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REVIEW

One-dimensional microstructure-assisted intradermal and intracellular delivery

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Abstract

The advancement in the materials manufacturing at micrometer and nanometer scales has already enabled numerous applications in electronics, optics, chemistry, biology and medicine. Biomedical devices carrying micro-/nanostructures are currently being widely used in drug delivery, drug release, biosensing and therapy. New clinical methods for disease diagnosis and treatments are being developed enabled by nanotechnology. One-dimensional (1D) structures are playing an important role in the direct drug delivery both in vivo and ex vivo among various micro-/nanostructures. Here, in this paper, we reviewed recent progresses made on next-generation intradermal and intracellular delivery strategies and applications with focus on 1D microstructure-based approaches.

Keywords Biomedical devices · Intradermal · Intracellular · 1D nanostructure · Drug delivery

Introduction

One-dimensional (1D) structures with sharp tips, such as needles, are capable of penetrating targets of interest such as skin, cells and other tissues as effective medication approaches, including drug/vaccine delivery, infusion and transfusions, although seemingly commonplace, play a key role in disease treatment and saving lives. The fast developing semiconductor industry has brought the advanced manufacturing technologies, such as photolithography, electron-beam lithography, soft lithography and nanosphere lithography [1–6]. As a result, the miniaturization of biomedical devices [7] was realized and it has brought tremendous opportunities to the modern medicine, for examples, microfluidic devices [8], wireless biosensors and drug delivery systems [9] and wearable healthcare devices [10].

Among the different micro-/nanoscale structures used in the variety of devices, the miniaturized 1D structures (microneedles, nanoneedles) have already been adopted in biological study and medical applications with primary focus on intradermal [11] and intracellular delivery applications [12] (Fig. 1). One-dimensional microstructures with different dimensions were used for medical applications at different scales. One-dimensional microstructure with lengths ranging from ~ 50 to ~ 500 microns is primarily used for intradermal delivery applications. For example, microneedles made of either rigid or soft materials were used as the key component in the emergent smart medical patch, which has been demonstrated to be successful in vaccine and drug delivery applications through physically penetrating through the skin [11, 13], as show in Fig. 1a, b [14, 15]. While smaller-scale 1D structures (with at least one dimension < 1 μm) are primary used for cellular level intracellular delivery applications. For example, nanoneedle and nanowire arrays can be used in intracellular delivery [12], as shown in Fig. 1c, d [16, 17]. In this review, we focus on the recent progress made on the intradermal and intracellular delivery strategies based on 1D micro-/nanostructures.

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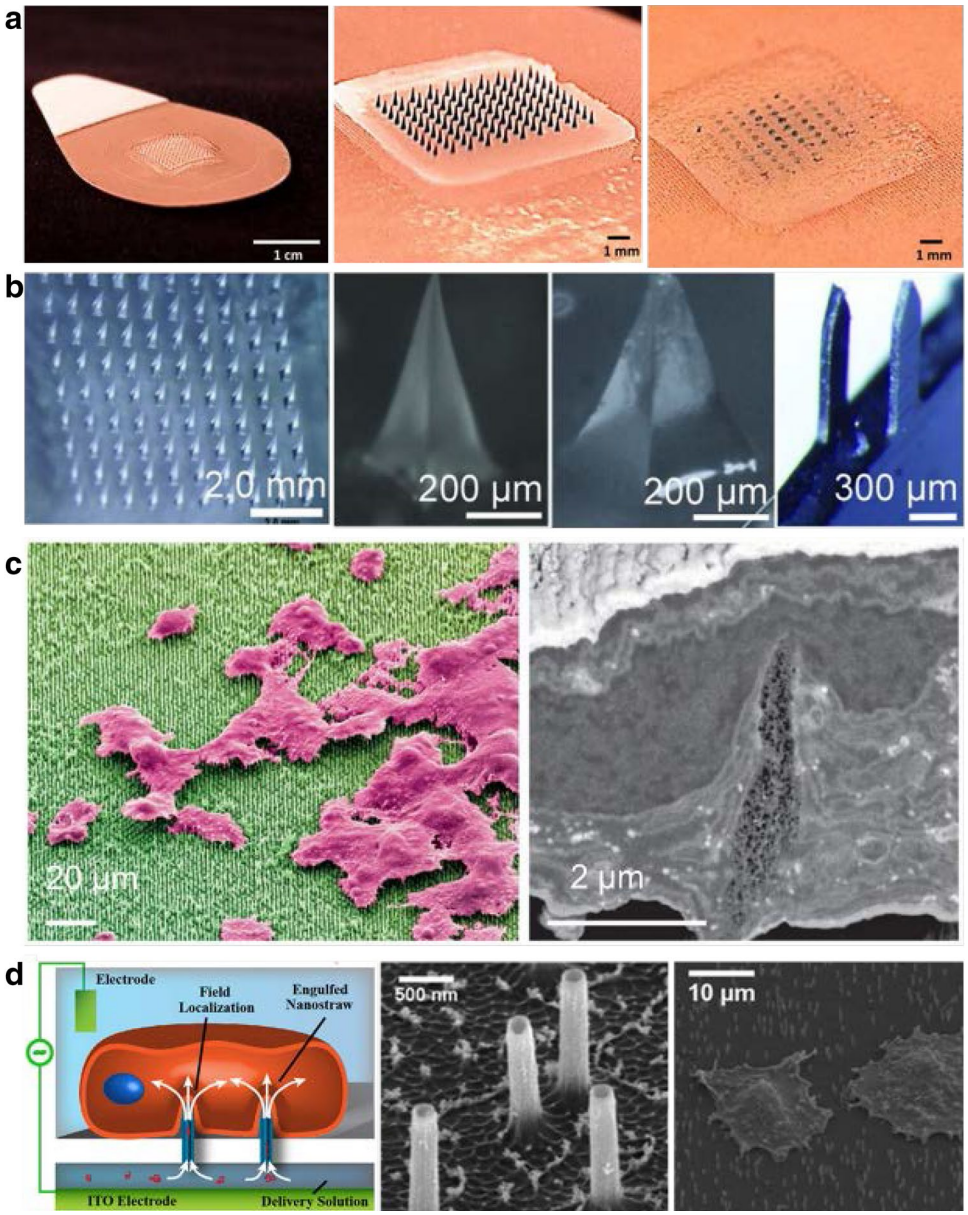

Fig. 1 **a** (left to right) Typical microneedle patch. Typical water-soluble microneedles-carrying drugs; after application to the skin, the microneedles dissolve and release drugs. Adapted and reprinted from the Ref. [14]. **b** (left to right) 10 × 10 array of hydrogel microneedle. Magnified views of a representative hydrogel microneedle before insertion into skin and after swelling in pig skin for 12 h; Typical stainless steel microneedles. Adapted and reprinted from the Ref. [15]. **c** (left to right) Cells deposited on an array of drug-coated porous silicon nanoneedles; the nanoneedles inserted into the cells to release biomolecules of interest. Adapted and reprinted from the Ref. [16]. **d** Representative nanostraw arrays for controlled intracellular delivery. Adapted and reprinted from the Ref. [17]

1D microstructure-assisted intradermal delivery

Skin is known as the largest organ in the body; it serves to protect the body from the outside environment. Small molecule-based medicine applied on the skin can diffuse through the skin barrier to the body. However, not all medicine can penetrate through the skin to reach the capillaries. Controlled release of particular drugs to the blood vessels and the desire for drugs to release locally remain as challenging tasks. Among the various recently developed techniques, 1D microneedle-based intradermal delivery is among the most promising next-generation intradermal delivery strategies [18–21]. Microneedles are 1D sharp structure, which can easily puncture the skin barrier to achieving proper drug penetration.

The precise microfabrication techniques are enabling flexible and customizable dimensions and materials of microneedles for specific applications. Materials for microneedles include semiconductors, metals and polymers [11, 22–25]. For examples, biodegradable porous silicon microneedle arrays were reported fabricated via combination of conventional photolithography with proper etching techniques. Polymer microneedle arrays of ~ 500 μm in length and ~ 5 μm in tip size were fabricated via a micro-molding process using a laser engineered molds [26]. Among different materials, polymer-based microneedles have several advantages over others. First, polymer microneedles can be manufactured at much lower cost due to scalable replica

molding process. Second, polymeric microneedles have lower risk as sharp biowastes [11, 22–24]. Third, polymers are a group of well-studied matrix materials for drug delivery which can themselves be bioactive [11, 27–29].

Microneedles have many advantages over the traditional syringes. First, it is a gentle and kid-friendly approach, because the micrometer-sized needles transverse the stratum corneum without stimulating the nerves, thus avoiding causing pain to the patients [11, 30–33]. Microneedles can be integrated to traditional patches to become microneedle patches. Small injection tasks can be done by the patients, thus reducing the frequency to visit a hospital. The microneedle patch can deliver drug locally at the skin tissue, which can bypasses the systemic circulation, thereby reducing the loss of efficacy and side effects [11]. Microneedle patches can deliver a wide selection of drugs, including proteins, antibodies and vaccines [20, 34–36]. For example, insulin [26] and growth hormone [37] have been successfully delivered through the animal skin using microneedles. Microneedle patches administrated vaccines (such as influenza and hepatitis B) can reach the skin tissue easier while avoid the systemic dosing [25, 38]. In addition, microneedle patches can be used to fight obesity, e.g., the microneedle carried degradable nanoparticles releasing browning agents that can transform white adipose tissue into brown adipose tissue that suppresses weight gain [39, 40].

Generally, based on the working mechanisms, polymeric microneedle patches can be categorized into following four representative types [11]. Type 1: Coated microneedles, as shown in Fig. 2a. Solid microneedles are coated with drug-carrying polymers. Different strategies can be used for coating drug molecules onto the surface of microneedles, including casting, dip coating and deposition [41, 42]. When applied, the microneedle array can be removed, while the drug-carrying coating is left in the skin for treating purpose. Type 2: Dissolvable microneedles, as shown in Fig. 2b. For this type, therapeutic drugs were mixed into the soluble polymeric matrix used to make the microneedles. Typical dissolvable materials include chitosan, maltose and polyvinyl alcohol [43–45]. Upon insertion into the skin, the polymeric microneedles can be fully dissolved and then release the drugs. The dissolving duration ranges from hours to days depending on the dissolution kinetics of the microneedles. Type 3: Degradable microneedles, as shown in Fig. 2c. A variety of therapeutic drugs, such as proteins, require continuous release over a desired period to maintain a constant dose [46]. To achieve this, both of the microneedles and the patch substrate were made of biodegradable polymers. The loaded drug can release upon the hydrolysis of the polymeric matrix. The release and diffusion rate of the drug molecules can be fine-tuned by using suitable polymer matrix with selected molecular weight and degradability. Degradable materials used in this type microneedles patch include silk, polyvinyl alcohol and poly(lactic-*co*-glycolic acid). For example, Hammond and coworkers use silk fibroin/poly(acrylic acid) composite to achieve high biocompatibility and biodegradability [47, 48]. Type 4: Bioresponsive microneedles, as shown in Fig. 2d. The bioresponsive microneedle patch can react to the specific biological signals or local environment changes, such as variations in glucose, pH, enzymes and reactive oxygen species [49, 50]. For example, blood glucose level of bioresponsive microneedle patch has been reported [26]. In which, the increase in H_2O_2 levels caused by the elevated glucose stimulates the release of the insulin embedded in the gel matrix in the "core"; the "shell", consisting of catalase, can then catalyze the excessive H_2O_2 to avoid severe inflammatory responses. Their results have suggested an effective control of blood glucose level in mice model. Such type offers options for on demand release in a relatively precise manner [49].

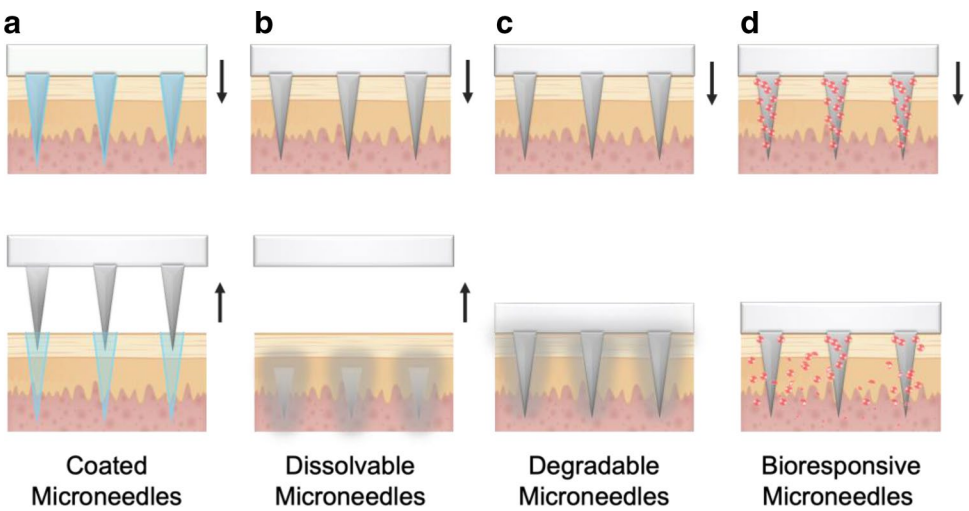

Fig. 2 Four common designs of microneedles for intradermal drug delivery. **a** Coated microneedles; **b** dissolvable microneedles; **c** degradable microneedles; **d** bioresponsive microneedles [11]

1D microstructure-assisted intracellular delivery

Effective and high throughput intracellular delivery systems are playing a critical role in many emergent medical applications ranging from cellular therapies to regenerative medicine [12]. Although vector-mediated approaches have been widely used for in vivo delivery, their biosafety remains a concern due to the use of toxic chemicals or viral vectors. Physical membrane disruption-based approaches are attractive candidates for next-generation intracellular delivery both in vitro and ex vivo [12], such as cell squeezing [51, 52], nanoneedle piercing [53] and hydrodynamic shear [53]. Among them, nanoneedle-mediated membrane disruption has a high delivery efficiency while causing little interruption to the cell's vital functions [53]. Nanoneedles can directly access the cytosol via cell membrane puncture, and nanoneedle-based platforms have been successfully used for intracellular delivery of molecular cargo such as DNA plasmids, RNA and proteins, in a *vector-free* manner. Thus far, three working mechanisms of nanoneedle-mediated intracellular delivery have been reported; they can be divided into the following three modes as shown in Fig. 3: passive puncture and delivery; active puncture and passive delivery; passive puncture and active delivery.

Passive puncture and passive delivery strategy

As shown in Fig. 3a, cells (usually adherent cells) are directly cultured on the substrate containing nanoneedles preloaded with biomolecular cargo. The cargo dissociates from the nanoneedles upon physical penetration through the cellular membrane. The delivery of molecules, like DNA, peptides, siRNA, proteins and impermeable inhibitors to universal cell lines, including challenging neurons and immune cells, has been demonstrated with this method. A recent study suggests that puncture does not occur upon initial contact between the cell and nanoneedle, but due to the active forces generated by cell spreading and formation of tension-promoting focal adhesions [54]. Certain criteria must be met by the nanoneedles, such as aspect ratio, pitch, sharpness and Young's Modulus, in order to achieve good cell membrane penetration [54, 55]. Vertical silicon nanowires being used as a universal platform for delivering biomolecules into living cells; [56, 57] these vertical silicon nanowire arrays can be fabricated by different means, such as template-assisted dry etching, metal-assisted silicon etching or conventional top-down lithography. Scalable nanofabrication techniques such as nanosphere lithography can be readily used to generate nanoneedles with sub-50 nm diameters or hierarchical nanotubes. The dimensions and pitches of the silicon nanowires are also tunable to generate different levels of force on the cells. This variable application of

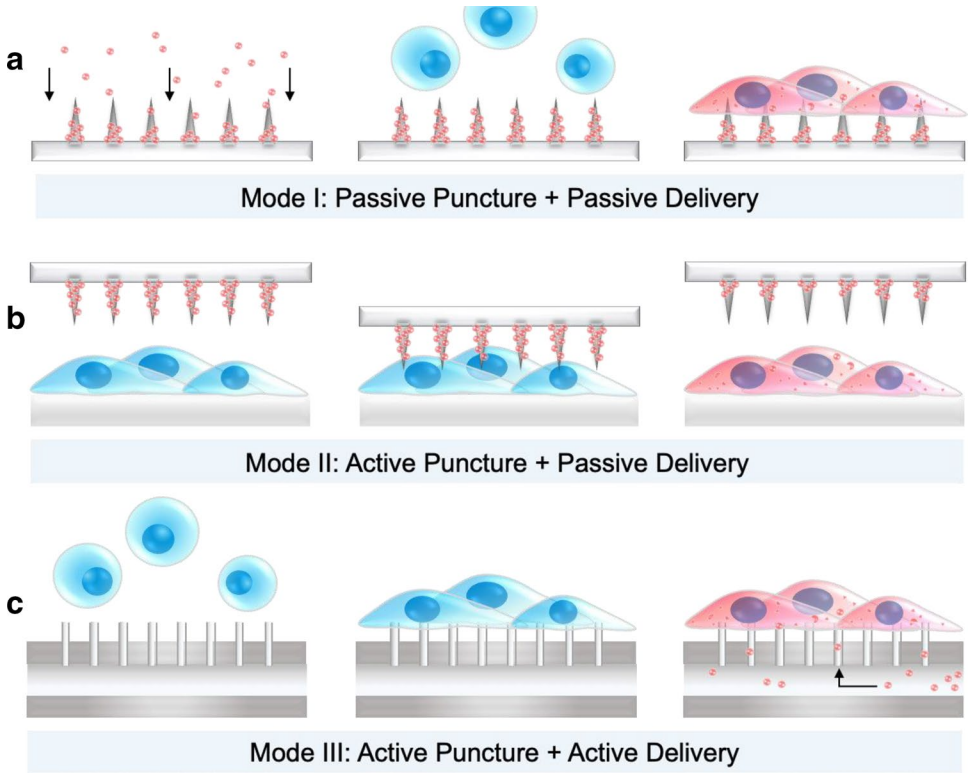

Fig. 3 Common modes for vector-free intracellular delivery enabled by nanoneedles: Mode I: Passive puncture and passive delivery; Mode II: active puncture and passive delivery. Mode III: Passive puncture and active delivery systems based on hollow 1D structures, e.g., tubes

forces has also been used to manipulate the growth of stem cells [58, 59].

Active puncture and passive delivery strategy

As shown in Fig. 3b, biomolecular cargos diffuse from the extracellular medium through the transient nanopores after withdrawal of the needles. It is worth noting that this mode is quite similar to other physical approaches such as electroporation and cell squeeze, which also generate transient nanopores on the cell surface. The cellular membrane recovers within a short period of time. In this method, a standard laboratory centrifuge was used to spin down a nanoneedle array substrate facing the adherent cultured cells, followed by withdrawal of the array and diffusive entry of cargo from the medium. A wide variety of cargos including DNA plasmids, RNA and proteins have been demonstrated to be successfully delivered to different cell types while maintaining > 80% viability [53, 60]. Biodegradable porous silicon needles were also developed to deliver different types of cargos. Since the active puncture is needed to facilitate entry of the molecules of interest, the required force for effective membrane penetration was investigated and estimated to be 2 nN per needle for needles with diameter of ~ 300 nm and height of ~ 4 μm [12]. Reduction in the needle diameter has been shown to help reduce the needed penetration force, thus reducing the required centrifuge speed.

Passive puncture and active delivery strategy

In Fig. 3c, hollow nanoneedles (also called nanotubes or nanostraws) are used to enable direct injection of target molecules (ionic species, DNA plasmids) into the cells after cellular membrane penetration with the nanostructures [17, 61, 62]. However, the passive puncture mode is still involved in this system and thus, a set of suitable nanotube mechanical parameters are needed for effective membrane penetration. The nanotubes or nanostraws can be fabricated using a porous membrane (such as porous polycarbonate) as the template [63]. These nanostraws allow for direct intracellular access without perturbing vital cell functions. A key benefit of this configuration is real-time control over delivery dynamics, volume and dosage concentration, as well as possible gating with electric fields.

Summary and perspective

In summary, the recent progresses on the precisely engineered 1D microstructures have shown great potential in revolutionizing both of the conventional intradermal and intracellular delivery. The microneedle patch enabled intradermal delivery brought unprecedent convenience to the patients as well as doctors with even improved efficiency in the drug deliver and release. Due to its simplicity, microneedle patch is expected to help improve the vaccine coverage in developing countries [64]. In addition, smart micropatch systems are also being developed to for the health monitoring and disease diagnose. The nanoneedle arrays are playing a critical role in the development of next-generation intracellular delivery technologies to greatly facilitate progress in multiple fields from cell-based therapies. They take us beyond routine nucleic acid transfection and enable robust manipulation of previously recalcitrant cell types. Including nanoneedles, other intracellular platforms based on exploding bubbles [65], microfluidic squeezing [66, 67] have been transformed into commercial ventures. Tackling this up and coming age of issues may depend on our capacity to comprehend current conveyance components and to execute the scientific methodologies important to describe cell reactions. Notwithstanding the hindrances that remain, we foresee that cutting edge innovations will make an interpretation of past scholastic undertakings into versatile, customized, cell-based diagnostics and the utilization of clinical intracellular conveyance to design cell destiny for helpful advantage.

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Compliance with ethical standards

Conflict of interest WJ, L.M and X.X declare that they have no conflict of interest.

Ethical approval This review does not contain any studies with human or animal subjects performed by any of the authors.

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