Published in final edited form as:

J Mater Chem B Mater Biol Med. 2013 ; 1(14): 1878–1884. doi:10.1039/C3TB00048F.

Nano- and microfabrication for overcoming drug delivery challenges

Kimberly R. Kam and Tejal A. Desai

UC Berkeley and UCSF Joint Graduate Group in Bioengineering, USA

Tejal A. Desai: Tejal.Desai@ucsf.edu

Abstract

This highlight article describes current nano- and microfabrication techniques for creating drug delivery devices. We first review the main physiological barriers to delivering therapeutic agents. Then, we describe how novel fabrication methods can be utilized to combine many features into a single physiologically relevant device to overcome drug delivery challenges.

Introduction

Current advancements in the microelectronics industry have led to the creation of new nano and micro materials. These developments have laid the foundation for novel design possibilities that have been applied to the biomedical field. One important application of these materials is in their use for creating drug delivery devices at a high level of design control. The microelectronics industry is capable of providing nanometer structures in a wide variety of materials at high throughputs. In this highlight article, we discuss how an increased level of design, consistency, and precision can be achieved with nano- and microfabrication to overcome drug delivery challenges.

Nano- and microfabrication techniques

In general, microfabrication is a versatile and facile process that can be employed for the preparation of drug delivery devices. Microfabrication can produce surfaces with ordered structures such as pillars, pits, wells, and grooves at micrometer length scales. It offers a high level of control over the sizes and shapes of drug devices because it is a “top-down” fabrication process. This method is a combination of three major unit process steps: (1) thin film deposition, (2) photolithography, and (3) etching as shown in Fig. 1.¹ In the first step, a two dimensional layout of the features is designed using computer aided design (CAD) software and then printed onto a photomask template. Next, the pattern is transferred onto a substrate through photolithography. This technique exposes a photosensitive polymer to UV light according to the design of the photomask. The crosslinked polymer remains on the surface while the uncrosslinked polymer is washed away. Finally, the resulting pattern which is not covered by the photoresist layer is etched anisotropically or isotropically using wet or dry chemical etching processes. Microfabrication is a powerful tool to develop micron scaled features, but standard photolithography is limited by the wavelength of light and is only capable of generating features with a lower resolution limit between 0.5 and 1 μm .²

However, other techniques such as electron beam lithography followed by nanoimprint lithography are capable of generating features that are not limited by the diffraction limit of light. This technique is a stamping process capable of generating nanometer scale patterns as small as 10 to 25 nm and was developed by Chou *et al.* in the 1990's.^{3,4} In contrast to conventional photolithography, nanofeatures are generated by the direct mechanical deformation of a thermoplastic material using a mold with nanofeatures. The molds are fabricated using electron-beam lithography. Specifically, a polymer resist material such as PMMA is spin casted onto a silicon wafer. The electron beam rasters across the resist according to the pattern that is programmed into the computer. After developing away the unreacted resist material, reactive ion etching is employed to anisotropically etch the underlying silicon wafer. Using plasma ignited by an RF signal, ions bombard the underlying silicon through chemical and physical etching to generate a mold for NIL. Next, the nano-featured mold is brought into contact with a thermoplastic material, and the temperature is raised above the glass transition temperature while the pressure is raised above the elastic modulus of the material to transfer the nano pattern. Another simple method to generate nanowires and nanofibers is through templating which consists of synthesizing the material of interest (usually a thermoplastic polymer) within the nanopores of a membrane.⁵ After the polymer of interest is molded into the template, a dilute solution of sodium hydroxide is used to etch the template material. In addition, by using other methods such as electrochemical oxidation, phase separation, extrusion, and template synthesis, aligned arrays of nanowires made of organic or inorganic materials can be fabricated. These resulting nanostructured films can be integrated into drug delivery devices or stand alone as devices in themselves.

Other “top-down” fabrication methods include shaping spherical microparticles or nanoparticles into various geometries as payloads for drug delivery. For example, DeSimone's group recently developed a method called Particle Replication In Non-wetting Templates (PRINT).⁶ This technique is capable of fabricating monodisperse particles with precise control over the surface charge, geometry, and moduli of the resulting particles.⁷ Another method reported by Champion and Mitragotri employs spherical particles as starting materials and manipulates the morphologies to generate non-spherical particles on the micro and nano length scale. This technique consists of embedding the polystyrene spheres in a polymer film before stretching the film to create ellipsoidal particles. Through this technique, they were able to produce twenty different three dimensional shapes with 200 nm polystyrene spheres as the starting material.⁸ The advantage of these fabrication methods is that highly controlled monodisperse particles can be generated as drug payloads, allowing for control and sustained release of the therapeutic over time. Clearly, there is a plethora of nano- and microfabrication techniques to precisely design and prepare drug delivery devices.

Challenges to drug delivery

Tight junctions and cell membranes

There are many challenges to delivering therapeutic agents, ranging from physiological properties of the tissue to physicochemical properties of the drug as outlined in Table 1. Within the physiological category, the hydrophobic membranes of cells form a highly impermeable barrier to most polar and charged molecules. The plasma membrane is composed of a fluid mosaic of tightly packed lipid molecules interspersed with proteins that are in specific conformations for structural support.⁹ This selectively permeable membrane presents a physical barrier to drug absorption and limits absorption to specific routes and mechanisms.

In addition, the barrier function of the epithelia has important implications for drug delivery. Epithelial tissues compartmentalize the body into cavities and are composed of specialized cells that are sealed together by tight junction proteins in the intercellular space. The paracellular pathway around the cells is one of the main routes for drug diffusion. However, the tight junction pores are small and are approximately 0.5–2 nm, while the hydrodynamic volumes of conventional therapeutics range from few to several hundred nanometers.¹⁰ Therefore, the tight junctions are a major barrier to drug delivery, particularly for large molecular weight therapeutics.¹¹ In order to deliver large molecules systemically, hypodermic needle injections are administered which have several disadvantages including: low patient compliance, accidental needle-sticks, and the associated medical personnel costs.

Mucosal layer

Drugs that are delivered through mucosal epithelia such as respiratory and alimentary tracts, encounter a mucosal membrane that acts as a significant barrier to drug penetration. Mucus is composed of long, entangled glycoprotein molecules called mucins that are arranged in a block copolymer-like structure of branched and un-branched blocks. They serve as a lubricant and protective layer for the body. Mucus is composed of 95% water, 4% mucin, and 1% inorganic salts, carbohydrates and lipids.¹² The viscoelasticity of mucus acts as a mechanical barrier to drug passage. Penetrating this layer would be beneficial to the delivery of drugs across mucosal tissues such as gastrointestinal tract, buccal, vaginal, or lung routes for example.

Clearance

Many drug delivery devices are plagued by the short residence times that they have in contact with the underlying absorption surfaces.¹³ For example, in the respiratory tract, mucus is involved in the process of mucociliary clearance. In this process, mucus entraps substances (dust and microorganisms), within a viscoelastic mucus layer. It is then propelled by the cilia towards the throat and the particulates are swallowed.⁹ Although this is a beneficial process for hazardous substances, drug delivery devices are cleared in this way. Additionally, in the gastrointestinal tract, the residence time is affected by the peristaltic environment which leads to rapid clearance. Therefore, increasing the residence times for devices is essential for improving drug delivery.

Nano- and microfabrication solutions to drug delivery challenges

There have been numerous papers that have reported biochemical approaches to mucosal adhesion using agents such as lectins and chitosan.^{14,15} These chemicals have shown to be effective, but they are limited by batch-to-batch repeatability and are expensive to incorporate into a device. Recently however, a new area in bioadhesion has emerged which relies purely on nanostructural and geometrical cues. This structure-mediated approach is an elegant solution to improve the drug residence time in contact with the epithelium, particularly with mucosal tissue. Specifically, a chemical vapor deposition (CVD) method was reported in which silicon nanowires were grown from silica microbeads (30–50 μm in diameter).^{16–18} It was observed that these fabricated devices significantly increased bioadhesion to mucosal tissues *in vitro* and *in vivo*. Nanowires were also shown to upregulate the mRNA expression levels of PKC which regulates the tight junctions by controlling the contraction of the acto-myosin filaments. Therefore, these nanostructures induced cellular restructuring *via* mechano-transduction pathways by interacting with cells at previously unattainable length scales as supported by the morphological changes in tight junction proteins (ZO-1 and Claudin 1).¹⁶ Therefore, these nanoengineered microparticles were able to significantly enhance bioadhesion, thereby increasing the residence time for model drugs.¹⁶ Additionally nano- and microfabrication allowed for the combination of two advantageous components: a large-scale payload on the microscale with a surface

topography on the nanoscale (see Fig. 2). In contrast, other fabrication processes that are not necessarily “top down” generate either microparticles or nanoparticles, but combining both length scales enables more control over the design of drug delivery devices.

Others have investigated how gecko-inspired nanostructures can increase adhesion to tissues. Geckos stick to a variety of surfaces through their hierarchically structured foot pads, which are finger-like spatulas made of beta-keratin. They adhere to surfaces because of their high surface area to volume ratio of nanosized spatulae, which together adhere to surfaces arising from weak secondary bond forces called van der Waals forces.¹⁹ Although most of the research has investigated dry adhesion, Izadi *et al.* has developed nonsticky fluoropolymer films that have exhibited remarkable adhesion in both dry and wet conditions due to van der Waals forces, electrostatic attractions, and hydrophobic effects.²⁰ Lee *et al.* has also developed a reversible adhesive material that is effective in both wet and dry environments.²¹ They fabricated a regular nanopillar array on a substrate material of poly(dimethyl-siloxane) (PDMS) elastomer using electron-beam lithography. Combined with a mussel-mimetic polymer layer, the device was able to reversibly adhere under wet conditions. In another report, a biocompatible and biodegradable elastomeric material was used for fabricating gecko-inspired adhesives using a silicon template made from photolithography and reactive ion etching. The polymer was cast into the mold and cured by UV light to create nanoscale pillars. Like all micro- and nanofabrication techniques, this method was highly controlled and tunable, allowing them to systematically vary the dimension of the nanoscale pillars. They investigated the ratio of the tip diameter to pitch distance to determine the parameters that lead to enhanced wet tissue adhesion.²² These gecko-inspired fabrication techniques are novel approaches that could be applied to drug delivery devices for enhancing the drug residence time by improving adhesion to mucosal tissues. Therefore, both nanowires and gecko-inspired materials in general are approaches to increase cytoadhesion and combat the mucosal barrier. This increased bioadhesion may also improve drug dosing and efficacy compared to other approaches because it allows for targeted delivery to epithelial tissues. Thus, a patient may not need as high of a drug dose to reach a therapeutic effect which may potentially decrease toxic side effects associated with certain therapeutics (*i.e.* for cancer).

A strategy to overcome the challenge of fast clearance has been addressed by a number of different groups. For example, Adriani *et al.*²³ recently reported the fabrication of mesoporous nanoparticles in disk-like and rod-like shapes to improve particle adhesion under microvascular shear flow conditions. The mesoporous nanoparticles were fabricated using a three-step process consisting of (1) porosification of the silicon film through electrochemical etching, (2) photolithography to pattern the film, and (3) reactive ion etching to form particles. They discovered that the intermediate sized disk-like particles and the largest rod-shaped particles adhered the strongest compared to other sizes. This microfabrication strategy of precisely tuning the size and shape of particles to enhance adhesion could have significant benefits for drug delivery by increasing the drug residence time with the epithelium.

Another innovative strategy to overcome the barrier function of the epithelium, and in particular stratified epithelium, is through the use of microneedles. Microneedles pierce the skin in a non-invasive and painless way. They penetrate the outer 10–20 μm of the skin, creating shunts to the dermis for delivering drugs topically or systemically. Microneedles are fabricated with a wide range of materials and are typically fabricated as an array of up to hundreds of microneedles over a substrate. The first microneedles were produced from silicon wafers by photolithography followed by deep reactive ion etching.²⁴ Other production methods have recently been developed for creating less expensive and biocompatible materials such as metal, polymer, and sugar-based microneedles. Metal

microneedles are mainly produced through laser cutting from sheet metal and bending them perpendicularly out-of-plane. Polymeric microneedles can be biocompatible and because of their viscoelastic properties, they are less prone to breakage once in the skin. Drugs can also be incorporated into biodegradable polymeric microneedles for controlled delivery.

Microneedles have proven effective throughout the literature. While many groups have reported how microneedles enhance the permeability of low and high molecular weight compounds,^{25,26} recent papers have also found microneedles to be effective for vaccine delivery. In one recent study by Matsuo *et al.*, a dissolving hyaluronate microneedle array was used as a transcutaneous immunization device to improve protective immune responses for vaccine antigens.²⁷ There have also been reports on improving upon the traditional design of microneedles. For example, Chu *et al.* recently investigated separable arrowhead microneedles, which is a novel approach to the current limitations of polymer and metal microneedles.²⁸ The arrowhead tips contain encapsulated drug and immediately separate from the metal needle shaft once inserted into the skin. This strategy allows for a quick, safe, and self-administered therapy as an alternative to hypodermic needle injections. The metal shafts provide strong mechanical integrity which allows for skin insertion, and the detachable arrowhead tips eliminate biohazardous sharp waste enabled by dissolving microneedles. Therefore, microfabrication has enabled the systemic design of microneedle arrays in order to overcome the barrier of stratified epithelium for transdermal drug delivery. Furthermore, microneedles incorporated into transdermal patches offer the advantage of reduced pain from drug administration since the needles are so small and short and do not reach nerve endings. This is a huge advantage over hypodermic needle injections because it would lead to subsequently increased patient compliance. Additionally, this route of administration reduces the associated costs of medical personnel who would normally administer injections to patients and it would also improve the safety of healthcare workers by reducing the biohazardous waste.

Finally, to overcome the tight junctional complex of the epithelium, we have recently reported that nanostructured topography loosens the barrier function of epithelial tissue by interacting with the tight junctions.²⁹ An aligned array of low aspect ratio nanopillars fabricated through NIL is capable of reversibly remodeling the tight junction proteins of simple epithelia. This loosening of the tight junctions allows for paracellular transport of high molecular weight therapeutics that are up to four orders of magnitude greater than the current molecular weight limits of the epithelium. Unlike harsh chemical permeabilizers which oftentimes damage epithelial cells, this nanofabricated approach has limited side effects due to the apparent reversibility in the tight junction remodeling. The ability to deliver high molecular weight biologics without the need for hypodermic needle injections has great potential in the field of drug delivery, and specifically in biotechnology.

Microfabrication enables the rational design of nano- and microparticles with different shapes and geometries in order to probe the cell membrane barrier. The shape of the particle influences a number of behaviors associated with drug delivery. For example, recent studies have reported that particles of various non-spherical geometries influence the rates of cellular internalization. In one study, HeLa cells were reported to internalize nonspherical particles made by PRINT processing that resemble rod-like bacteria with dimensions as large as 3 μm .³⁰ However, other studies by this group showed that cubic particles with lengths of 3–5 μm were not internalized. They reported how the particles with higher aspect ratios seemed to exhibit higher internalization rates than the lower aspect ratio cubic particles. Interestingly, it appears that aspect ratios that are too high have greater difficulty being internalized by macrophages. For example, studies by Geng *et al.* and Sharma *et al.* reported that particles with aspect ratios greater than 23 exhibited reduced phagocytosis, and studies by Champion *et al.* also show that aspect ratios greater than 20 are not

phagocytosed.^{8,31,32} The observed trends in particle aspect ratio could be explained by the thermodynamic and kinetic modeling and analyses reported by Li *et al.*³³ They constructed an endocytosis phase diagram of radius *versus* particle aspect ratio for the rational design of drug delivery nanoparticles. In this paper, Li *et al.* reported that the optimal nanoparticle radius for the fastest endocytosis rate is 25 nm. This minimum radius decreases as the aspect ratio increases which would explain the empirical results from the studies by Geng *et al.*, Sharma *et al.*, and Champion *et al.* Nano- and microfabrication allows for a high level of control to tune the size, aspect ratio, and shape of particles for drug payloads. With this precise control, monodisperse particles can be generated which allows for more accurate control over drug release rates from particle payloads. This is a tremendous advantage over the more established self-assembly or “bottom-up” approaches which generate a distribution of particle sizes and shapes.

In other studies, nano- and microtopographical cues were discovered to modulate cellular internalization rates. For example, Teo *et al.* cultured mesenchymal stem cells and monkey kidney fibroblasts on nano- and micropillar substrates that were fabricated with electron beam lithography followed by nanoimprint lithography.³⁴ After FACs analysis, they reported higher cellular internalization of fluorescently labeled dextran when cells were cultured on the micron sized pillars. They hypothesized that these larger pillars were able to induce more actin-dense regions compared to the smaller nanopillars. This phenomenon would translate into higher intracellular contractility, upregulation of Rho GTPases, and higher rates of macropinocytosis.

Furthermore, another advantage of microfabrication is that it is capable of creating microdevices with precisely controlled modular components that are tailorable for specific drug delivery applications. For example, Bernards *et al.* recently reported a novel fabrication method to create nanoporous thin films for the zero-order release of a small molecule and protein.³⁵ The theory behind this approach is that the nanopores were made to be on the same length scale as the drug of interest so that single-file, concentration-independent transport was achieved. Bernards *et al.* grew zinc oxide rods hydrothermally and used the resulting nanorods as a template. They then spin casted a solution of biodegradable polycaprolactone onto the nanostructured template. After etching away the ZnO in a weakly acidic solution, a nanoporous PCL thin film was achieved. Others have reported similar nanoporous membranes fabricated through different methods. For example, nanoporous PCL thin membranes were fabricated for zero-order drug release of IFN- α , an antitumor agent. This group recently reported a hot embossing technique followed by phase inversion to create nanoporous membranes with pores of approximately 20–60 nm.³⁶ These nanofabrication methods allow for controlled nano-pore sizes to achieve zero-order drug release. This nanofabrication approach precludes the need for continuous therapeutic injections since a nanoporous delivery device can sustain release up to 6 months *in vitro*.³⁷ This is a remarkable advantage over the gold standard treatment for diseases such as wet macular degeneration, for example, where patients currently receive eye injections once a month. Therefore, with only one surgery to implant the nanoporous device, this approach would lead to improved patient compliance since it would reduce the pain associated with frequent injections.

Future directions

Nano- and microfabrication will continue to be explored for therapeutic delivery applications because of their ability to combine modular components such as shape, geometry, and size into a single device. Future work may include a further understanding of how the device influences biological processes on the nano- and microscale that could be

harnessed for drug delivery applications. This would enable the exploration of a spectrum of subtle interactions that were previously unattainable.

Although this field has demonstrated promising results, it's important to recognize that it is still in its infancy. Most studies have been performed with *in vitro* and *ex vivo* models. *In vivo* considerations such as biodistribution, toxicity, protein adsorption, and device removal have had limited exploration. Furthermore, affordable scale-up methods should be explored to enable translation of these devices to commercial markets. This area of nano- and microfabrication research has considerable room for growth and offers exciting possibilities for the future of drug delivery.

Biographies

Kimberly Kam received her Ph.D. from the UC Berkeley and UC San Francisco Joint Graduate Group in Bioengineering. Her work investigated how nanotopographical cues loosen the epithelial barrier function for the transport of high molecular weight biologics. She received her M.S. in Materials Science & Engineering from UC Berkeley and her B.S. from the Massachusetts Institute of Technology (MIT). She currently works as an Associate Scientist at Genentech in South San Francisco.

Dr. Tejal Desai is currently Professor of Bioengineering and Therapeutic Sciences at the University of California, San Francisco. She is also a member of the California Institute of Quantitative Biomedical Research and Chair of the UC Berkeley & UCSF Graduate Group in Bioengineering. Dr. Tejal Desai directs the Laboratory of Therapeutic Micro- and Nanotechnology at UCSF. Her research uses micro- and nanofabrication techniques to create implantable biohybrid devices for cell encapsulation, targeted drug delivery, and templates for cell and tissue regeneration. In addition to authoring over 150 technical papers and delivering over 150 invited talks, she is the co-editor of an encyclopedia on Therapeutic Microtechnology. Her many innovations have awarded her a number of accolades, including a 2000 National Science Foundation CAREER Award; a 2003 Eurand Grand Prize for outstanding research in oral drug delivery, and 2006 Grand Prize for innovative approaches to drug delivery.

References

1. Ainslie KM, Kraning CM, Desai TA. Microfabrication of an asymmetric, multi-layered microdevice for controlled release of orally delivered therapeutics. *Lab Chip*. 2008; 8:1042–1047. [PubMed: 18584077]
2. Qin D, Xia Y, Whitesides GM. Soft lithography for micro- and nanoscale patterning. *Nat. Protoc*. 2010; 5:491–502. [PubMed: 20203666]

3. Chou SY, Krauss PR, Renstrom PJ. Imprint lithography with 25-nanometer resolution. *Science*. 1996; 272:85–87.
4. Krauss, PR.; Chou, SY. Sub-10 nm Imprint Lithography and Applications; 1997 55th Annual Device Research Conference Digest; p. 90-91.
5. Tao SL, Desai TA. Aligned arrays of biodegradable poly(epsilon-caprolactone) nanowires and nanofibers by template synthesis. *Nano Lett*. 2007; 7:1463–1468. [PubMed: 17488047]
6. Perry JL, Herlihy KP, Napier ME, DeSimone JM. PRINT: a novel platform toward shape and size specific nanoparticle theranostics. *Acc. Chem. Res*. 2011; 44:990–998. [PubMed: 21809808]
7. Canelas DA, Herlihy KP, DeSimone JM. Top-down particle fabrication: control of size and shape for diagnostic imaging and drug delivery. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol*. 2009; 1:391–404. [PubMed: 20049805]
8. Champion JA, Mitragotri S. Role of target geometry in phagocytosis. *PNAS*. 2006; 103:4930–4934. [PubMed: 16549762]
9. Hillery, AM.; Lloyd, AW.; Swarbrick, J. Drug Delivery and Targeting: For Pharmacists and Pharmaceutical Scientists. CRC Press; 2002.
10. Kitchens K, Kolhatkar R, Swaan P, Eddington N, Ghandehari H. Transport of poly(amidoamine) dendrimers across Caco-2 cell monolayers: influence of size, charge and fluorescent labeling. *Pharm. Res*. 2006; 23:2818–2826. [PubMed: 17094034]
11. Niessen CM. Tight junctions/adherens junctions: basic structure and function. *J. Invest. Dermatol*. 2007; 127:2525–2532. [PubMed: 17934504]
12. Peppas NA, Huang Y. Nanoscale technology of mucoadhesive interactions. *Adv. Drug Delivery Rev*. 2004; 56:1675–1687.
13. Baloglu E, Senyigit ZA, Karavana SY, Bernkop-Schnürch A. Strategies to prolong the intravaginal residence time of drug delivery systems. *J. Pharm. Pharm. Sci*. 2009; 12:312–336. [PubMed: 20067707]
14. Woodley J. Bioadhesion – new possibilities for drug administration? *Clin. Pharmacokinet*. 2001; 40:77–84. [PubMed: 11286325]
15. Smart JD. Recent developments in the use of bioadhesive systems for delivery of drugs to the oral cavity. *Crit. Rev. Ther. Drug Carrier Syst*. 2004; 21:319–344. [PubMed: 15638469]
16. Uskoković V, Lee PP, Walsh LA, Fischer KE, Desai TA. PEGylated silicon nanowire coated silica microparticles for drug delivery across intestinal epithelium. *Biomaterials*. 2012; 33:1663–1672. [PubMed: 22116000]
17. Fischer KE, et al. Biomimetic nanowire coatings for next generation adhesive drug delivery systems. *Nano Lett*. 2009; 9:716–720. [PubMed: 19199759]
18. Fischer KE, et al. Hierarchical nanoengineered surfaces for enhanced cytoadhesion and drug delivery. *Biomaterials*. 2011; 32:3499–3506. [PubMed: 21296409]
19. Autumn K, et al. Evidence for van der Waals adhesion in gecko setae. *PNAS*. 2002; 99:12252–12256. [PubMed: 12198184]
20. Izadi H, Zhao B, Han Y, McManus N, Penlidis A. Teflon hierarchical nanopillars with dry and wet adhesive properties. *J. Polym. Sci., Part B: Polym. Phys*. 2012; 50:846–851.
21. Lee H, Lee BP, Messersmith PB. A reversible wet/dry adhesive inspired by mussels and geckos. *Nature*. 2007; 448:338–341. [PubMed: 17637666]
22. Mahdavi A, et al. A biodegradable and biocompatible gecko-inspired tissue adhesive. *Proc. Natl. Acad. Sci. U. S. A*. 2008; 105:2307–2312. [PubMed: 18287082]
23. Adriani G, et al. The preferential targeting of the diseased microvasculature by disk-like particles. *Biomaterials*. 2012; 33:5504–5513. [PubMed: 22579236]
24. McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles for gene and drug delivery. *Annu. Rev. Biomed. Eng*. 2000; 2:289–313. [PubMed: 11701514]
25. Mikszta JA, et al. Improved genetic immunization *via* micromechanical disruption of skin-barrier function and targeted epidermal delivery. *Nat. Med*. 2002; 8:415–419. [PubMed: 11927950]
26. McAllister DV, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *PNAS*. 2003; 100:13755–13760. [PubMed: 14623977]

27. Matsuo K, et al. A low-invasive and effective transcutaneous immunization system using a novel dissolving microneedle array for soluble and particulate antigens. *J. Controlled Release*. 2012; 161:10–17.
28. Chu LY, Prausnitz MR. Separable arrowhead microneedles. *J. Controlled Release*. 2011; 149:242–249.
29. Kam K, et al. Nanostructure-mediated transport of biologics across epithelial tissue: enhancing permeability *via* nanotopography. *Nano Lett*. 2013
30. Gratton SEA, et al. The effect of particle design on cellular internalization pathways. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:11613–11618. [PubMed: 18697944]
31. Geng Y, et al. Shape effects of filaments *versus* spherical particles in flow and drug delivery. *Nat. Nanotechnol.* 2007; 2:249–255. [PubMed: 18654271]
32. Sharma G, et al. Polymer particle shape independently influences binding and internalization by macrophages. *J. Controlled Release*. 2010; 147:408–412.
33. Li X. Size and shape effects on receptor-mediated endocytosis of nanoparticles. *J. Appl. Phys.* 2012; 111 024702.
34. Teo BKK, et al. The effect of micro and nanotopography on endocytosis in drug and gene delivery systems. *Biomaterials*. 2011; 32:9866–9875. [PubMed: 21924770]
35. Bernards DA, Desai TA. Nanotemplating of biodegradable polymer membranes for constant-rate drug delivery. *Adv. Mater.* 2010; 22:2358–2362. [PubMed: 20376851]
36. He H, et al. Use of a nanoporous biodegradable miniature device to regulate cytokine release for cancer treatment. *J. Controlled Release*. 2011; 151:239–245.
37. Bernards DA, Lance KD, Ciaccio NA, Desai TA. Nanostructured thin film polymer devices for constant-rate protein delivery. *Nano Lett*. 2012; 12:5355–5361. [PubMed: 22985294]

Fig. 1.
 (a) A general overview of “top-down” microfabrication involving the steps of thin film deposition followed by UV photolithography using a template mask to generate a PEGMA laden SU-8 microdevice. (b) A micrograph of the resulting hydrogel-filled microdevices. Figure was reproduced from Ainslie *et al.*¹

Fig. 2.

Nanoengineered microparticles demonstrate the modular and highly-controllable nature of “top-down” nano- and microfabrication. By combining large microparticles with nanowires, the drug delivery device is able to optimize a large drug payload with good cytoadhesion to epithelial cells. Figure was reproduced from Fischer *et al.*¹⁷

Table 1

An outline of the various drug delivery challenges that are overcome by nano and microfabrication. (a) Zonula Occluden-1 tight junction fluorescence has a ruffled morphology when in the presence of the nanostructured topography, indicating a remodeling in the tight junction to allow for drug transport across the epithelial barrier (scale bar is 20 μm).²⁹ (b) Nanowires significantly enhance bioadhesion due to the higher surface area to volume ratios for higher van der Waals interactions.¹⁷ (c) Microneedles penetrate the skin and disrupt the primary barrier in transdermal drug delivery, the stratum corneum.²⁸ (d) Nanoparticles of varying geometries lead to differences in cellular internalization rates.³⁰ (e) Nanoporous membranes with pore sizes on the same order as the therapeutic of interest enable zero-order drug release from a payload³⁵

Specific challenge	Nano- or microfabrication solution	Examples
(a) Tight junctions seal epithelial cells together (0.5–2 nm in width)	<ul style="list-style-type: none"> Nanostructures loosen the tight junctions (ZO-1 and Claudin 1) for paracellular drug delivery
(b) Mucosal layer and clearance	<ul style="list-style-type: none"> Nanowires enhance adhesion through van der Waals forces Gecko-inspired wet adhesive materials
(c) Overcoming the stratum corneum barrier for transdermal drug delivery	<ul style="list-style-type: none"> Microneedles provide shunts to the dermis, disrupting the dead corneocytes, for topical or systemic drug delivery
(d) Cellular internalization	<ul style="list-style-type: none"> Micro or nanoparticle geometry, shape, and surface chemistry influences endocytosis

Specific challenge	Nano- or microfabrication solution	Examples			
(e) Achieving zero-order release	• Nanoporous membranes for single-file diffusion	1.	2.	3.	4.