles could be classified into solid millineedles and hollow millineedles. Solid millineedles, such as acupuncture needle and suture needle, mainly are used as an effective tool to penetrate skin and tissue. Hollow millineedles, with channels inside, could transport drugs or extract samples *in vivo*. In this section, we will review the fabrication methods and applications of millineedles.

4.1 Fabrication of Millineedles

Millineedles are usually made from stainless steel, titanium alloy, and other metals with excellent mechanical property and biocompatibility. Typical fabrication process of millineedles include three steps, namely drawing, cutting, and grinding, as shown in Figure 4, which has been widely used in the manufacture of millineedles. The millineedles generally are disposable medical device, and the manufacturing process is very mature. Its

service life is little related to the manufacturing process, and mainly related to the skills of operator. Metal rods or pipes are firstly drawn with a special designed mold to the desired diameter. Subsequently, long rods or pipes are cut into small pieces. The end of the needle pipe is sharp by grinding with a high-speed rotating grinding tool. Grinding is the key step that significantly influences on the surface quality and sharpness of millineedle tip. Needle angles significant determine the penetration force [134]. The grinding tools and fabrication parameters should be optimized to obtain high surface quality and reduce burrs on the tip. Custom designed grinding wheels were employed to fabricate acupuncture needles and suture needles with special designed structures. During the fabrication process of suture needle, various techniques, such as mechanical micro-drilling, laser, electro discharge machining, electro chemical machining, laser micro-drilling, electron beam machining, end-milling and punching, are used for drilling of micro sized holes. These manufacturing methods are also promised to be used to fabricate suture needle used in clinical practice. For example, Siddiquee et al. [135] used a novel micro-drilling technology to form the micro sized hole in suture needle, which can quickly form hole with smooth surface. In here, a prior countersinking operation was introduced to form the counter sunk hole, which provides a lead-in for accurate location of center and arrests the chatter in the subsequent drilling operation.

Researches also reported that some micro/nano machining technology, such as laser cutting, rolling forming, and so on, were used to fabricate the micro-texture in the surface of millineedles. Figueredo et al. [136] reported that the laser cutting can be used to form internal flexures in the surface of puncture needle. This internal flexure enables tissue to enter the hollow needle and then be severed from the surrounding tissue when the needle is withdrawn. Wang et al. [137] also used laser cutting to manufacture micro-channels on millineedles, which can serve as ultrasound wave reflectors to improve the positioning accuracy during needle insertion. What's more, they reported a least squares support vector machines, which can predict the machined geometry or depth during laser beam machining processes. Wang et al. [138] used laser cutting and rolling forming fabricate three non-smooth surfaces, pitted, wavy and zigzag, on the puncture needles, which can reduce the pain and resistance during puncture effectivity. These technologies were used to improve the function by changing surface morphology features of millineedles. Further experiments should be carried out to verify the application in clinical practice, because the fabricated morphology features may increase the difficulty of sterilization and foreign body removal.

During the fabrication of millineedles, the process parameters also affect the needle quality. For example, Wang et al. [139] used cubic boron nitride (CBN) and silicon carbide (SiC) wheels to grind a 15° bevel angle tip on thin nickel titanium and stainless steel wires respectively. The results show that cubic boron nitride grinding has a lower grinding force and smaller wire deflection than that of the silicon carbide. Compared to stainless steel wire, the nickel titanium exhibits a larger grinding force and wire deflection, lower surface roughness.

4.2 Applications of Millineedles

Millineedles are usually employed for tissues or organs penetration. Different millineedle structures can be applied in various biomedical fields. Puncture millineedles can extract or sample blood, secretions and tissue for clinical testing, and deliver therapeutic drugs to human circulatory system. Acupuncture millineedles are usually used to stimulate acupuncture points. Suture millineedles are mainly used to suture tissues during surgery. Table 10 shows some typical millineedles and their applications.

4.2.1 Millineedles for Puncture

Puncture needle, solid millineedle, is an indispensable medical device in medical surgery [140], which have been widely used for tissue puncture, tissue extraction and so on [141]. For example, doctors can get an accurate and reliable diagnosis by puncturing the tissue with puncture needle to analyze the intrinsic differences in tissue cells [142]. Miyazaki et al. [143] used a puncture needle with a side trap to obtain tissue and cytological specimens for

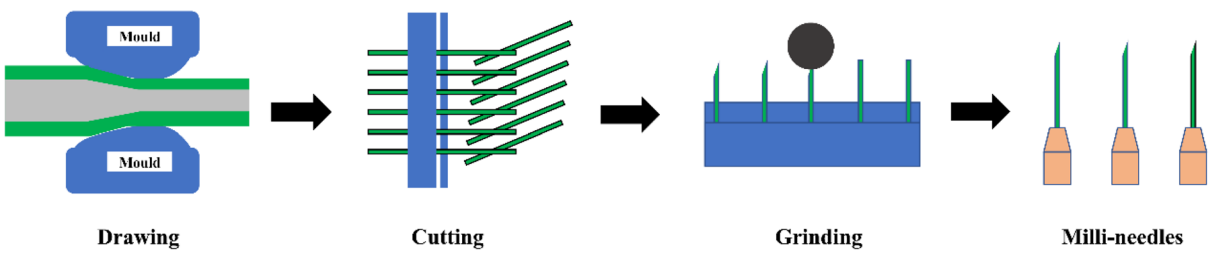
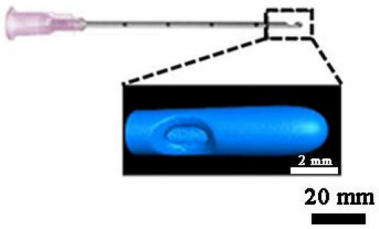
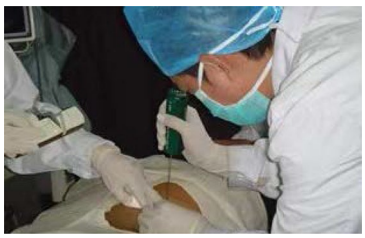
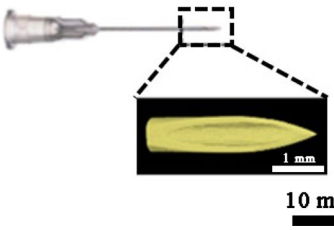
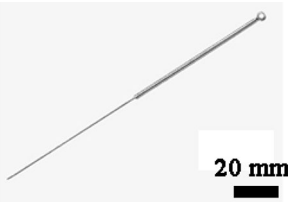
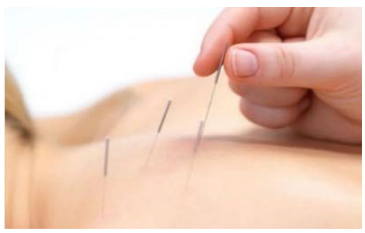
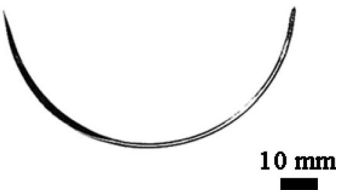
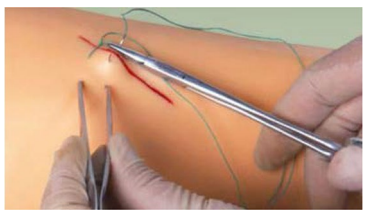


Figure 4 Fabrication process of millineedles

the diagnostic of thoracic lymphadenopathy. This puncture needle can receive the sample during fine-needle aspiration through the needle tip, and is considered to obtain core biopsy for histologic evaluation rather than only cytological material, as in other needles. Kyoshima et al. [144] reported a newly designed puncture needle for aspirating large or giant aneurysms. This puncture needle is designed to prevent blood from leaking when the internal needle is removed and has a lateral tube for aspiration. For example, Gupta et al. [145] used an 18-gauge needle puncture the right subclavian vein under fluoroscopic guidance, and realized the percutaneous recanalization of a chronic occlusion of the junction between the right subclavian vein and the right brachiocephalic vein. Besides, some researches also reported some novel puncture, which can effectively ensure the safety of medical worker during using puncture needle [146] (Table 10).

What's more, puncture needle also can be integrated with other technology realize robot-assisted percutaneous therapies, where instrumented tools or cooperative robots can enhance the human ability to recognize events during needle puncture by providing additional visual, aural, or haptic feedback. Simone et al. [147] reported a robot-assisted puncture system, where the time, position of the puncture needle, and puncture force were recorded to accurately model the needle puncture. This puncture system can ensure the puncture needle stop at a specified location, and is promising to be used in some specific applications, such as liver ablation and prostate brachytherapy. Hing et al. [148] also reported a novel robot-assisted puncture system, which improve the accuracy of needle guidance and deployment of radioactive seeds within the prostate gland. In this system, two C-ARM fluoroscopes were used to record the puncture fiducial movement, which can provide accurate haptic feedback for needle puncture tasks. Furthermore, this haptic feedback system can also reduce the needle deflections. Besides, for the flexibility during operation, some

Table 10 Applications of millineedles

Millineedles types	Millineedles images	Applications images
Puncture		
Syringe		
Acupuncture		
Suture		

researchers have reported some puncture needle with multiple DOF (Table 10). For example, Farooq et al. [149] proposed a new vein puncturing solution during vitreoretinal surgeries, where the puncture needle possessed 4-DOF motion. In this solution, the puncture needle divided into two segments: cannula and stylet. The stylet can be used to puncture the veins, while cannula can be used as a vessel for on-site drug delivery. Besides, this puncture needle can produce repeatable motions with accuracy in submillimeter, which can effectively reduce the damages of operation to the eye.

In conclusion, the puncture needle can be used to extract samples, and has been used in more and more areas. Besides, along with the development of technology and medicine, the puncture needle also can be used to meet more medical requirement. For example, the puncture needle also can be integrated with other technologies, such as CT, fluoroscopes, ultrasound and so on, to improve the accuracy of puncture.

4.2.2 Millineedles for Syringe

Syringe needle is one of hollow millineedle, and researches have reported a number of syringe needle with different size (Table 10). Syringe needle can be divided into two types: sharp needles and blunt needles [150]. The sharp needles possess sharp top, and can pierce into the skin easily, which often cause larger tissues damage [151]. The blunt needle does not have sharp top, which is difficult to pierce into skin. Comparing with sharp needle, it causes less tissues damage [152]. Generally, the syringe needle is used to deliver drugs, where it can pierce the skin to deliver drugs directly into the body. Besides, the syringe needle could also be used to extract biopsy samples to detect diseases [153].

The process of syringe needle piercing into skin is very complicated, which can be divided into three stages [154]. Firstly, when the needle contacts with the skin, the skin deforms elastically. In this process, the piercing force increases with the increase of the needle displacement. Secondly, the skin is rupture suddenly, when the piercing force increase to a certain value. In this stage, the interaction force between needles with skin reaches its peak point. Thirdly, the needle enters into skin, and the piercing force increases first and then decreases. In this stage, the crack cause by needle extends in the skin along with displacement of needle. Besides, due to skin inhomogeneity, the crack often extends uncontrollably during needle entering into skin, which may cause some dangerous accident. Now, some studies reported that the faster motions of needle can cause less uncontrolled crack extension [155].

Researches have reported that the syringe needle can delivery liquid drugs into different tissue of body directly, including muscle, vein, subcutaneous, intradermal, artery, and so on, and possess the advantages of convenient, fast and effective during application [156]. In here, the sharp needle is used more often, because the needle needs to pierce skin easily. The syringe needle also can delivery biological agent, such stem cells, protein, gene, and so on, to treat disease [157]. Zhu et al. [158] used a puncture needle to treat persistent elbow pain. In here, a puncture needle was advanced into the calcification foci under sonographic guidance, and then inject of 25 mg prednisone acetate and 1% lidocaine. Besides, puncture needle also can be used to initiate percutaneous recanalization. The specification can affect the activity of biological agent. For example, researchers reported that the survival ratio of adipose granule cells decreased with the decrease of the inner diameter of the syringe needle [159]. Besides, the syringe needle can delivery drugs to acupoint, which combine with acupuncture. Hua et al. [160] found that the acupoint injection relieve the pain of the primary osteoporosis patients effectively, whose clinical efficacy was superior to the intramuscular injection. What's more, the syringe needle also can be used in the area of cosmetic, and it is often used to delivery filler, such as hyaluronic acid. In here, the blunt needle can avoid inject filler into blood vessel to some extent.

In conclusion, there are various types of syringe needle, and it can be used to delivery various drugs. Along with the development of medicine, the syringe needle can realize other functions of millineedles, such as puncture, acupuncture, and so on.

4.2.3 Millineedles for Acupuncture

Although, drugs are essential to treat diseases in modern medicine, it has also been associated with many adverse effects, such as drug resistance, low effectiveness for some diseases, and so on [161]. For example, when antipsychotic medications are used to psychiatric disorders long time, it can result in serious complications including intestinal obstruction, colon obstruction, intestinal ischemia and so on [162]. Acupuncture, a complementary and alternative therapy, has been used in China for thousands of years and has become increasingly popular in western countries because of its significant effect and few side effects [163].

Acupuncture needle, solid millineedle, possess a number of types, which can be divided into *Chan Zhen*, *Yuan Zhen*, *Di Zhen*, *Feng Zhen*, *Pi Zhen*, *Yuan Li Zhen*, *Hao Zhen*, *Chang Zhen* and *Da Zhen*, according to "*The Yellow Emperor's Classic of Internal Medicine*"

[164, 165]. The modern medicine is also shown that the acupuncture needle can be used to treat illnesses and injuries for extend lifespan by inserting into the specific acupoints of body (Table 10) [166, 167]. For example, some meta-analyses showed that acupuncture is a safe and effective method for the treatment of depression, with relatively few adverse effects [168]. Among all types of needles, filiform needle is one of mostly used acupuncture needles [169] due to it resulting in minor tissue damage [170]. Comparing with filiform needle, the elongated needle can enlarge the depth of stimulation, which is suited to stimulate the acupoint in deeper site [171]. Besides, in order to meet some specific goals, some acupuncture needles with particular structure also have been reported. For example, the acupotomy, the combination of needle and knife, can release and strip adhesion structures or soft tissues through the little knife at the tip of the needle [172].

What's more, some novel acupuncture needles, combined traditional acupuncture needles with physical energy field, such as electric field [173], heat field [174] and so on, also have been reported. Electric acupuncture needles which can stimulate the acupoints with micro current after inserting into the acupoints, and can continuously provide stimulation to the acupoints to generate nerve electrical signals. It can provide better treatment effect comparing with traditional acupuncture needles [175]. Fire needle therapy is a special type of acupuncture, which uses specially heated and burned-red needles inserted into the acupoints or affected body region to improve symptoms. Combining heat and acupuncture, it can improve blood circulation with the help of thermal stimulations, promote the reduction of chronic inflammation, and enhance tissue regeneration, effectively relieving tissue edema and muscle spasm [176].

In conclusion, along with the development of medicine and technologies, the acupuncture needle has attracted increasing attention. On the one hand, with the discovery of new theory of acupuncture, the acupuncture needle has been used to treat more miscellaneous diseases, such as cancer, stroke, and so on. On the other hand, studies have reported more and more novel acupuncture needles, which extend the application areas and improve curative effect greatly of the acupuncture needle.

4.2.4 Millineedles for Suture

Suture needle is another solid millineedle, and has been used in various surgery to suture soft tissue, such as skin, blood vessels, nerves, heart, etc. (Table 10) [177]. According to the overall shape of suture needles, they can be divided into straight suture needles and curved suture needles. Curved suture needles can be simply divided into J-shaped suture needles and arc suture needles. In clinical practice, the appropriate suture needle is generally selected according to the space range of the suture. The straight needle is suitable for suture in wide or shallow operation sites, such as skin and gastrointestinal mucosa, liver and so on. The J-shaped suture needles possess better flexibility than straight suture needles, which fit for skin suture or deep wound suture with poor access. The curved suture needle is the most widely used in clinical applications, and is suitable for suturing narrow or deep tissues [178].

Now, there are a number of researches reported the application of suture needle. Lukas et al. [179] evaluated the forces required for suturing selected skin wounds by numerical analysis and in vivo experiments, which provided the guidance for the application of robot-assisted surgery and tissue adhesive. Li et al. [180] studied the interactive action between suture needle and liver tissue, and indicated that the suture needles with triangle cross-section tip and larger diameter size, showed higher resistance against the surrounding tissue. This result is useful for automatic suture technique, because it can optimize the suture path according to the puncture force. What's more, in order to expand the application of suture needle, it has been combined with other technology. Frick et al. [181] combined the suture needle with virtual reality technology, which is an effective educational tool. In order to improve the force feedback, they examined the resistance forces encountered during the passage of a straight suture needle through sheepskin and sheep tendon, which provide input data for haptic virtual reality surgical simulation.

In conclusion, suture needle can be used to suture wounds, which is benefit for the healing of wound. Now, there are a number of types of suture needle, which can meet the different requirement during suture. Besides, the suture needle also can integrate with other technology, such as robot-assisted technology, organize welding technology, and so on, to extend the application of suture needle.

5 Conclusions and Outlook

5.1 Conclusions

Disease diagnosis and therapy are two main issues of clinical medicine. Needles, as some of the most widely used clinical devices, can penetrate the cell membrane and skin barrier of the human body to realize biosensing and drug delivery. Needles can be classified as either solid needles or hollow needles. According to the dimensions, these can be further classified into nanoneedles, microneedles, and millineedles. Typical fabrication methods and applications of three types of needles were introduced and highlighted in this review. Nanoneedles are effective tools that can easily penetrate the cell

membrane due to their nanoscale dimensions and high aspect ratios. The typical strategies for fabricating nanoneedles include bottom-up and top-down fabrication. Intracellular applications of nanoneedles, including cell sensing and drug delivery, were highlighted. Microneedles are microscale biomedical tools that can painlessly penetrate the stratum corneum layer and create microchannels on the skin. The fabrication strategies of microneedles include substrate manufacturing, formative manufacturing, and additive manufacturing. Microneedle-based electrodes exhibit biosignal recording performances that are similar to those of commercial wet electrodes. Microneedle-based transdermal biosensing methods and drug delivery strategies were summarized. Millineedles are some of the most common biomedical devices and play a significant role in clinical medicine. Typical procedures for fabricating millineedles include drawing, cutting, and grinding. Millineedles can be classified as puncture needles, syringe needles, acupuncture needles, or suture needles based on their biomedical applications.

5.2 Outlook

Human pursuits for a better life emphasize the progress of modern clinical medicine. Although tremendous progress in the fabrication and biomedical application of nanoneedles, microneedles and millineedles has been achieved, significant challenges remain to be solved. First, the materials used in needles should be further investigated; these include smart materials and responsive materials, which are essential for patient self-administration and remote medicine. Second, novel needle structures, such as bioinspired needles, honeybee stinger needles, cactus needles, and combined needle structures of multiple sizes, require exquisite engineering to lower their friction coefficients and improve their penetration performance. Third, novel fabrication techniques that can achieve mass production of needles, especially metal nano/micro needles with excellent mechanical properties and biocompatibility, are urgently required. Furthermore, it is necessary to apply needles to some modern clinical techniques such as vaccine injection, macromolecule delivery, and cell drug screening to further benefit humans.

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Author contributions
BL wrote the original draft of nanoneedles and microneedles sections; XY wrote the original draft of millineedles section; YZ drawled pictures of nanoneedles and microneedles sections; ZSY, JBY, JY and XY modified the paper, LLJ and CYW organized and reviewed this paper. All authors read and approved the final manuscript.

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