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Microneedle Technologies for Food and Crop Health: Recent Advances and Future Perspectives

Zahra Faraji Rad

The global food supply constantly faces the threats of emerging crop diseases initiated by pathogens such as bacteria, fungi, and viruses. Plant diseases can cause significant economic and production losses in the agriculture industry, and early disease detection significantly mitigates losses. Monitoring the food quality and detecting pathogens during the food supply chain is essential in confirming the food's safety and reducing crop loss. This results in lowering production costs and increasing average yield in the agriculture industry. Considering the significant development of nanotechnology in biomedicine for human health monitoring, diagnostics, and treatment, there is an increasing interest in using nanotechnology in crop production, health, and plant science. This technology can allow continuous monitoring of plant health and on-site diagnostics of plant diseases. While many microneedle-based devices are previously reported for human health monitoring, diagnostics, and treatment, the application of this technology to agriculture started relatively recently. This review investigates the recent development of microneedle technology in food and crop health, where the most state-of-the-art microneedle-based devices are utilized for plant drug delivery, disease monitoring, and diagnosis. Finally, the current challenges and future directions in developing microneedle technology for food and crop health are discussed.

1. Introduction

Agriculture and food science are significant elements of the worldwide economy. In the long term, the continuous development and application of new technologies and knowledge are crucial to maintaining sustainability, food security, and competitiveness in this sector. With current population growth, the global demand for food is growing significantly. It is anticipated that by 2050, food production needs to be increased by 100% to meet the growing population's demand.[1] In addition, plant diseases result in 220 billion dollars of crop losses globally, where on average 30% of worldwide crop production loss is due to pathogens such as bacteria, viruses, and fungi.[2] Pathogens and pests are annually responsible for 17%–30% loss of five major crops (rice, potatoes, maize, wheat, and soybean) worldwide.[3,4] The agricultural land area decreases due to population growth globally, further stressing crop supply chains. Plant diseases negatively affect agricultural productivity, and crop failure due to pathogen infection is one of the most common issues in the industry. Current diagnostic technologies such as DNA amplification by polymerase chain reaction (PCR) and its variants (quantitative PCR (qPCR), multiplex PCR, nested PCR, digital PCR (dPCR)),[5] loop-mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assays (ELISA), immunofluorescence (IF), fluorescence in situ hybridization (FISH), and flow cytometry (FCM) are laboratory-based detection technologies that need skilled personnel for operation, cannot provide continuous monitoring, and are often unavailable in remote locations.[6] As a result, developing small, rapid, cost-effective, and field-portable disease detection, monitoring, and treatment devices capable of providing preliminary information on crop health is essential for increasing productivity and crop protection without the delays of laboratory extraction and testing of samples. Sensitive, rapid, and early diagnosis of plant disease is vital to maintaining sustainability, food security, and competitiveness in agriculture and crop health. Micronanotechnology has the potential to develop and transform the agriculture and food industries by offering opportunities to increase global food production, biosecurity, and safety, as well as reduce waste and improve nutritional value. This technology can bring science to farmers and enable them to address the causes of crop failures in their fields and consequently increase their productivity. Some of the applications of micro–nano technology in this field include using nanomaterials as fertilizers and bactericides,[7–9] microsensors for pathogen detection, and microneedle-based technologies for drug delivery, monitoring plant health and soil quality,[10] and rapid extraction of DNA from plant leaves.[11]

This review paper discusses the emerging microneedle technology, previously concentrated on medical applications, applied to the field of food and crop health. There are only a few studies on the application of microneedles for plant-related work. This technology has great potential to provide novel solutions to many

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challenges facing the agriculture industry. In medical science, microneedles have been proven as minimally invasive devices, initially developed for drug delivery to overcome the skin's stratum corneum (SC) barrier.[12–14] The technology gained interest due to its pain-free penetration and the ability to deliver low- and high-molecular-weight active therapeutic ingredients, which enables targeted delivery of therapeutic agents. In addition, the technology can be used for testing a small sample size of biofluids such as interstitial fluid or capillary blood for diagnostics and monitoring purposes such as monitoring the level of blood glucose in diabetic patients.[12] In recent years, there has been an exponential increase in the development of microneedle technologies for a wide range of applications such as drug delivery,[15] optogenetic study,[16] and biosensing.[17] This is mainly due to the high demand for easy-to-use in situ point-of-care (POC) diagnostic tests, advancements in microfabrication technology, and the ability to easily and quickly produce polymeric microneedles via micromolding methods.[18–20] In addition to biomedical applications, microneedle technology enables minimally invasive, instantaneous access to the epidermis of leaf tissue for various applications, providing the capability for real-time, in situ analysis and monitoring of specific pathogens within the plant or delivering a therapeutic compound such as fertilizers. This paper highlights the most recent microneedle technology development in crop and plant health, enabling rapid detection, identification, treatment, and eradication of harmful plant diseases. The design, geometry, materials, and fabrication of microneedle arrays are reviewed. Moreover, the research trends and challenges associated with the implication of microneedle technology in agricultural research are discussed to guide and assist future studies.

2. Microneedles Technology

The concept of the microneedle to noninvasively bypass the outermost layer of the skin, known as SC with 10–20 μm thickness and deliver drugs, was first presented in 1976. The development of experimental research was delayed until the late 1990s due to the lack of microfabrication tools at the time.[12] Microneedles are micrometer-sized projections that are sharp and robust enough for skin penetration without causing pain due to their small dimensions. These microstructures were initially tested as a transdermal drug delivery system to replace the traditional hypodermic needle injections for delivering therapeutic agents to the human body.[12,21] Calcein, a low-molecular-weight dye, was the first compound experimentally delivered using microneedles.[22] Since then, the concept, design requirement, manufacturability, and application for painless transdermal drug delivery and POC diagnostics of microneedles have been studied widely, as reported in the literature, for biomedical applications.

Upon application of microneedles, micropores will be created on the skin surface, which allows the transport of substances, including drugs, micro- and nanoparticles, and macromolecules to the skin, consequently overcoming the limitations associated with traditional transdermal drug delivery systems.[20,23] The interest in applying the technology for POC diagnostic is a relatively new topic and has increased considerably in the last few years. Microneedles have been shown to penetrate the SC and reach the viable epidermis, eluding contact with blood capillaries and fluids in the skin's dermal layer. The current goal of POC diagnostics is to integrate microneedles into a compact system capable of real-time sampling of biofluids to provide an automated and accurate readout of a patient's biomarkers. Traditional testing requires access to laboratories and equipment and must be performed by clinical personnel; however, POC diagnostics enables quicker and in situ measurements of diseases without skilled personnel and laboratories, therefore, significantly accelerating the route to treatment and management of diseases. **Figure 1** shows examples of microneedle arrays.

2.1. Types and Materials of Microneedles

Generally, microneedles are divided into five categories: solid, coated, dissolving, hollow (or open-channel), and hydrogel-forming polymer microneedles (**Figure 2**).[24] A solid microneedle array creates micropores on the surface of the tissue, allowing the therapeutic agents to be infused through the micropores and reach the sample's deeper layers. A coated microneedle is a solid microneedle precoated with a drug of interest before insertion. The amount of drug that can be loaded depends on the microneedle surface area and the thickness of the coating layer.[25] Similar to hypodermic needles, hollow microneedles contain

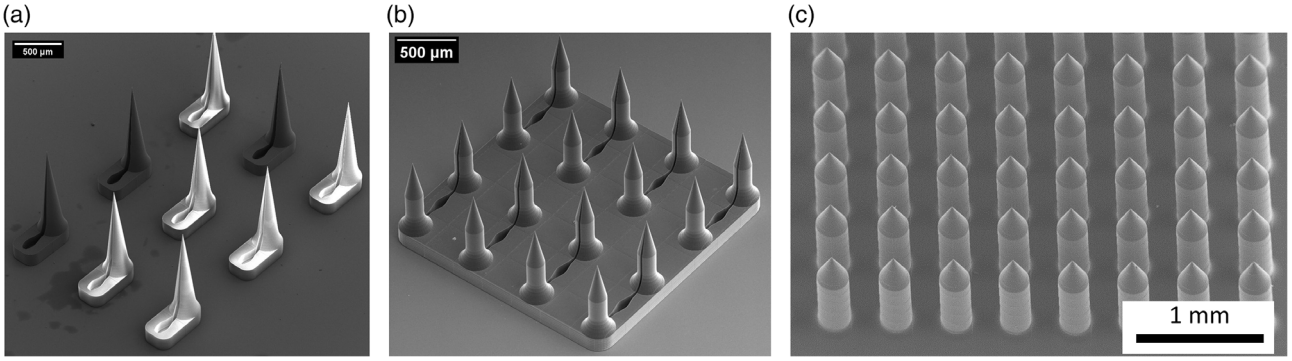

Figure 1. a) Ultrasharp side-opened channel microneedle patch connected to microfluidic reservoirs. Reproduced with permission.[93] Copyright 2021, Elsevier. b) A microneedle array fabricated by 3D laser lithography. Reproduced under the terms of the CC-BY 4.0 license.[18] Copyright 2017, Springer Nature. c) An array of solid microneedles. Reproduced with permission.[94] Reproduced under the terms of the CC-BY 4.0 license. Copyright 2022, the Beilstein Institute for the Advancement of Chemical Sciences.

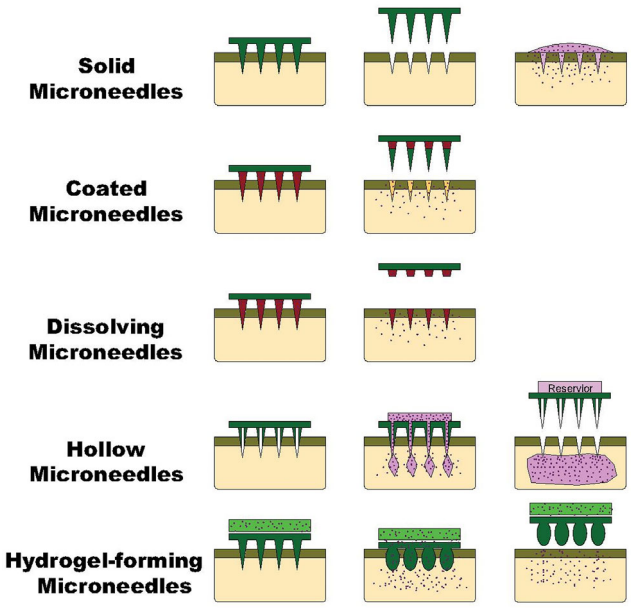

Figure 2. Schematic illustration of different types of microneedles.

an internal channel for pressure-driven fluid transport through the skin and microneedle. Open-channel microneedle design is a relatively new concept.[26,27] Like hollow microneedles, the open-channel design provides 2D flows of fluids that can be used to deliver drugs and extract biological fluid. One primary concern of hollow microneedles is the blockage of the channels due to entrapped dermal tissue; thus, another advantage of the open-channel design is minimizing or altogether avoiding the blockage of tissues inside the microneedle lumen due to its side-opened design.[18] In addition, open-channel microneedles are more easily manufactured than hollow microneedles.[28] Dissolving microneedles are made with biodegradable polymer materials where the drug of interest is encapsulated into the polymer. The dissolution occurs upon the insertion of microneedles into the skin; thus, the drug will be released.[29] Hydrogel-forming polymer microneedles are made from superswelling materials (crosslinked hydrogels). This type of microneedle has hydrophilic properties, enabling it to uptake a large volume of water into its polymeric network. In addition to sampling, hydrogel-forming microneedles can be used in drug delivery applications if the drug is encapsulated into its polymeric structure.[30]

Microneedles are made from various materials; the most commonly used materials are silicon, metals, ceramics, and polymers. Among all, silicon has been most extensively used for the fabrication of microneedles. The first microneedles were also made from silicon. However, silicon microneedles require a time-consuming and complex multistep manufacturing process, limiting their usage for real-world applications. An additional concern is that silicon is a brittle material which can break during insertion and leave residuals in the skin, resulting in foreign body reactions.[12] Microneedles are also fabricated from metals such as titanium, stainless steel, nickel, and palladium. Compared to silicon, metals have better biocompatibility and mechanical properties; however, it is challenging to manufacture complex microneedle structures from metals. Due to their high compression resistance and chemical properties, ceramics such as alumina, organically modified ceramics, calcium phosphate dihydrate, and calcium sulfate dihydrate have also been used to fabricate microneedles.[14,31] Polymer materials are receiving more interest from the medical industry due to their good mechanical properties, excellent biocompatibility, low toxicity, and lower fabrication costs compared to other materials. Polymer microneedles are manufactured from hydrogel-forming, dissolving, and nondissolving polymers. A wide range of polymers such as polylactic acid (PLA), poly (vinyl alcohol) (PVA), poly (methyl methacrylate) (PMMA), polyglycolic acid (PGA), poly (lactic-co-glycolic acid) (PLGA), poly (vinylpyrrolidone) (PVP), poly (carbonate), cyclic-olefin copolymer, polystyrene (PS), SU-8 photoresist, and poly (methyl vinyl ether-co-maleic anhydride) is used for microneedle fabrication.[14,32] Generally, hydrogel-forming and dissolving microneedle arrays are fabricated from these polymers. In dissolving microneedles, the drug is usually encapsulated in the polymer structure or coated on the surface of the polymer material, which will completely or partially dissolve or release from the surface upon insertion. For sample collection, microneedle structures can be designed with nonporous structures or loaded with functional nanoparticles to allow sample extraction. Hydrogel microneedles are a relatively new category of microneedles; when inserted, the swelling properties of hydrogel cause the materials to uptake the sample from the tissue and swell.[30] The extracted sample can be removed from the polymer structure by methods such as rinsing or centrifuging. The sample can be further used for disease monitoring and diagnostics.

2.2. Manufacturing of Microneedles

Microneedles generally range from 100 to 2000 μm in height and are fabricated in different geometries using a variety of materials and manufacturing methods. Features, including height, diameter, tip sharpness, and the array's overall dimension, must be considered when designing microneedles for a specific application.[12] However, microneedle geometry is constrained by the fabrication method used to manufacture the device. In addition, a microneedle geometric specification will be needed to consider the characteristics of the sample, material selection, its mechanical stability, and the fluid dynamics for the transport of fluid across the sample via microchannels.[28] For effective insertion, the microneedle tip must be sharp and robust to withstand forces applied laterally by tissue.[33,34]

With the advancement of high-precision microfabrication tools, it has become possible to manufacture microneedle arrays in various geometries, lengths, densities, and sizes directly from 3D designs for specific applications.[35,36] In addition, recent developments in micronanotechnology and manufacturing techniques enable the precise fabrication of microneedle arrays with submicrometer resolution. The manufacturing approaches can be classified into two major categories: 1) subtractive manufacturing methods, such as dry and wet etching, lithography, and laser ablation, and 2) additive manufacturing methods, such as 3D printing and 3D laser lithography based on two-photon polymerization (TPP). The detailed manufacturing procedures of microneedles change for different

Table 1. A summary of materials, manufacturing techniques, and types of microneedles.[12,101,102]

Materials	Manufacturing techniques	Types of microneedles
Silicon	Wet etching, dry etching, lithography	Solid, hollow, coated
Metals	Laser cutting, laser ablation, electroplating, electropolishing	Solid, hollow
	Deep X-ray lithography, drawing lithography	Solid, dissolving, hydrogel forming
Ceramics	Micromolding, sintering lithography, TPP	Solid
Polymers	3D printing, TPP	Solid, hollow
	Micromolding and casting	Solid, dissolving, hydrogel forming
	Droplet-born air blowing	Dissolving, hydrogel forming
	Hot/soft embossing	Solid
Silicon, metals, ceramics, polymers	Dipping, spraying, atomized spraying process	Coated

geometries, materials, and applications. **Table 1** provides an overview of different materials, manufacturing methods, and types of microneedles.

2.2.1. Subtractive Manufacturing

Subtractive manufacturing methods such as reactive ion etching (RIE) and deep RIE (DRIE) require clean-room facilities for operation. In these techniques, the unwanted materials are removed from the substrate top–down. Etching processes require a prerequisite step known as photolithography for pattern creation before etching. The pattern is transferred from an original photomask to a photosensitive material on a substrate by photolithography using UV light exposure; silicon wafers are typically used as substrates. After a structure is defined on the silicon substrate, materials deposited on substrates are removed using chemically reactive plasma consisting of high-energy ions which attack the surface and react with it. As the electrons are accelerated into the etching chamber, ions are formed from the collision of stray electrons with reactant gas molecules such as SF_6 (sulfur hexafluoride).[37] The relative amounts of ions' over-reactive radicals are controlled by adjusting the gas pressure, affecting the degree of isotropy of etching. An electric field (bias) accelerates the ions and further increases the directivity of the etching.[38] The RIE method creates anisotropic etch profiles; thus, achieving high aspect ratios over etch depths of more than a few micrometers is difficult due to the relatively low etch rate and the anisotropic nature of the process. DRIE, or the so-called Bosch Process, invented by Laermer and Schilp at Robert Bosch GmbH in the mid-1990s[39] deeply etches silicon substrates while maintaining high aspect ratios and straight sidewalls and thus is more suitable for the fabrication of microneedles. The main difference between RIE and DRIE is the passivation steps in between the etching steps to create vertical sidewalls. DRIE uses fluorine-based chemistry by applying the concept of etching and passivation cycles in a time-multiplexed manner. The primary procedure in DRIE is to etch the bulk material vertically by protecting (passivation) the sidewalls throughout the etching. This is accomplished by continually alternating between an etching step and a polymer deposition step which passivates and coats the sidewalls uniformly. The etching step is an ion-assisted process using an etching gas like SF_6. The polymer deposition or sidewall passivation step uses a polymer layer, commonly a Teflon-like layer, polymerized from C_4F_8 (octafluorocyclobutane) with a thickness of a few tens of nanometers. The passivation layer protects the sidewalls from lateral etching in the following etching step. At the start of each cycle, the silicon substrate is exposed to fluorine radicals (reactive plasma species). A portion of the SF_6 etching step removes the passivation layer from the substrate base. In DRIE, the etch rate in the vertical direction is much quicker than the etch rate in the lateral direction, resulting in anisotropic profiles, thus generating vertical sidewalls.[38] This switching etch arrangement may create a scalloping pattern on the sidewalls. By continually alternating isotropic and anisotropic etching, 3D structures, particularly out-of-plane microneedle arrays, can be made. The etch rate achievable through the DRIE process is significantly higher than wet etching and RIE techniques; therefore, DRIE has become a widespread technology for microneedle fabrication. **Figure 3**a shows a typical

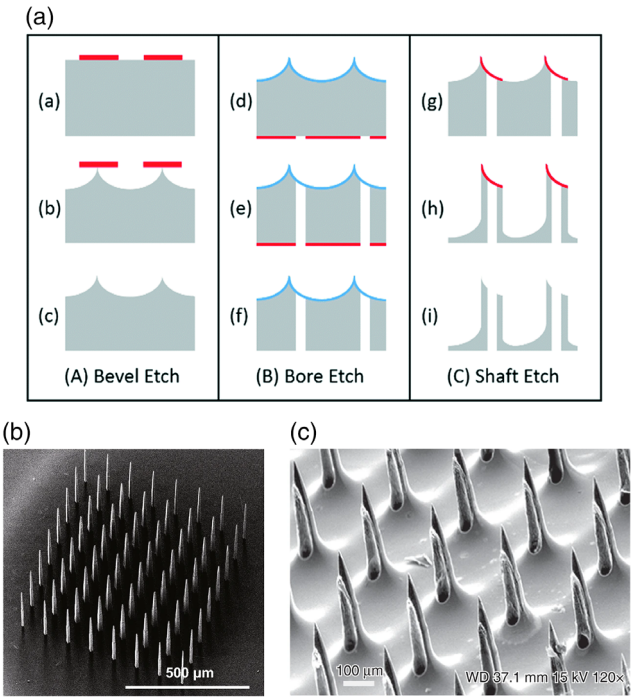

Figure 3. a) A typical process for fabrication of hollow silicon microneedles, starting with etching the microneedle tip (A), etching the inner channel (B), and etching the shaft of the microneedle (C), gray: silicon wafer, red: photoresist masks, blue: oxide stop layers. Reproduced under the terms of the CC-BY 4.0 license.[95] Copyright 2020, Royal Society of Chemistry. b) Scanning electron micrographs (SEM) images of a microneedle patch fabricated by two-step DRIE. Reproduced under the terms of the CC-BY 4.0 license.[96] Copyright 2022, Springer. c) Hollow microneedle array fabricated by plasma etching. Reproduced under the terms of the CC-BY 4.0 license.[97] Copyright 2019, Springer Nature.

process for the fabrication of hollow silicon microneedles, and Figure 3b,c shows an example of silicon microneedles. Although some studies have shown very smooth silicon sidewalls and high-aspect-ratio micropillars, access to DRIE facilities, and its high costs, are significant factors limiting parallel optimization of all critical process parameters, particularly when etching has a long duration, multiple steps, and multiple control parameters. In addition, controlling process parameters for etching structures with heights greater than ≈500 μm becomes increasingly challenging. Wet etching may result in smoother sidewalls; however, fabrication of high-aspect-ratio structures may not be possible because anisotropic etching properties are related to crystal structure orientation.

2.2.2. Additive Manufacturing

In recent years, additive 3D printing techniques have become promising tools for producing novel designs of microneedles. In contrast to subtractive manufacturing techniques, additive manufacturing methods such as 3D printing and 3D laser lithography based on TPP enable printing of more complex geometries that were not feasible to create with conventional microfabrication methods that require high-cost cleanroom operation facilities, harsh processing environments, and extensive technical expertise (**Figure 4**).[18,36] Other advantages of these methods include cost-effectiveness, less material usage, reduced number of fabrication steps, low requirements for manufacturing environments, rapid design modifications, the ability to integrate and print multiple components simultaneously, and general ease of use.[35] The process involves the fabrication of customized 3D microstructures directly from computer-aided design (CAD) drawings. TPP is a high-precision micro–nano fabrication technique enabling the printing of structures with submicrometer resolution.[35] In this technique, a picosecond or femtosecond laser is applied to a photosensitive material to start the polymerization process. The laser tightly focuses on a spot, and upon absorption, the liquid photoresist will be polymerized at a region called polymerization voxel. This voxel-by-voxel process enables the formation of precise microstructures without using a photomask.[35,40]

Most 3D printing techniques use polymers to manufacture microneedles. Fused deposition modeling (FDM) is the most accessible and affordable 3D printing technique based on the extrusion of hot-melt thermoplastic polymer filaments from the print head. In this method, the 3D printing of the pattern occurs layer by layer while the print head extrudes and deposits the polymer filament on the print station.[41–43] In the last few years, 3D printing methods have gained significant interest among researchers for the fabrication of microneedle arrays; however, due to the lengthy printing time and concerns regarding the biocompatibility of its materials, this manufacturing technique is mainly suitable for fabricating master microneedles for prototyping and molding.[24] In the replica molding or

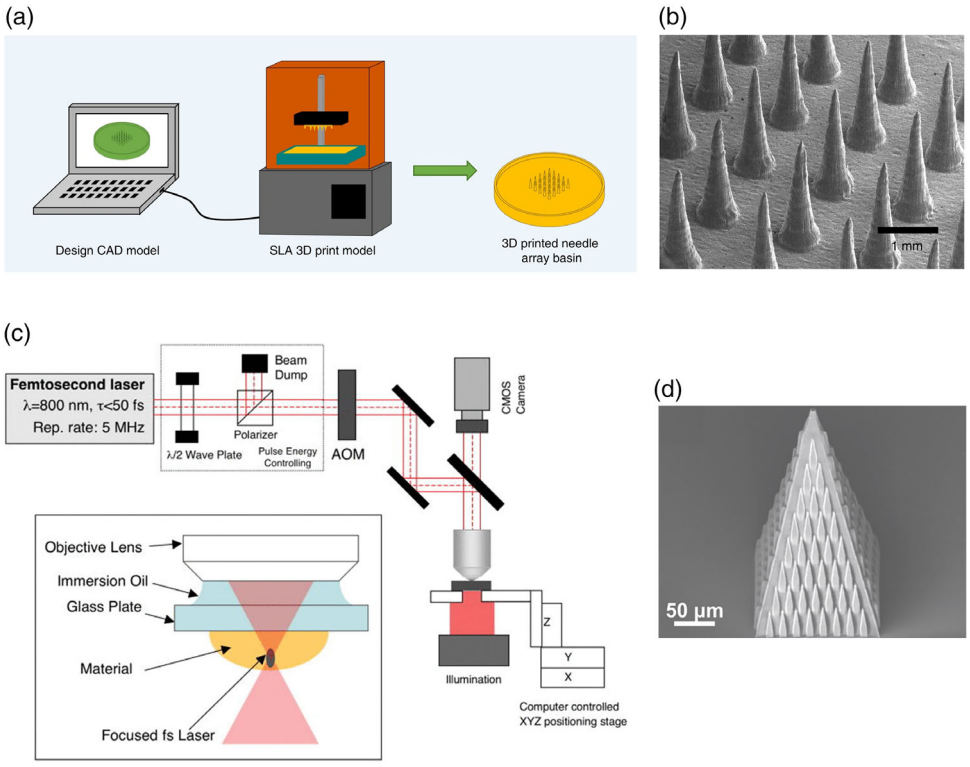

Figure 4. a) Schematic illustration of the process setup for 3D printing microneedle arrays. b) Microneedles fabricated by a desktop stereolithography (SLA) 3D printer. Reproduced under the terms of the CC-BY 4.0 license.[36] Copyright 2019, Springer Nature. c) Schematic diagram of the experimental setup for TPP technique. Reproduced with permission.[98] Copyright 2012, Laser Institute of America. d) Microneedles fabricated by TPP. Reproduced under the terms of the CC-BY 4.0 license.[99] Copyright 2019, Springer Nature.

micromolding process, a master mold manufactured from a subtractive or additive manufacturing technique is used to fabricate polymeric replicas by injecting or casting material into a soft or hard mold. To reduce the adhesion of the mold to the material, the mold surface can be chemically treated. A liquid polymer solution is then deposited on the mold to solidify by curing, cooling, or crosslinking. The processed polymeric microstructure is consequently peeled off from the mold. The mold can be reused repeatedly in this process to produce more replicas; therefore, this technique has the potential to produce batches of microneedles.

3. Plant Leaf Structure

Plant leaves are widely used for treatment and disease diagnostics. Even though plants are always at risk of infection due to the global presence of pathogens, to some extent, plants can inhibit infection and prevent the progress of invasion and establishment of disease through their defense mechanism. Pathogens need to bypass various highly effective chemical and physical defense mechanisms to disease plants. The most effective defense barrier is the plant cell wall, which comprises a network of cellulose microfibrils crosslinked by hemicellulose molecules and embedded in a highly crosslinked pectin matrix (**Figure 5**).[44] In the leaf tissue, cells are enclosed by various protective structures, for example, waxy cuticle layers and cell walls. The cell wall of a plant acts as an effective barrier where the delivery of new wall material by the actin cytoskeleton makes it even more reinforced at the infection site.[45] In the event of a pathogen attack, the cell wall is thickened and reinforced; it also releases antimicrobial compounds at the invasion site.[46] These protective structures must be degraded to extract the sample from the cells or deliver therapeutic materials. For nucleic acid amplification, high-quality DNA/RNA is always required to achieve consistent and reliable results, irrespective of the technique used for extracting the genetic materials. The presence of plant contamination, such as polysaccharides, proteins, and polyphenolics in the genetic materials, makes nucleic acid extraction more challenging.[47] Thus, to obtain a high-quality sample, it is required that all sorts of contaminations are removed from the extracted sample before amplification. Purifying the extracted DNA/RNA is one of the most common methods to increase the quality of the targeted sample and improve the sensitivity and specificity of the pathogen detection process. However, the technique is time-consuming and requires access to the equipment. The most efficient approach is to develop a technique that can eliminate contamination during nucleic acid extraction and eradicate the need for the purification step. A few studies have shown that microneedle arrays can directly sample DNA/RNA without purification by breaking the rigid cell wall and penetrating the plant cell, removing the need for sample purification.[11,48] The exclusion of this critical step can pave the way for the development of rapid POC diagnostic devices for detecting plant diseases.

4. Current Methods for Plant Disease Diagnosis

Plant diseases can be identified and detected by direct and indirect techniques.[6,49] In direct methods, plant pathogens such as bacteria, fungi, viruses, oomycetes, or their biomolecular markers (proteins, nucleic acids, carbohydrates, etc.) are usually isolated from plant tissue and analyzed. In indirect methods, the plant disease is detected via alterations in physiological or histological indications, including changes in growth rate, morphology, leaf surface temperature, or humidity.[49] Current diagnostic methods are mainly based on protein-based molecular assays and nucleic acid amplification (e.g., PCR, ELISA, LAMP, and RPA), which have been widely applied to detect the pathogen of interest through laboratory analysis of biomolecules. Despite the outstanding accuracy and detection sensitivity, these established molecular diagnostic tests require lengthy sample preparation and assay time, expensive instruments, skilled personnel to conduct the analysis, and are limited to laboratory settings.[50] In addition, these methods cannot be performed in remote locations. Therefore, the samples must be collected from the field and transferred to the laboratory for testing.[51] The process typically takes days, which could result in a pandemic of the pathogen and partial or complete loss of crops. Plant diseases, such as late blight, can damage the whole field of crops in a few days if not treated immediately.[52]

A typical nucleic acid-based plant diagnostic test covers three main steps: 1) nucleic acid extraction, 2) amplification, and 3) amplification detection. The nucleic acid extraction step is the most challenging step when translating laboratory molecular assays into POC tests.[53,54] Cetyltrimethylammonium bromide (CTAB)-based extraction is the most extensively used nucleic acid extraction method from plant tissues. This complicated multistep, 40-year-old technique, is still considered a "gold standard" protocol for DNA extraction.[55] The overall process involves 1) mechanical grinding of plant tissues, such as leaves, using mortar and pestle, 2) chemical cell lysis for breaking cells and releasing DNA, and 3) DNA precipitation to separate DNA from other cellular debris mixed with the DNA during the lysis step, and 4) purification with alcohol to eliminate any remaining and unwanted cellular debris, polysaccharides, and proteins (**Figure 6**). This process generally takes hours, requires access to the labs and equipment, and must be performed by skilled personnel.[11] Following the DNA or RNA sample extraction, techniques such as LAMP or PCR amplification and its variants will be performed using specific primers to detect the target sequence of the pathogen.

5. Microneedle-Based Point-of-Care Diagnostic Methods

Plant diseases initiated by pathogens create economic losses, extensive environmental problems in natural ecosystems, and lead to loss of food production.[56] Traditionally, testing was performed in laboratories by a skilled person, thus restricting the roll-out of rapid diagnostics. POC diagnostics devices based on microneedles and in vivo agricultural biosensors can provide non-destructive, in situ, real-time data on plant health to increase agricultural productivity and prevent widespread infection or complete loss of crops. This technology will support the rapidly developing area of precision farming, improve food quality, and reduce crop losses. Some benefits of POC analysis, when compared to standard laboratory testing, include 1) conducting the

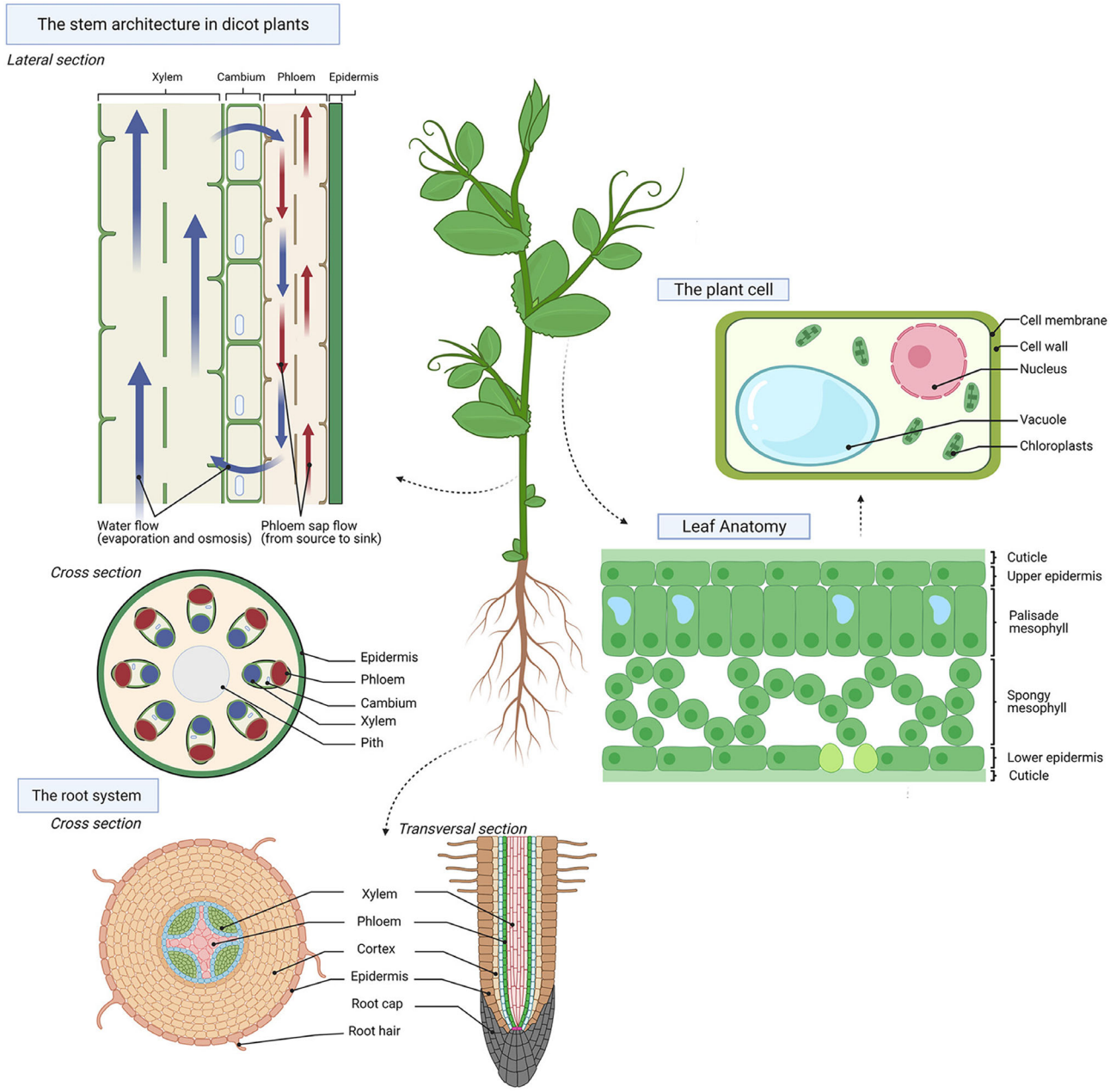

Figure 5. An overview of plant anatomy, demonstrating the stem architecture, root system, plant cell structure, and leaf anatomy. Adopted under the terms of the CC-BY 4.0 license.[100] Copyright 2021 The Authors, American Chemical Society.

diagnostic test by a nonspecialist in the field for rapid analysis and turnaround of results, 2) faster crop management and treatment, 3) lower risks of sample contamination and deterioration, 4) biosecurity applications including surveillance of imported food and plants, 5) no need for a laboratory setting, equipment, and skilled personnel, 6) early detection resulting in prevention of pandemic of plant disease, 7) no loss of samples in transit and transportation, 8) no need for sample storage, and 9) lower number of steps for analysis.[53]

POC devices include technologies that integrate collection systems, sensors, data units, and displaying units to provide information about food and crop health, assisting in the decision-making and management of plant diseases. In particular, for optimum usage in the field, the POC devices should be simple and require as little equipment as possible. Most POC analysis devices developed to date use visual or optical tools such as optical detection of amplicons like chemiluminescence, fluorescence, and colorimetric visualization.[57] The signals are read by smartphones, CCD cameras, light detectors, or even the naked eye.[53] Among all the options, fluorescence-based detection systems have been most extensively used due to their high sensitivity, simplicity, and ability to be integrated with microfluidic

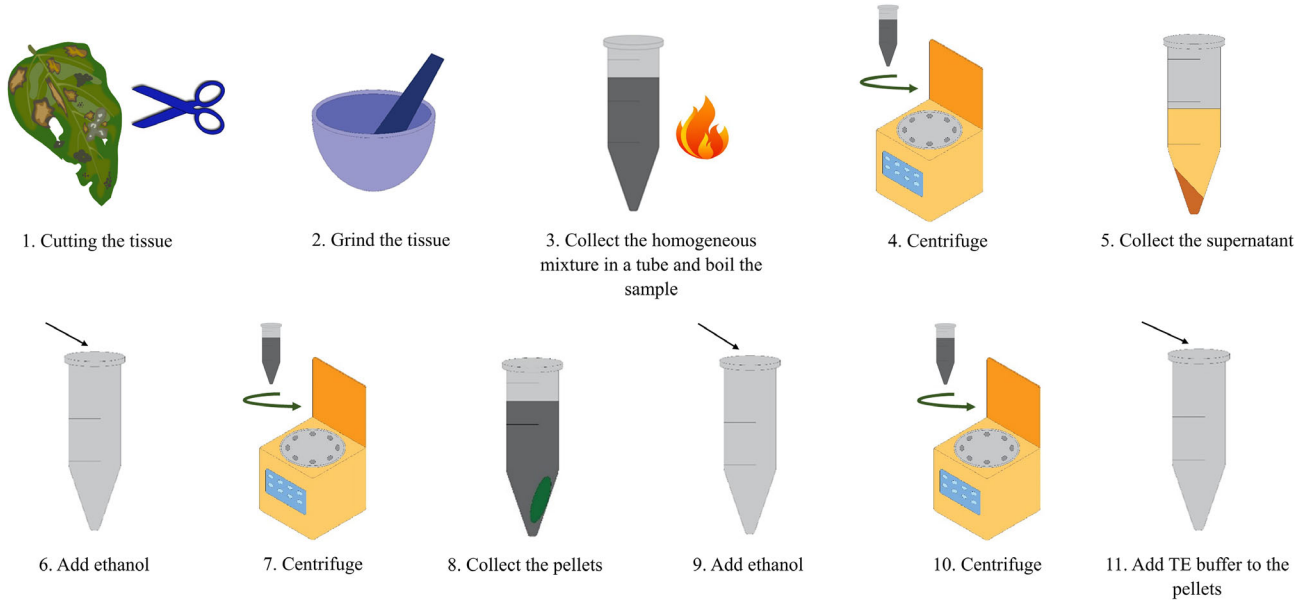

Figure 6. Schematic illustration of laboratory-based CTAB extraction method, which takes a few hours.

devices.[58] Researchers have combined fluorescence-based detectors with different amplification techniques, such as LAMP[59] and qPCR[60] for POC plant pathogen analysis. PCR tests may not be the best option when a simple and rapid POC amplification is needed; in this method, the need for a thermal cycler makes it difficult to miniaturize and integrate it into a POC device.[53] The LAMP technique has a short reaction time and requires simpler equipment when compared to PCR-based methods allowing the amplification reaction to perform at a constant temperature.[61,62] Therefore, the technique has been implemented in several POC pathogen detection devices.[63–65]

By integrating microneedle patches with molecular detection systems utilizing amplification assays (biosensors), rapid, cost-effective, simple, and field-deployable sample extraction, amplification, and diagnosis of plant pathogens will be possible. Despite the high potential of microneedle technology, few studies have reported the use of microneedles for agricultural applications. Microneedles are a great alternative to conventional nucleic acid extraction methods as they provide a simple and cell lysis-free DNA extraction technique for diagnosing a wide range of plant diseases in the field. Because the microneedles sample from a small area on the plant, minimal tissue is damaged, allowing samples to be taken from plants without destroying the plant (e.g., plants being tested in quarantine or young plants with only one set of true leaves or those in tissue culture facilities being cleaned up from viruses). A study examined plant DNA extraction by disposable microneedle patches made from PVA, a water-absorbing polymer. After extracting the sample, the collected DNA sample was analyzed with a real-time PCR technique to detect *Phytophthora infestans*, an oomycete plant pathogen responsible for causing late blight disease on tomato and potato plants. In this study, deploying a microneedle patch as a DNA extraction tool significantly reduced the sample preparation time from hours to seconds. This is mainly due to elimination of complex extraction protocols. Thus, no grinding and purification steps were required. The robust microneedle array broke the cell wall and collected the plant DNA directly from the cells (**Figure 7**a).[11,48] Another study demonstrated that the microneedle device could isolate fragile RNA molecules for RT-LAMP or RT-PCR analysis.[55] However, despite great achievement in both studies, the fidelity of the microneedle mold was not reported. The microneedle mold was purchased from Blueacre Technology Ltd., Ireland, which sells each mold for hundreds of dollars. In addition to nucleic acid extraction, microneedles have been used for other applications, such as sampling from soil and food and bioimpedance measurements. Conventionally, soil properties are measured via coarse sampling of soils and testing at remote laboratories; this strategy may not be adequate to show variation at the proper spatial and temporal resolution.[66] Various soil conditions, such as moisture, soil gas, pests and insects, nutrients and fertilizers, pH variation, temperature, and pollutants in the soil, play a critical role in crop growth and productivity.[67] Monitoring the soil conditions provides vital information to minimize environmental damages, improve resource utilization, and provide essential information regarding soil conditions, plant growth, and their relationship.[66] Therefore, novel technologies with sufficient spatiotemporal resolutions are required for in situ sampling and monitoring of the biological, chemical, and physical properties of soil for efficient agriculture management. O'Flynn et al. developed microneedles-based electrochemical sensors to study nitrate levels in soils to the detection limits of 100 nM. A gold-coated microneedle electrode integrated with a copper passivation layer on top of the gold was used in this study.[68] Recently Kim et al. used microneedle technology for sampling and testing *Escherichia coli* contamination in fish fillets (Figure 7b). The silk-based porous microneedle patch was applied to the commercial food packing without

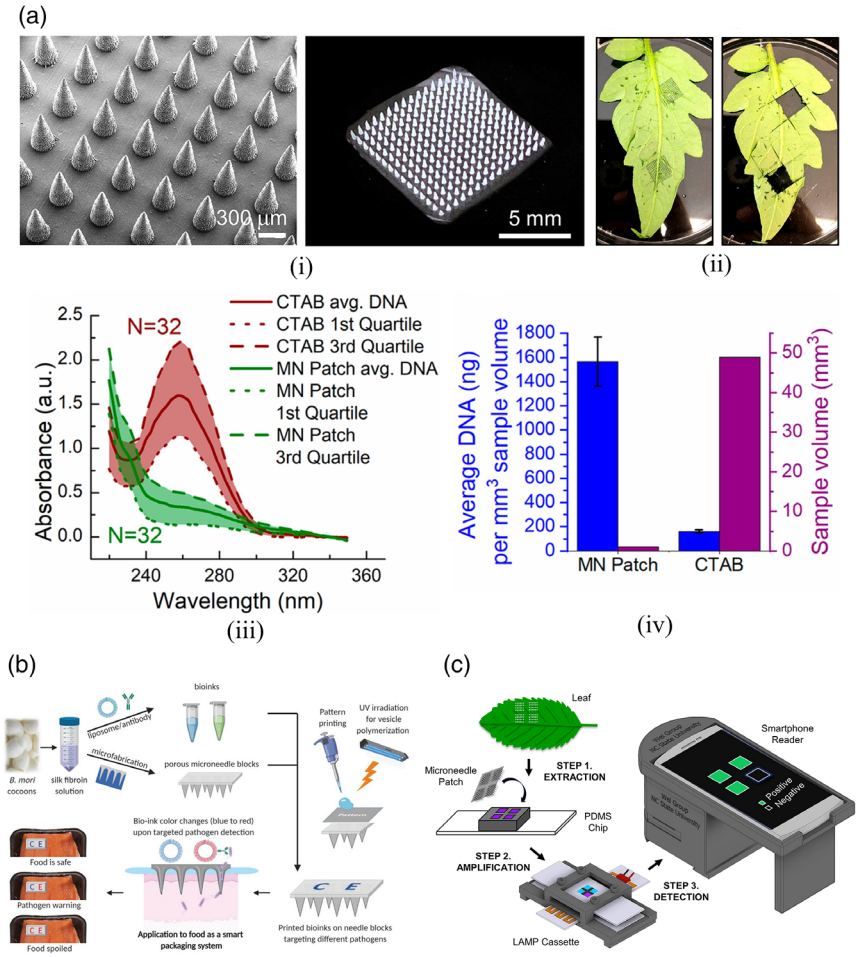

Figure 7. a) Microneedle patches for extraction of DNA, (i) a microneedle patch used to isolate DNA from the plant, (ii) images of tomato leaf after insertion of a microneedle patch, (iii) a comparison of DNA extraction efficiency by Nanodrop UV absorption spectra of samples for microneedle and CTAB extraction techniques, and (iv) a comparison of the total amount of DNA isolated by each method. Adapted with permission.[11] Copyright 2019, American Chemical Society. b) Schematic representation of the food quality monitoring device based on silk microneedle patch using printed bioinks as colorimetric sensors. Reproduced with permission.[69] Copyright 2021, Wiley-VCH. c) A microneedle-smartphone nucleic acid amplification system to detect *Phytophthora infestans* DNA and tomato TSWV RNA. Reproduced with permission.[55] Copyright 2021, Elsevier.

opening the package. The sample collected was consequently transferred to an embedded colorimetric sensor functionalized with antibodies for detecting pathogenic bacteria or an unfunctionalized sensor for monitoring the pH to detect food spoilage. The study showed that the microneedle patch, with 1600 µm-high microneedles, was strong enough to penetrate the commercial polyvinylidene chloride (PVDC) packaging and take samples from fish.[69]

In a recent study, a microneedle device and a 3D-printed smartphone LAMP amplification and detection system were used to detect tomato spotted wilt virus (TSWV), an RNA plant virus, and *Phytophthora infestans* from the infected tomato leaves (Figure 7c).[55] To monitor ion species inside the tomato stem via the impedance measurement method, Jeon et al. created an implantable silicon microneedle device integrated with a micropatterned impedance measurement sensor (11 mm × 5 mm total device size) for direct and real-time measuring of the electrical conductivity of the tomato plant sap. Electrical conductivity provides information regarding the concentration of the nutrient solution in plants. This study created a long in-plane microneedle with a 5 mm length to reach the xylem.[70] The same group later developed a microneedle sensor for real-time measurement of the electrical conductivity of cucumber stems.[71] In another example, Bukhamsin et al. used a microneedle electrode to record the bioimpedance of barley leaves.[72] A study used a tungsten microneedle to quantify the actin response of *Arabidopsis* plants. This work quantified the duration and magnitude of mechanical forces that can stimulate a structural defense response in a plant cell, representing the ultrastructural changes in response to pathogen invasion.[73] Dhanjai et al. fabricated a stainless steel microneedle electrode by the layer-by-layer assembly for real-time monitoring plant polyphenolics such as chlorogenic acid and gallic acid. In order to create a highly stable, sensitive, and conductive surface for antioxidant oxidation, the microneedles were manufactured with layers of carbon nanotube-cellulose nanocrystal and polyaniline conductive polymer.[74]

6. Microneedles as Wearable Devices

Wearable devices, including electronic devices that can be attached to the body or worn on the body, like smartwatches, have been widely used in healthcare for continuous monitoring of biometric data such as blood pressure, temperature, and blood oxygen. However, the concept has not been fully extended for constantly tracking plants' pathological and physiological parameters. Plant wearables can provide precise, simple, and large-scale tracking of plant health compared to techniques like nanobionics,[74] Raman spectroscopy, and IR fluorescence-based measurements, which need more advanced tools for off-site analysis. Despite other wearable devices, such as the adhesive thin-film system which attaches to the surface of plants, microneedle wearable devices can noninvasively and nondestructively reach plants' vascular system and simultaneously sample sap and analyze its physiochemical properties and composition, including electrical conductivity and pH levels. For example, Miller et al. fabricated a metal microneedle patch to estimate plant rehydration and drought conditions by inserting the patch into the sorghum tissue and measuring the impedance of the leaf, root crown, or stalk in reference to the soil.[75] Baek et al. developed a microneedle thermal probe based on a modified Garnier sap flow technique for the noninvasive measurement of sap flow through the xylem in greenhouse tomato plants for monitoring water transportation.[76] Measurement of sap flow is essential for monitoring plant reactions to environmental parameters, including humidity, sunlight, and soil water content; however, the established methods, such as the heat dissipation method, are limited to large woody plants, and small plants like tomatoes cannot survive the conventional invasive procedures, where a large thermal probe is used for measurement.[76] In addition, the heat dissipation method requires heating the macroscale probes 8–10 °C above the ambient temperature, which will interrupt the plant's growth.[77] Thus, the small size and simplicity of the microneedle sap flow sensor provide a reduction in the disruption and damage compared to the current methods.

7. Microneedle-Based Therapeutic Delivery

To enhance food safety and productivity, it is essential to improve agrochemical delivery, including pesticides and fertilizers. Methods such as soil drench,[78] foliar spray,[79] trunk injection/petiole feeding, and root application[80] are the standard techniques used to deliver agrochemicals. However, these established methods can adversely affect the soil microbiome or the environment due to agrochemical runoff. They could have low delivery efficiency due to the presence of the epidermis and cuticle, which act as plants' barrier tissues. Methods such as petiole feeding and trunk injection can address the challenges associated with the plant barrier tissues by mechanically removing the barriers and directly accessing vasculature for higher and more efficient delivery. However, due to the invasive nature of these methods, they are better suited for large plants.[80] Other methods, like pressurized bath infusion and foliar infiltration, are commonly used in labs, but these methods have shown a low delivery efficiency, mainly because the materials are left in the intercellular space of leaves.[81]

The plants usually take up a small percentage of the applied agrochemicals.[82] Technologies such as drones and in-field sensors for automated spraying are promising new tools developed in recent years to improve food production and the negative environmental implications of standard techniques. However, these technologies can be expensive and require a significant investment for implementation.

Microneedle technologies that have been studied to treat human diseases can be translated for treating crop and plant infections caused by pathogens. Microneedle devices have expanded the scope of subcutaneous drug delivery and vaccination across human skin. Applying microneedle arrays to the skin creates micrometer-sized pathways for transporting molecules, such as biomedical antigens and cells, without stimulating the pain nerves.[83] The amount of the therapeutic load that a microneedle patch can deliver relies on the properties of the microneedle device, including insertion capability, size and the number of microneedles, geometry, mechanical integrity, and the amount of loaded dose.[20] Solid and dissolving microneedle geometries are commonly used for therapeutic delivery purposes. In agricultural treatments, microneedle devices can deliver a wide range of therapeutic payloads into plant tissues, from small to large molecules. In this application, it is crucial to control the penetration depth of the microneedle devices so that the patch can reach pathogens residing in hard-to-reach locations of the plant tissue. With the advancement of microfabrication technologies, it is possible to accurately customize the microneedle geometries to ensure agrochemicals can reach plants' targeted areas, such as stems, for a low-cost and more efficient delivery. For instance, *Xylella fastidiosa* bacteria, which causes citrus variegated chlorosis, exists in the xylem of the plant tissue,[84] whereas *Candidatus Liberibacter asiatcus*, which causes citrus greening, exists in the phloem tissue.[85]

Kundu et al. used 5×5 array of stainless steel microneedles with a base width and height of 500 μm to deliver a zinc-based antimicrobial (Zinkicide) model drug into the stem of citrus saplings. This study investigated the uptake mechanism of the therapeutic cargo in the leaves, stems, and roots of the plant. The study showed that the microneedle patch could puncture the targeted xylem and the phloem regions and increase the therapeutic uptake by 7.5 times in the stem and 6 times in the plant leaves. There has been no increase in zinc concentration in roots after treatment, which is of great importance when treating diseases such as Huanglongbing (HLB), also known as citrus greening (**Figure 8**a–c).[86] In another study, Cao et al. designed new silk fibroin-based biomaterials for manufacturing microneedle devices for delivering a variety of cargos, including small molecules and large proteins, into tomato plant xylem and phloem (Figure 8d–g).[80] **Table 2** provides a detailed list of recent studies on microneedles used in food and crop health.

8. Conclusion and Future Perspectives

In recent years, the application and transition of new technologies initially developed in the human medical field have found their way into agriculture and plant health. To address the agricultural industry's challenges, plant treatment and precision agriculture innovations are vital for improving crop productivity,

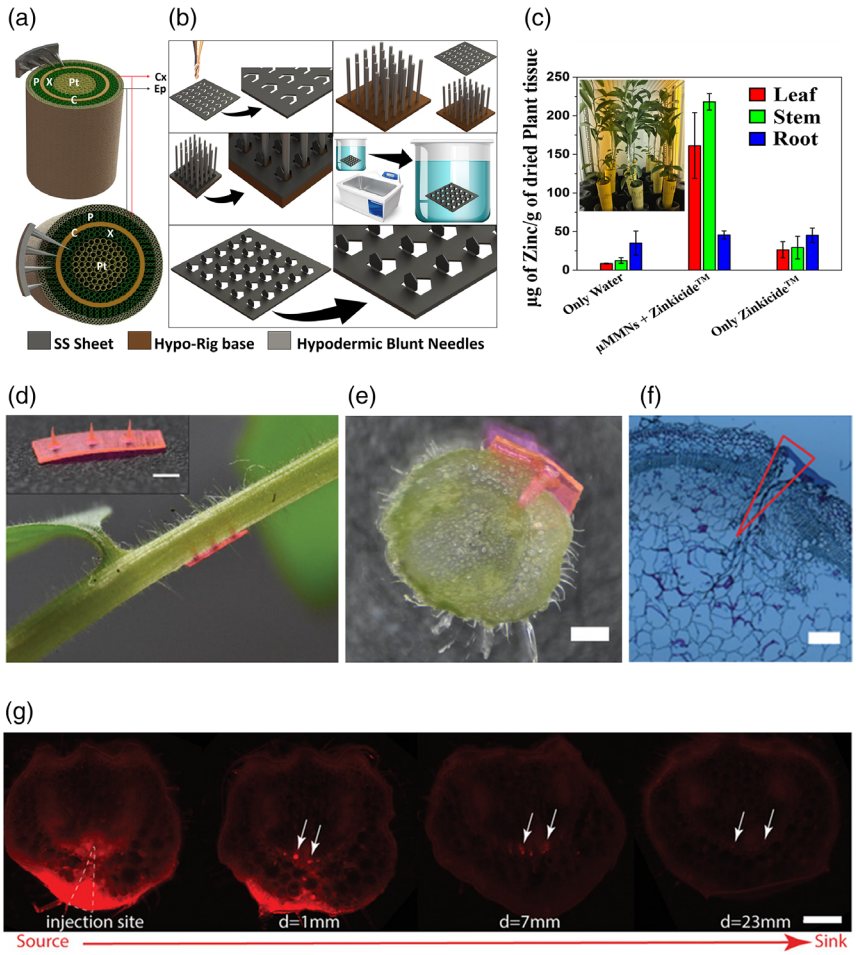

Figure 8. Schematic of a) microneedle array and plant stem tissue and b) the fabrication process of micromilled microneedle arrays. c) Graph showing the Zink concentration in different parts of the plant after the application of Zinkicide. Adapted under the terms of the CC-BY 4.0 license.[86] Copyright 2019, Springer Nature. d) Insertion of a microneedle patch loaded with rhodamine 6G into a tomato plant (scale bars represent 1 mm). e) A section view of the site of injection. f) A histological section of the stem shows the microneedle's penetration for payload delivery. g) Fluorescent microscope images showing the delivery of rhodamine 6G in the tomato phloem. Reproduced under the terms of the CC-BY 4.0 license.[80] Copyright 2020, Wiley-VCH.

increasing plants' resistance to stresses and diseases, and enhancing crop production sustainability. Due to rapid advances in nanotechnology and micro–nanofabrication techniques, significant progress has been achieved in developing emerging platforms to protect crop and plant health.

The application of microneedles for medical purposes has made major developments and provided new solutions to many health problems. Microneedles have gained great interest as high-value technology for drug delivery, vaccination, and diagnostics, targeting various diseases.[87] The design of plant-specific microneedle devices provides new opportunities to enhance crop health and biosecurity, create new tools for diagnostics, and enable new plant engineering developments. For example, common techniques used for the chemical analysis of plants for measuring ionic concentration are destructive and require sacrificing the plant. In contrast, microneedle technology enables noninvasive plant health monitoring or uses microneedles for sample extraction, thus reducing the extraction time from hours to minutes. The extracted samples can either be used for PCR amplification without purification or can be transferred into a microsensor for in situ pathogen detection. This can overcome the problems associated with conventional molecular diagnostic methods based on nucleic acid amplification, enable the farmers and field workers to detect plant disease in the field, and implement treatment strategies in situ without the need to ship the samples to the laboratories for testing. In addition, the modular design of the biosensor will ensure that the device is adaptable and upgradeable, as the industry requires. The agricultural microsensor can replace the traditional, expensive, time-consuming laboratory test procedures without compromising the sensitivity or interfering with crop growth.[88] By integrating microneedle patches with miniaturized amplification assays, fast, economical, simple, and field-deployable amplification and diagnosis of plant pathogens will be possible. Research studies demonstrate that agricultural sensors can accurately detect pathogens that cause various infections in crops and plants.[89]

Table 2. A list of recent studies on microneedles used in food and crop health.

Sample type	Microneedle structure/material	Application	Ref.
Tomato leaves	PVA microneedle patches	DNA extraction for detection of late blight disease in tomatoes from field-infected leaf and laboratory-inoculated samples	[11]
Tomato leaves	PVA microneedle patches	Extraction of *Phytophthora infestans* DNA from infected tomato leaves	[48]
Tomato leaves	An integrated PVA microneedle-smartphone amplification platform	Isolation and detection of *Phytophthora infestans* (DNA-based pathogen) and tomato spotted wilt virus (RNA-based pathogen) from field-collected and laboratory-inoculated tomato leaves	[55]
Soils	A gold-coated microneedle-based electrochemical sensors	Detection of nitrate levels in soils based on microneedles and electrochemical sensors	[68]
Fish fillets	Silk-based porous microneedle patch	Detection of *Escherichia coli* contamination in fish fillets	[69]
Tomato stems	Silicon microneedle device integrated with a micropatterned impedance measurement sensor	Direct and real-time measurement of the electrical conductivity of the tomato stem	[70]
Cucumber stems	Microneedle sensor with an electrode array	Real-time measurement of the electrical conductivity in greenhouse-grown cucumber	[71]
Barley leaves	Microneedle electrodes	Monitor the bioimpedance of Barely leaves	[72]
Arabidopsis plants	Tungsten microneedle	Study the duration and magnitude of mechanical forces that can stimulate a structural defense response in a plant cell	[73]
Orange and kiwi fruits	Stainless steel microneedle electrode	Real-time monitoring of plant polyphenolics such as chlorogenic acid and gallic acid	[74]
Greenhouse tomato tree	Microneedle thermal probe	Noninvasive measurement of sap flow through the xylem for monitoring water transportation	[76]
Citrus seedlings (Citrus reshini, Cleopatra mandarin)	Stainless steel microneedles	Delivery of a zinc-based antimicrobial (Zinkicide) model drug into the stem of citrus saplings	[86]
Tomato and tobacco plants, citrus tree	Silk fibroin-based microneedle devices	Delivery of a variety of cargo to the xylem and phloem, including small molecules and large proteins	[80]

Intelligent material utilization and advances in nanotechnology fabrication allow a low-cost product to be developed. Due to these advantages, research on agricultural biosensors has advanced in the past few years.[90,91] This technology has been extensively applied in nanomedicine for sensing different biomarkers. However, few studies are available for POC diagnosing of food and plant diseases. A POC plant pathogen detection system enables farmers and field workers to detect plant disease in the field and implement treatment strategies in situ without shipping the samples to laboratories for testing. This technology will assist the fast-emerging field of precision farming, enabling more efficient use of resources and reducing the environmental impact of the agriculture industry.[92] Less sample usage without compromising the assay's sensitivity, rapid turnaround of results, cost-effectiveness, quick analysis time, and the potential of developing disposable devices are a few advantages of micronanotechnology implication in POC diagnostics of plants. However, developing reliable miniaturized devices for treating, monitoring, and detecting plant diseases is still a growing field of research.

This review article has highlighted the latest development in microneedle technology for plant disease detection and drug delivery. Rapid POC diagnostics of plant diseases can prevent a pandemic, such as late blight, which can quickly spread throughout the crop field in a few days[52] and enable immediate corrective actions. Early diagnostics promises to improve crop health, quality, yield, and affordable management of diseases, by designing devices for non-specialist use, with minimal instrumentation and training. This technology can potentially bring science to the farmers and assist them in performing their testing. The new transition allows many conventional laboratory tests to be carried out in the crop field at a more cost-effective and faster pace, screen-wide cultivated areas or imported crops and plants for the presence of dangerous pathogens and implement prevention strategies. Enabling farmers to do their testing will increase the amount of testing done and, consequently, help significantly reduce plant diseases. This will also help engage the field workers and farmers in the scientific process. However, despite tremendous development in the field, the technology has not yet been commercialized in any area. This is mainly due to the difficulty in cost-effective scale-up manufacturing of microneedles. Despite the challenges associated with microneedle device development, this miniaturized and convenient technology has remarkable potential to enhance plant and crop disease monitoring, diagnostics, and health management.

The future of smart agricultural biosensing relies on the sensors' sensitivity, reliability, and specificity, as well as the device's functionality, low cost, and miniaturized size. In POC, reliability, and reproducibility are the most critical factors in sampling the analytes, whereas the amplification and detection methods should be fast, sensitive, and easy to use.

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Conflict of Interest

The author declares no conflict of interest.

Keywords

crop health, drug delivery, microneedles, nanotechnology, point-of-care diagnostics

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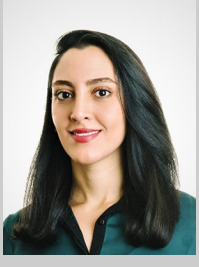

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