Advanced Materials and Nanotechnology for Drug Delivery

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Abstract: Many biological barriers are of great importance. For example, stratum corneum, the outmost layer of skin, effectively protects people from being invaded by external microorganisms such as bacteria and viruses. Cell membranes help organisms maintain homeostasis by controlling substances to enter and leave cells. However, on the other hand, these biological barriers seriously restrict drug delivery. For instance, stratum corneum has a very dense structure and only allows very small molecules with molecule weight of below 500 Da to permeate while most drug molecules are much larger than that. A wide variety of drugs including genes need to enter cells for proper function but cell membranes are not permeable to them. To overcome these biological barriers, many drug delivery routes are being actively researched and developed. In this review, we will focus on two advanced materials and nanotechnology approaches for delivering vaccines through the skin for painless and efficient immunization and transporting drug molecules to cross cell membranes for high-throughput intracellular delivery.

1. Introduction

Humans are surrounded with endless hazardous agents like viruses, bacteria, gases, chemicals and physical agents. The fine and sophisticated biological barriers in the human body can strongly prevent the access of these hazardous agents into the human body and thus minimize the potential health risk towards humans. Epithelia of the skin, the gastrointestinal tract and the respiratory system are three anatomical primary barriers to isolate the human body from external environment.[1] For example, skin – the largest organ of the human body – contains three layers including stratum corneum, dermis, and hypodermis. The highly packed stratum corneum layer consisting of dead cells acts as a strict and powerful barrier to prevent external agents from invading the human body. While these powerful barriers effectively protect the human body from invading risk agents, they also block the routes for transdermal drug delivery, which is an alternative route for oral and parenteral administration and has tremendous advantages such as pain free, eliminating the first-pass effect, no needle stick injuries, no cross infection and high efficacy. However, the nature of the skin only allows very small molecules with a molecule weight of below 500 Da to permeate skin while most drug molecules are much larger than that.[2] Besides skin, there are also many other biological barriers which seriously limit delivery of drugs to desired sites, including mucosal membranes, the blood-brain barrier and the cell/nuclear membrane.[3] The cell membrane, the biological barrier that isolates and protect cell from outside environment, restricts intracellular delivery of drugs and biomolecules into the cell cytoplasm. These biological barriers seriously limit the delivery of drugs into the desired sites within the body, resulting in low delivery efficacy, poor therapeutic efficacy, and high cost. To address these issues, many biological, chemical and physical strategies have been developed to overcome the biological barriers for greatly improved drug delivery. Herein, we will highlight two of the recent advanced physical approaches for transdermal and intracellular delivery.

2. Microneedle arrays for transdermal delivery

2.1. Silicon and metal microneedle arrays

Needle and syringe injection has been widely used for delivering drugs and vaccines to the subcutaneous layer and muscle. Although working effectively, this approach has many shortcomings such as causing pain and leading to infection and needle stick injuries. To deal with the problems, a great alternative is to deliver drugs to skin for ultimate absorption into the systemic circulation. For this purpose, microneedle arrays are particularly attractive because of their simple administration, cheap cost, minimal invasive property and lower risk of infection.[4-8] At the early stage of the development, microneedle arrays were made of silicon and metals.[4, 5] Silicon microneedle arrays were firstly reported in 1998 and they were fabricated through deep reactive ion etching (DRIE).[9, 10] These microneedles are often a few hundred micrometers in length and with very sharp tips to ensure reliable penetration into the epidermal and upper dermal layers of skin for drug delivery. With these solid microneedles, drugs are often dry-coated on their surface for subsequent delivery to the skin. Desirably, the coating should be mainly on the surface of the distal part of the microneedles because only this part can enter the skin during microneedle penetration (**Figure 1**a and 1b). Such coating is very challenging due to the micro-dimensions of the microneedles,[11] particularly for highly densely packed microneedles.[2] To cope with this, various approaches have been developed for dry-coating a range of molecules to the surface of the microneedles. After coating, the dry-coated microneedles can be applied to the skin and the coating will be dissolved in the wet cellular environment in the skin, indicated by the red signal in Figure 1c and 1d, within a short period of time, generally a few minutes.[2, 11-13] The densely packed microneedles (over 20,000 microneedles per cm^2) were designed for "depositing antigen directly to high populations of antigen presenting cells residing within the skin layers" for ultimately dramatically improved vaccine efficacy.[14] These microneedles can be applied to the skin by using a spring based applicator.[15] By controlling the application speed (strain rate), the penetration depth of a same microneedle patch to the skin can be controlled.[15] Due to the

high density, when the patch was applied to the skin by hand,[11, 16, 17] it led to variable and shallow penetration depth into the skin.[15] With patch application, a study showed that these densely packed microneedles were able to directly deposit antigen to more than 50% of the epidermal and dermal immune cells.[14] In Figure 1c, it can be clearly seen that the coating (red) has been transported to the vicinity of a great number of immune cells (green). Another study showed that, after microneedle delivery of vaccines, the number of the immune cells under the patched area displayed a dramatic reduction of 66% within one day.[18] This indicates the immune cells might have drained to the lymph nodes for generating immune responses. [18] When microneedle arrays were tested for vaccine delivery in animal models, in general, with reference to needle and syringe injection, it is possible to achieve improved immune responses with same doses of vaccines or similar immune responses with reduced doses. For example, when a Macroflux® microneedle array system (330 μm in length, 190 microneedles per cm^2, 1 cm^2 in area) was used to deliver dry-coated ovalbumin (OVA) protein to hairless guinea pigs, at 1 and 5 μg doses, the microneedle administration induced immune responses of up to 50-fold greater than those observed after subcutaneous or intramuscular injection of the same doses, determined by the anti-OVA antibody titers.[19] When another type of densely packed microneedles (110 μm in length, over 20,000 microneedles per cm^2, 4x4 mm per patch, 2 patches per mouse) was used to deliver vaccines in mouse models and compared with intramuscular injection, to induce similar immune responses, the dose sparing was as high as over 100 and 10 folds for influenza vaccine[13, 14, 20] and HSV2-gD2 DNA vaccine,[21, 22] respectively. Microneedle arrays have now been tested to be suitable for delivering a range of vaccines including ovalbumin protein,[12] human papillomavirus (HPV) vaccine (Gardasil® by Merck),[23] inactivated whole chikungunya virus vaccine, DNA-delivered attenuated west nile virus vaccine,[18] inactivated rotavirus vaccine,[24] hepatitis C DNA vaccine, [25] and Bacillus Calmette-Guerin vaccine.[26] Except vaccines, this technology has also been widely applied to deliver insulin,[27, 28] and

hormone.[29] Microneedle technology provides a non-invasive way for insulin delivery and requires minimal training. In addition, reports also show microneedles can help gene transfection and siRNA-based gene silencing.[30, 31] Overall, extensive results have demonstrated successful delivery of a wide range of biomolecules including proteins, DNA, fluorescent probes, viruses, virus-like-particles, drugs, hormones to skin, often with improved efficacy. In addition to enhanced efficacy and minimal pain, microneedles offer other advantages such as excellent thermostability at room temperature compared with liquid vaccine administrated by needle and syringe: influenza vaccine coated microneedle arrays remain stable for at least 6 months at 23 ^{o}C, indicated by its capacity of inducing statistically equivalent immunogenicity with freshly coated patches.[13] In contrast, the vaccine in liquid form has a shelf-life of only 14-20 weeks defined by having above 2/3 of the original hemagglutinin content remained.[32] The thermostability is very important for reducing cost of vaccination and for reaching areas lacking of "cold-chain" for vaccine storage and transport. It is believed that the addition of trehalose and viscosity enhancer in coating solution was beneficial to the improved stability.[33]

2.2. Dissolvable polymer microneedle arrays

Despite of great results of silicon and metal microneedle arrays for transdermal delivery, there are safety and cost concerns including: (1) the fabrication of these microneedles often requires expensive materials and production technologies such as deep reactive ion etching (DRIE) and laser cutting; and (2) the possible breakage of the small microneedles in skin leads to safety problems. To overcome these disadvantages, one approach is to choose biocompatible and biodegradable polymers as the materials for manufacturing microneedle arrays. Such microneedles may be designed to incorporate drugs/vaccines in the interior of the microneedles which can be rapidly dissolved within the skin during application for drug release.[16, 17, 34, 35] Compared with dry-coated solid microneedle arrays, dissolvable ones have superior advantages including: 1) greater drug/vaccine loading capacity; 2) increased control

of release; 3) reduced cost and safety concern because cheap biocompatible and biodegradable materials and cost-effective molding technology can be used in fabrication.[34] This type of microneedle arrays was firstly reported by Prausnitz and co-workers in 2008. [35] In the work, microneedles were made using in-situ UV polymerization of monomeric vinyl pyrrolidone. This kind of microneedles had been used to deliver proteins,[35] influenza prophylaxis[36] and influenza vaccine.[36] For the influenza vaccine, it was found that robust antibody and cellular immune responses were generated. Carboxymethylcellulose (CMC) was also used to fabricate dissolving microneedle arrays.[15, 34, 37] Demuth *et al.* reported composite dissolving microneedles composed of ovalbumin vaccine and silk hydrogel tips for sustainable vaccine release. This technology demonstrates programmable 1-2 weeks vaccine release through which enhanced cellular and humoral immune responses were achieved.[38] Omenetto and co-workers also reported a type of dissolvable silk protein microneedles with tunable release kinetics.[39, 40]

Although dissolvable microneedles possess many advantages and have been demonstrated to work effectively, these dissolvable polymers generally have relatively weak mechanical properties and it is highly desirable to improve their mechanical strength to ensure reliable, consistent and reproducible penetration in human skin for future practical applications. Currently, majority of the research was performed in mouse models, but human skin is mechanically much stronger and thicker than mouse skin. The weak mechanical strength of polyvinylpyrrolidone (PVP) microneedles had been attempted to be increased by forming poly(vinylpyrrolidone-co-methacrylic acid) (PVP-MAA) co-polymer microneedles. However, with the increase of the mechanical strength, the co-polymer microneedles dissolved much slower than the pure PVP ones. In comparison, it took PVP and PVP-MAA (25% of MAA) microneedles to dissolve within 15 min and 2 h, respectively.[35] Very recently, we reported to add nanomaterials to a dissolvable polymer to greatly improve the mechanical properties but without sacrificing the dissolution rate in the skin. **Figure 2**a shows that the Young's modulus

of CMC increases with the addition of layered double hydroxides (LDH) nanoparticles from 0.993±0.065 (Sample "A": pure polymer) to 1.489±0.036 (Sample "B": 2% LDH) and 2.878±0.123 (Sample "C": 5% LDH) GPa. With the mechanically strengthened CMC-LDH nanocomposite, microneedle arrays with very sharp tips can be conveniently fabricated using molding technique (Figure 2b). During fabrication, it is possible to only incorporate drugs/vaccines to the microneedles while the base is only made of polymer (Figure 2c). In the figure, green fluorescence shows the surrogate of vaccines has been predominantly in the microneedles while the base is dark (non-fluorescent). Attractively, these microneedles can rapidly dissolve in the skin within only 1 minute after application (Figure 2d). Confocal microscopy images confirm that the microneedles can successfully pierce pig skin (Figure 2g) and deliver the incorporated fluorescent dyes to the skin (Figure 2h). More importantly, **Figure 3a** indicates that the CMC-LDH nanocomposite microneedles are capable to uniformly pierce pig skin across the whole patch area, while only the central part of the CMC microneedle patch can penetrate into skin (Figure 3b). Furthermore, even in the penetrated area, the penetration depth of nanomaterial strengthened microneedles is greater (71±7 v.s. 46±12 μm for that created by pure CMC microneedles). The application of these microneedles on human skin was also performed with similar findings. In vaccination test in mouse model, microneedle arrays worked much better than needle and syringe injection. After primary immunization, subcutaneous injection of 20 μg of ovalbumin did not generate noticeable antibody titers while microneedle delivery induced already a very strong immune response (Figure 3c). If we compare pure CMC and CMC-LDH microneedles, it can be easily found that CMC-LDH microneedle delivery leads to stronger immune response with a lower dose (Figure 3d). The introduction of nanotechnology to strengthen dissolvable polymer microneedles can greatly increase the drug delivery efficacy and design flexibility and ensure the reliability of this transdermal delivery technique. In the long run, the nanomaterials may

also be used to aid gene delivery and DNA vaccination. This work is an important step toward using dissolvable microneedle arrays for potential use in clinical applications.[41]

3. Nanoneedle arrays for intercellular delivery

3.1 Nanowire arrays for passive penetration into cells for intracellular delivery

Delivery of biomolecules (e.g., cell probes, genes, peptides, drugs, and proteins) into living cells is of great importance to not only basic cell biology but also medical applications. For example, gene and cell therapy is promising for curing many diseases which are otherwise difficult to be treated. In the therapy, genes such as DNA, siRNA, miRNA, and shRNA need to be delivered to cell cytoplasm or nuclei to be functional.[42-45] It is also often neccessary to deliver fluorescent probes into cells for studying biological processes at molecular level inside living cells. However, these molecules have low capability of penetrating cell membranes because of a variety of reasons including large size, surface charge, instability, hydrophilicity, etc.[42, 43] Many efforts have been made to increase intracellular delivery efficacy using techniques such as viral vectors,[45, 46] cell-penetrating peptide vectors,[47] and nanoparticles.[48] Although these methods work effectively with some advantages, their wide applications are hindered by many limitations. For example, there are safety concerns for viral vectors[49, 50]. Nanomaterials and cell-penetrating peptides have the problems of cell-type specificity, limitation of *in vivo* applicability, and cytotoxicity.[51, 52] Except these biological and chemical approaches, physical routes like microinjection and electroporation are also actively developed for intracellular delivery. However, microinjection is time consuming and requires expensive equipment and trained personnel and electroporation often leads to high cell toxicity. Kim *et al.* introduced using silicon nanowire (SiNW) arrays for intracellular delivery. In the work, HEK 293T cells were cultured on the SiNWs array pre-coated with plasmid GFP DNA. During cell culturing, the nanowires were able to pierce into cells and the pre-coated DNA was able to express protein.[53] Shalek *et al.* further demonstrated surface-modified

SiNWs which can be spatially localized for an efficient and universal delivery of biomolecules into immortalized and primary cells. Similarly, the cells also need to be cultured on the nanowires in order for them to pierce into cells for subsequent intracellular delivery. With this method, a wide range of biomedical applications can be achieved. For example, it is able to deliver small molecules to guide neuronal progenitor growth, siRNAs to knock down transcript levels, peptides to inhibit apoptosis, and introduce targeted proteins to specific organelles.[54] Recently, alumina hollow nanostraw arrays were fabricated, which can also penetrate into cells during culturing and then proteins and genetic materials are able to be injected into cells through an integrated device.[55] Also these hollow nanostraws can be equipped with nanoelectroporation platform to achieve highly efficient molecular delivery and high transfection yields with excellent uniformity and cell viability.[56] However, in all of these approaches, cells need to be cultured on the nanowires or nanostraws to allow them to be passively incorporated into cells for subsequent intracellular delivery of various molecules, drugs, genes and fluorescent probes. It is expected that it needs to take a relatively long period of time for cell culturing before intracellular delivery can be performed. The other issue is whether the cells in which intracellular delivery has been achieved by the technologies can be removed from the growing substrate and used for any further applications.

3.2 Nanoneedle arrays to actively disrupt cell membranes for intracellular delivery

Inspired by the success of employing microneedle arrays for drug delivery, we first reported using a nanoneedle array to actively and mechanically disrupt cell membranes for intracellular delivery. Transdermal and intracellular delivery has one common point. For transdermal delivery, the outmost layer of skin, stratum corneum limits the diffusion of most materials, molecules, genes and drugs to the skin. For intracellular delivery, cell membrane plays a similar role, so since microneedles can be used to pierce stratum corneum to make transdermal delivery achievable, one should be able to use a similar approach for intracellular delivery. However, if microneedles are applied to cells, due to their large geometry, cells

might be easily killed. Therefore, nanoneedles were designed for intracellular delivery. To allow the nanoneedles with an extremely small diameter to have enough strength to mechanically disrupt the cell membranes, we used diamond, the hardest material in nature, to produce the nanoneedle patch (**Figure 4a)**.[57] This diamond nanoneedle array was fabricated by bias-assisted reactive ion etching (RIE) of a silicon substrate with a pre-deposited diamond film. Different from time-consuming culturing cells on SiNWs substrates, a suspension containing a large number of cells was rapidly applied onto the diamond nanoneedles. It is expected that the cell membranes will be disrupted during the process so high-throughput intracellular delivery is permitted. Figure 4b and 4c shows two groups of cells in which one group was treated by the nanoneedle array (Figure 4b) and the other group was left untreated. A luminescent iridium(III) polypyridine complex was added in the cell suspension. This luminescent complex is special because it shows negligible nuclear uptake.[58] The results show that nanoneedle treated cells display very strong fluorescence in the cytoplasm of cells (Figure 4b) while the untreated group shows very weak signal (Figure 4c). If we compare the fluorescence signals in the nuclei of the representative cells in the two groups after normalizing their fluorescence intensities in the cytoplasm to be the same value (Figure 4d and 4e), it is easy to find that there is much stronger fluorescence in the nuclei of the nanoneedle treated cells. Because the fluorescent probe is not able to enter the cell nucleus by diffusion, it is reasonable to conclude that the nanoneedle treatment has played an important role in aiding direct nucleus delivery. Other than using fluorescent probes, an anticancer drug, cisplatin, was also tested. Cisplatin needs to enter cells to effectively kill them. In the experiments, it was found that either nanoneedle array or 1 μM of cisplatin treatment led to negligible cancer cell death compared with untreated cells. In great contrast, the viability of the group of cells which was treated by both nanoneedles and 1 μM of cisplatin was dramatically dropped to 39.9±6.5% (Figure 4f). The corresponding optical images of the untreated cells and the 3 groups of cells which were treated under different conditions are

shown in **Figure 5**a-d. These images clearly demonstrate the stunning effect of the diamond nanoneedles in improving intracellular delivery. In another study, with similar mechanism, a densely packed diamond nanocone array was able to improve the delivery of differentiation medium to MC-3T3 cells to speed up their differentiation ability.[59] This novel technology of using nanoneedle/nanocone arrays for intracellular delivery has many superior advantages compared with prior approaches. It is expected to be convenient, highly efficient, high-throughput, universal, safe and cost-effective. This facile approach paves the way for potential high-throughput delivery of genes, drugs and fluorescent probes into cells.

4. Conclusion and outlook

We have reviewed the recent advanced materials and nanotechnology development of drug delivery crossing biological barriers mainly including microneedle arrays for transdermal delivery and nanoneedle arrays for intracellular delivery. Microneedle arrays are expected to play a very important role in skin disease treatment, efficient vaccination and drug delivery. This technology possesses overwhelming advantages of being pain-free, reduced risk of infection and needle-stick injuries, ease of administration, improved efficacy, and minimal requirement of 'cold-chain' facility and trained personnel. The similar principle has now been extended for convenient, highly efficient, high-throughput, safe and universal intracellular delivery. For microneedle arrays for transdermal delivery, relatively systematic studies have been performed and a number of clinical trials are undergoing to bring the technology for clinical use. Further technological development will be needed to ensure the safety profile and further reduce the cost as well as increase the convenience. The approach of applying nanoneedle arrays for intracellular delivery is currently still at its starting stage. More systematic studies are required to demonstrate the advantages of the technology. In addition, it is essential to carry out researches to optimize the nanoneedle array manufacturing and design parameters for optimal and reproducible results. In summary, the purpose of this research

news is to highlight the important progress in the field of advanced materials and nanotechnology for drug delivery crossing biological barriers including microneedle arrays for transdermal delivery and nanoneedle ones for intracellular delivery. We hope to excite the interest to explore more solutions from advanced materials and nanotechnology perspective to overcome the difficulties which we are currently facing and to encourage more *in vitro* and *in vivo* studies, and clinical trials of drug delivery with the developed approaches.

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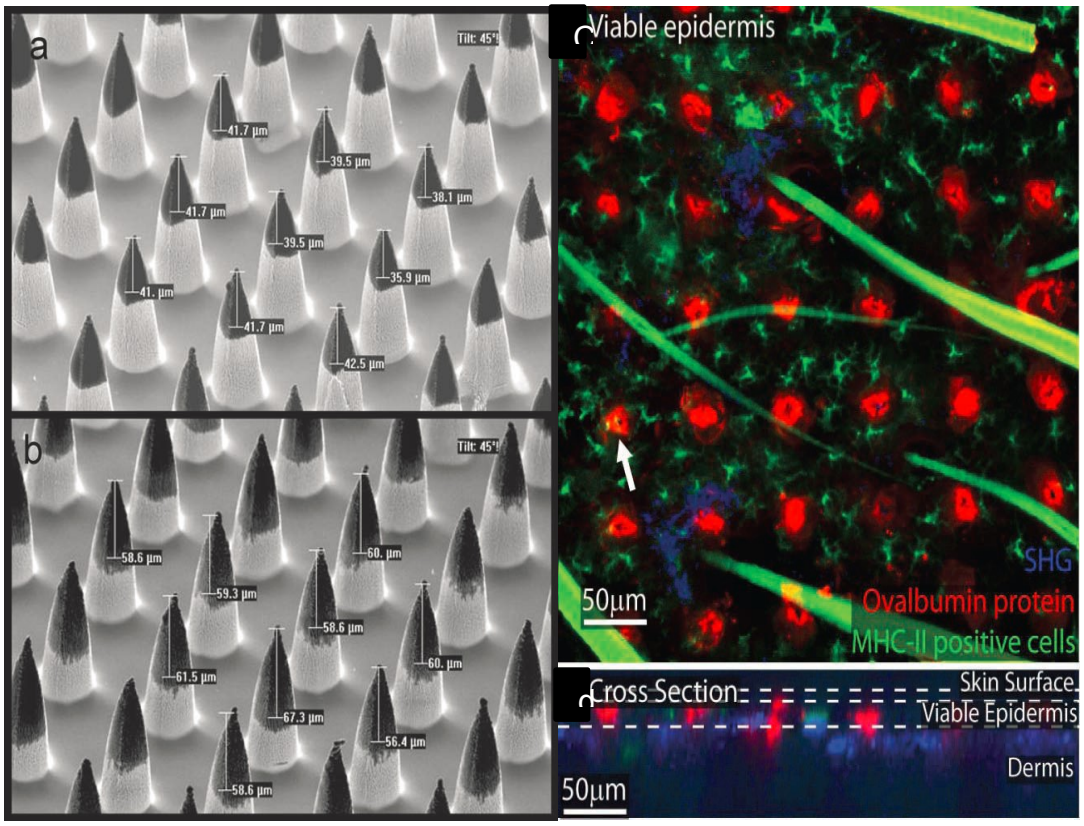

Figure 1 (a) and (b) SEM images of silicon microprojections coated with ovalbumin (OVA) protein. The dark signal on the tips of microprojections indicates dry coated DNA vaccine which can be dissolved and released in the epidermal and dermal layers once the microprojections are inserted into skin. (c) and (d) Fluorescence microscopy images of the release of Cy5-OVA (red) from the microprojection patch applied to murine skin. The image shows a 0.176 μm^2 region of the patched area consisting of 36 projections sites. MHC-II positive cells were stained using an FITC (green) stain. The second harmonic generation of collagen is also shown (blue). d) A cross-sectional view of six coating delivery sites. Colocalization of MHCII positive cells and rhodamine dextran is shown (arrow heads).

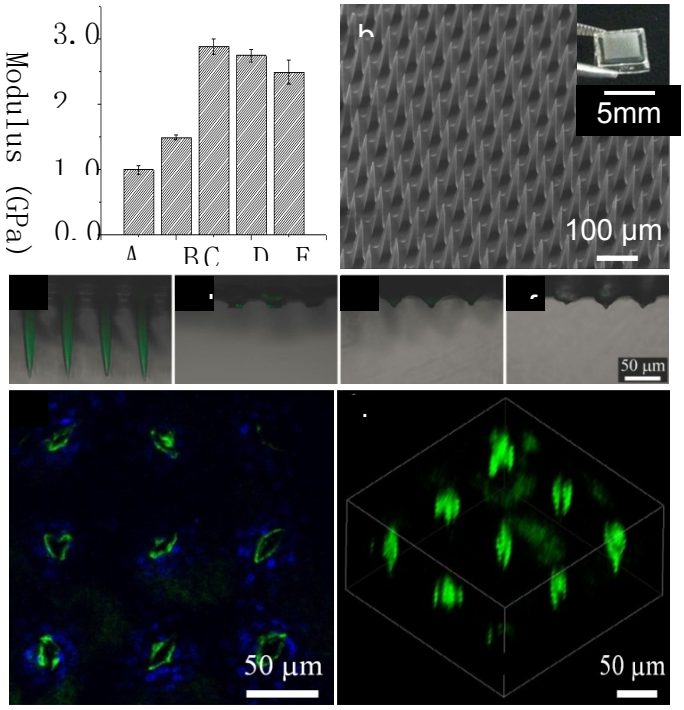

Figure 2 (a) Elastic modulus of CMC polymer films with different LDH concentrations: A) 0 wt%; B) 2 wt%; D) 10 wt% and E) 5 wt% with centrifugation. (b) Scanning electron microscopy images of CMC-LDH nanocomposite dissolvable microneedles (inset: digital camera image of a polymer microneedle array). (c-f) Merged fluorescence and reflectance confocal microscopy images of CMC–LDH nanocomposite microneedles: c) before application, d) 1 min, e) 2 min, and f) 5 min after application to pig skin. (g-h) Laser scanning confocal microscopy images of pig skin after 5 min microneedle application; g: top view and h: z-view.

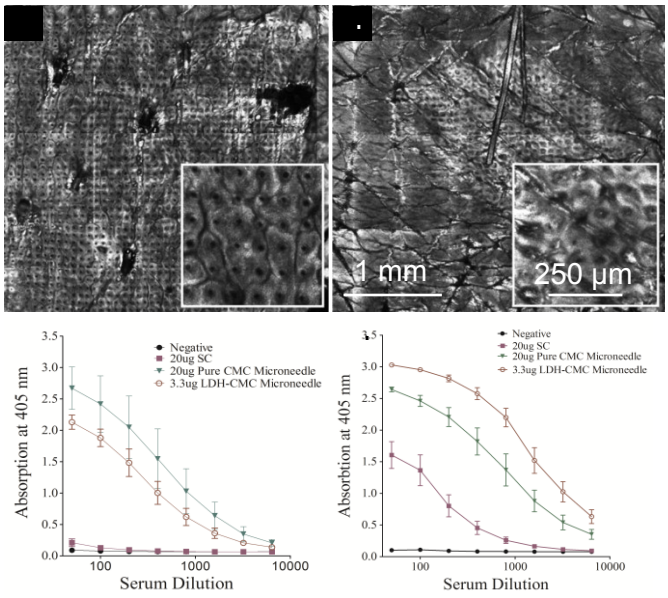

Figure 3 (a-b) Reflectance confocal microscopy images of pig skin after 5 min CMC–LDH nanocomposite microneedle application. (c-d) Total ovalbumin lgG levels at 14 and 38 d after vaccination. Five mice were subcutaneously injected with 20 μg of OVA protein to be the positive control. Four unimmunized mice were negative control. For microneedle immunization, either pure CMC or CMC–LDH nanocomposite microneedle patches containing different amounts of OVA protein were used to vaccinate the mice. Each group has four mice. Mice were immunized at day 0 and boosted at day 17. At days 14 and 38, sera were collected and assayed for antibody titer measurements. The antibody titers at different dilutions of each group of mice were shown in the figure.

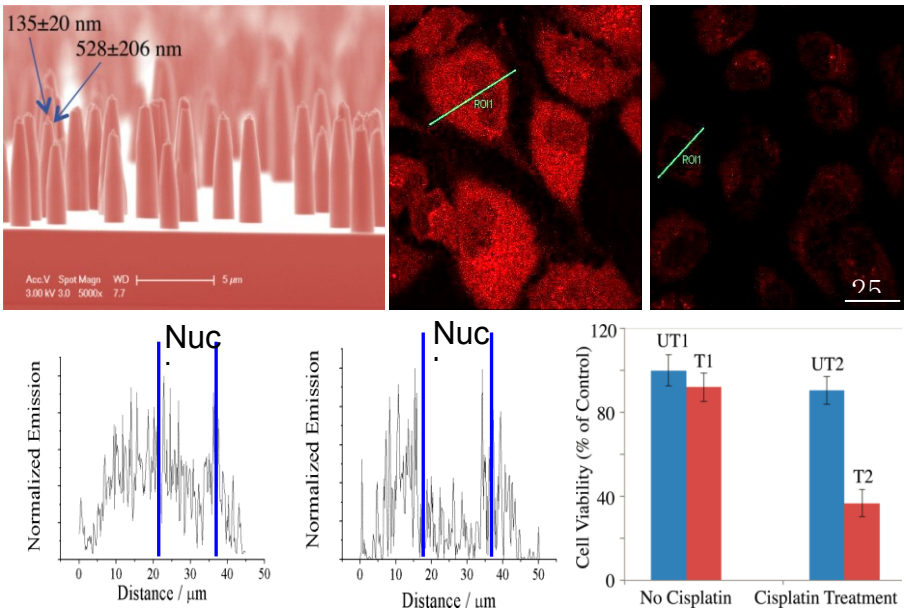

Figure 4 (a) SEM image of diamond nanoprojections; (b) and (c) are the confocal microcopy images of diamond nanoneedles treated cells and untreated cells, respectively, after 19 hours incubation with luminescent iridium (III) polypyridine complex; (d) and (e) show the normalized emission intensity over the lines drawn crossing over cells in (b) and (c), respectively; The scale bars in (b), (c) indicate 25 µm. (f) The viability of cells at 72 hours post plating. The cells were treated with diamond nanoneedles, cisplatin or none or both. UT (shown in blue) and T (shown in red) indicate that the cells were untreated or treated with nanoneedles, respectively. UT1: the cells were treated by neither nanoneedles nor cisplatin; T1: the cells were treated with nanoneedles but not cisplatin; UT2: the cells were treated by cisplatin but not nanoneedles; T2: the cells were treated by both nanoneedles and cisplatin.

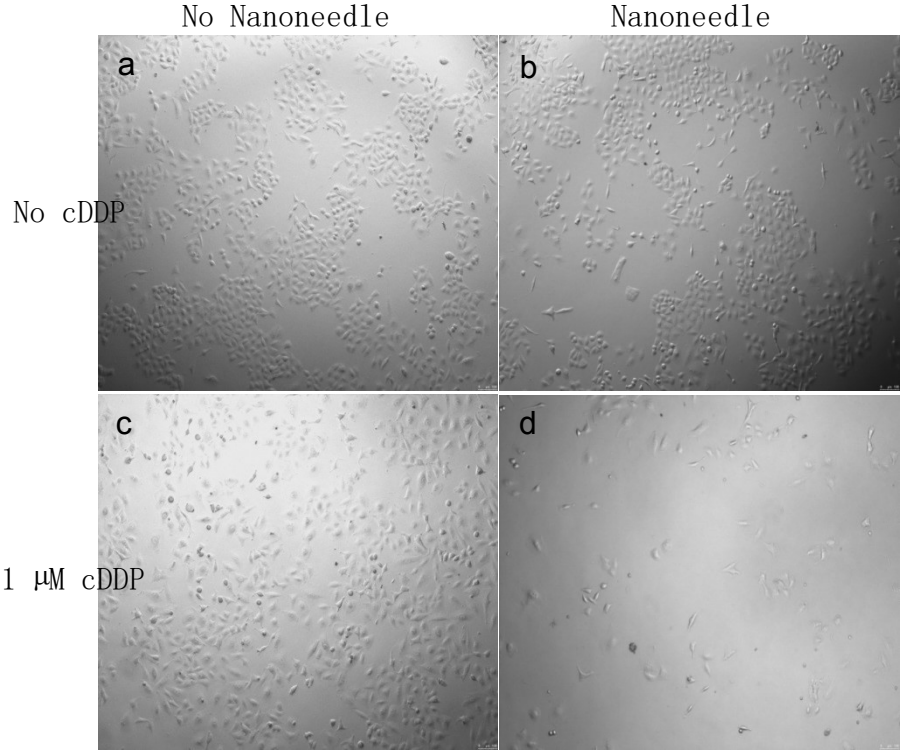

Figure 5 Bright field images of cells at 72 hours post plating. (a) Cells were treated with neither diamond nanoneedles nor cisplatin (cDDP); (b) Cells were treated with diamond nanoneedles but not cisplatin; (c) Cells were treated with cisplatin but not diamond nanoneedles; and (d) Cells were treated with both diamond nanoneedles and cisplatin.

The table of contents entry should be 50−60 words long (max. 400 characters), and the first phrase should be bold.

Biological barriers including stratum corneum and cell membranes pose a difficulty in transdermal and intracellular delivery of fluorescent probes, biological molecules, genes, etc. Various approaches have been or are being developed for tackling the problem. Herein, we review the current state of applying advanced materials and nanotechnology for drug delivery crossing biological barriers by highlighting recently published novel and important results.

Keywords: drug delivery; microneedle arrays; nanoneedle arrays; biological barriers

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Advanced Materials and Nanotechnology for Drug Delivery