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Engineering Micro-Nanomaterials for Biomedical Translation

Yaping Chen†, Maria Alba†, Terence Tieu†, Ziqiu Tong, Rajpreet Singh Minhas, David Rudd*, Nicolas H. Voelcker*, Anna Cifuentes-Rius*, Roey Elnathan*

Dr. Y. C., Dr. M. A., T. T., Dr. Z. T., R. S. M., Dr. D. R., Prof. N. H. V., Dr. A. C-R., Dr. R. E.
Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia
E-mail: david.rudd@monash.edu, nicolas.voelcker@monash.edu, anna.cifuentesrius@monash.edu, roey.elnathan@monash.edu

Dr. Y. C., Dr. M. A., R. S. M., Dr. D. R., Prof. N. H. V., Dr. R. E.
Melbourne Centre for Nanofabrication, Victorian Node of the Australian National Fabrication Facility, 151 Wellington Road, Clayton, VIC 3168, Australia

Dr R. E., Prof. N. H. V.
Department of Materials Science and Engineering Monash University, 22 Alliance Lane, Clayton, VIC 3168, Australia

T. T, Prof. N. H. V.
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Clayton, VIC 3168, Australia

Prof. N. H. V.
INM-Leibniz Institute for New Materials, Campus D2 2, Saarbrücken 66123, Germany

† These authors contributed equally.
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Abstract

Engineered nano–bio interfaces – driven by vertical micro-/nanoneedles, nanoparticles, organ-on-chip devices, and a diversity of nano-substrates for mass spectroscopy imaging – are spurring scientific and technological progress, from fundamental to transnational biomedical research. Each class has its own characteristic features, which is critical for their translational uptake; but they broadly share the same range of functionality and applicability at the forefront of modern research and medicine.

The review provides insights into unique attributes of microneedle technology and its ability for efficient transdermal transport of therapeutic compounds. We highlight the use of nanoneedle technology in precise manipulation of increasingly complex cellular processes at the cell–material interface, and their potential for major improvements for many fundamental research applications and ex vivo cell-based therapies. We provide a snapshot in the use of FDA-approved nanoparticle therapeutics and their applications in nanomedicine. We cover achievements in organ-on-chip technology, particularly at the pre-clinical stage, and its potential to efficiently screen diverse types of therapeutics. The final section is dedicated to the use of nanomaterial-enhanced mass spectrometry in drug discovery and imaging. Overall, with this review we aim to highlight those main rules in the design of bio-nano interfaces that have successfully achieved translation into the market.
1. Introduction

The rise of micro-nanotechnologies enabled by the cutting-edge advances in micro-nano-fabrication has allowed researchers to manipulate materials at the nanoscale with high degree of precision.[1] These micro-nanomaterials have shown promise in tackling some of the most urgent biomedical unmet needs in the treatment and diagnosis of diseases like cancer, infectious diseases or neurological disorders.[2]

The interactions of such nanomaterials and biology – from molecules to tissues – are paramount to the success of the field.[2o,3] From nanostructures (nanoparticles,[4] micro-/nanoneedles,[5] and their electroactive analogues[2n,6]) to organ-on-chip devices,[7] their interface with biological settings[8] – known as nano–bio interface – have to be not only well understood but also engineered to maximise their performance.[9] In some cases, biomimicry has played a central role in leveraging the power of nanomaterials in biomedicine.[10] Trying to replicate what nature is excellent at doing using advanced micro and nanotechnologies have been proven really effective and the way to move the field forward. For example, nanoparticles with virus-like sizes and properties have resulted in effective nanocarriers for gene and drug delivery, nanoneedles can emulate the perfect mechanical, chemical and electrical cues to trigger neuronal responses,[11] and organ-on-chip technologies aim to replicate natural processes found in our bodies.[12] But the field does not stop here. Boundaries are pushed to materialize new sophisticated technologies that enable more precision and better outcomes.[1d-e,13] For example, self-applicable and pain-free microneedle patches have shown promise in making vaccines more accessible,[14] and nanoparticles can improve sensitivity and selectivity in mass spectroscopy-based analysis given their optical properties and high surface area.[15]
Altogether, these are exciting times for micro-nanomaterials for biomedical applications. Here we cover the most recent advances in the field from fundamental concepts all the way to clinical translation. We specifically focus on micro- and nanoneedle systems, nanoparticles, organ-on-chip devices and nanomaterial-enhanced mass spectrometry approaches. These areas all have a common component: the nano–bio interface. With this review, we highlight their individual journeys and what can we learn from their failure and successes to advance the field.

2. Microneedle systems

Microneedles were first conceptualized by Gerstel and Place in 1971, but it was not until the development of microfabrication technologies in the late 1990s that they were realized. Microneedle arrays consist of microscopic projections ranging from 10 to 900 µm in height, designed to painlessly by-pass the stratum corneum – the most external layer of the skin – and reach the viable layers. Upon application on the skin, microneedles create microchannels that facilitate the diffusion of molecules otherwise impermeable to the skin. Hypodermic needle and syringe have been for more than 100 years the gold standard for the administration of biomolecules. They are cheap and provide extraordinary bioavailability of almost any type of pharmaceutical. But they also require specialized healthcare professionals, cause needle phobia, and risk of injury. Microneedle arrays are ideally placed to find the balance between therapeutic efficacy, patient compliance, and financial viability. They are a minimally invasive drug delivery system that combines the benefits of skin patches (painless, convenient, low risk of infection, etc.) with the capabilities of traditional needle and syringe (delivery of macromolecules with great bioavailability). Microneedle systems are playing a central role in the development of minimally invasive technologies suitable for the administration of biopharmaceuticals. A ClinicalTrial.gov search reports 113 studies for the keyword ‘microneedles’, 74 of which have been completed (October 2020). This has been greatly motivated by the enormous increase in the number
of biomolecules as drugs in recent years. The fact that more than half of the current top 20 blockbuster drugs are biopharmaceuticals and 21% of the FDA approved drugs in 2019 were biomolecules\textsuperscript{[18]} illustrate the projected market growth, which is expected to reach $388 billion by 2024.\textsuperscript{[5c]}

A variety of microneedle designs have been explored. Depending on the method used to be applied to the skin, they can be categorized into solid, coated, hollow and dissolving microneedles (see Figure 1A).\textsuperscript{[3d]} Academic and industrial research and development have largely focused on the delivery of vaccines using microneedle patches. Vaccines appear as ideal pharmaceuticals for their transdermal delivery: they are potent, their administration typically needs less than three doses, and vaccine is largely populated by immune cells.\textsuperscript{[3d]} Commercial translation, however, was pioneered by the use of solid microneedles in cosmetics, when microneedles were first proposed for the stimulation of collagen and elastin production in the bottom layers of the skin.\textsuperscript{[19]} Other applications include the delivery of pharmaceuticals for the treatment of cancer, skin conditions, bacterial infections and osteoporosis.\textsuperscript{[20]} Microneedles have also been applied for local anesthesia, pain management and contraceptive purposes. More recently, microneedles have been used to sample skin fluids for diagnosis and health monitoring.\textsuperscript{[21]}

2.1. Design considerations

The appropriate design of the microneedle arrays is critical for the correct performance of the delivery system, and it will be strongly influenced by the nature of the formulation to be delivered. Aspects of the design such as material, geometry, density, stability, and uniformity of the content need to be carefully considered by microneedle developers early in the development process for a successful translation into commercial products.

2.1.1. Materials
The material employed in the fabrication of microneedles will determine a variety of attributes, from their mechanical stability and ability to pierce the skin to biocompatibility to manufacturing approaches.

The limited availability of technologies able to shape microscopic designs constrained the crystallization of Gerstel and Place’s microneedle concept until the development of silicon microtechnology in the 1990s. First microneedle patches were produced in silicon and were used to demonstrate the concept.\textsuperscript{[22]} Silicon microneedles are typically made using (deep) reactive ion etching combined with pre-patterning methods such as UV-photolithography. Microneedles fabricated by Si etching techniques can be designed with an exquisite precision and excellent sharpness, but they also require extensive optimization. Silica glass microneedles were also manufactured following similar approaches with comparable features.\textsuperscript{[23]} A disadvantage that both silicon and glass high aspect-ratio microstructures present is their brittleness, which increases the risk of fracture upon skin application. Silicon and glass microneedle biocompatibility have been extensively investigated. They have been proven biocompatible and non-toxic, but because they are brittle, there is the possibility of breakage upon insertion.\textsuperscript{[24]} In most cases, these microscopic pieces will be expelled within a few weeks during the natural renewal of epidermal layers. However, there have been safety concerns due to the potential formation of silicon and glass related subcutaneous granulomas.\textsuperscript{[25]}

The development of more complex manufacturing methods such as micromachining and laser-drilling enabled the fabrication of microneedles using various types of metals. Microneedles made of stainless steel, titanium, palladium and their alloys have been reported, all of which display great amenability and reduced risk of fracture.\textsuperscript{[26]} These metals are generally regarded as biocompatible although obviously influenced by their precise composition.\textsuperscript{[27]} Metals have been widely used in many other medical applications, including long-term orthopedics and stents which require superior biocompatibility and safety profiles.
Micromold casting and injection molding are techniques typically employed in the fabrication of ceramic, sugar-based and polymeric microneedle patches. A liquid solution, which may contain the drug, is casted onto an inverse mold of microneedles. After drying, the patch is peeled out of the mold to make dissolving or, in some cases, coated microneedles. Ceramic microneedles have good mechanical strength, especially to compression forces, but can be brittle under tensile stress. Ceramics such as alumina and calcium phosphate have demonstrated suitable biocompatibility in medical devices such as implants and bone regeneration.[28] Sugar-based microneedles have been fabricated using various carbohydrates, including maltose, sucrose, galactose and trehalose.[29] Such microneedles have the ability to dissolve when in contact with the skin, releasing their cargo. Sugar-based needles are regarded as inexpensive and biocompatible. The main disadvantage of this type of microneedles is their limited strength to puncture the skin and the need for post-casting thermal treatments, which limits the number of drugs that can be loaded into these microneedles.

Microneedle technology has undoubtedly benefited from the great progress that polymer science has experienced over in the last two decades. Polymeric materials have attracted interest in both academic and industry settings for microneedle fabrication due to their advantageous properties: tunable mechanical properties, improved safety, and low cost.[30] The vast majority of polymers that have proposed for the fabrication of microneedles have demonstrated a favorable biocompatibility.[31] Polymers such as poly(carbonate), polystyrene, SU-8 photoresist and poly(methyl methacrylate) have been extensively used in FDA approved medical products (e.g. contact lenses, syringes, bone cements, cell culture plates). Some concerns still exist regarding long-term applications where dissolution products may induce adverse effects.[32] Other polymers employed for microneedle manufacture are not only biocompatible, but also biodegradable. Poly(vinyl alcohol) (PVA) and poly(vinylpyrrolidone) (PVP) were the first polymers used in the fabrication of microneedle arrays that dissolve upon piercing the skin.[33] Both PVP and PVA have an extraordinary solubility in water and very low cytotoxicity. Aliphatic polyesters such as
polylactic acid (PLA) and polyglycolic acid (PGA) have also shown good biocompatibility and biodegradability as microneedle arrays.\textsuperscript{[34]} Their degradation rate may be tuned by adjusting the PLA to PGA ratio in PLGA co-polymers.\textsuperscript{[34b]} The main drawback of these water dissolvable polymers is that they need to be stored under controlled temperature and humidity to avoid degradation. Innovative functional materials such as thermo-\textsuperscript{[35]}, electro-\textsuperscript{[36]}, light-\textsuperscript{[37]}, chemo-\textsuperscript{[38]} and bio-responsive\textsuperscript{[29b]} composites have been recently employed in microneedle fabrication, adding versatility and advanced features to transdermal systems. Among them, glucose-responsive polymers and hybrid materials that have the ability to release insulin on-demand have arguably focused the most intensive research efforts.\textsuperscript{[1b, 1c]}

2.1.2. Geometry

The geometry of the microneedles in terms of height, arrangement, density, and sharpness will determine their penetration depth and interaction with skin tissue and fluids. Microneedles have a length between 100 and 1,000 µm. Longer needles facilitate more reliable skin insertion,\textsuperscript{[39]} but these can cause pain and increase the risk of infection.\textsuperscript{[40]} Other geometric parameters than can affect the penetration depth are aspect ratio, base diameter, and sharpness. In general, wider microneedles and smaller aspect ratios provide an increased resistance to fracture or deformation.\textsuperscript{[41]} But they are harder to penetrate the skin and may cause pain. Tips of 1 to 10 µm are sharp enough to puncture the skin as long as the mechanical strength is sufficient.\textsuperscript{[39]} The microneedle density can vary from a few microneedles to thousands of them per cm\textsuperscript{2}. A higher density will facilitate the delivery of higher dose with smaller patches, but they can also increase pain.\textsuperscript{[40-41]} The penetration ability can also be affected by the density. High densities may induce a “bed-of-nails” effect and prevent skin puncture.\textsuperscript{[41]} But low densities may require larger patches, making skin insertion less reliable due to the non-planar nature of the skin. The skin thickness and elastic properties, however, can vary depending on many factors including age, gender, ethnicity and body area\textsuperscript{[42]} and these also need to be considered when determining the penetration depth.

2.1.3. Other considerations

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The uniformity of the content is one of the most common pharmacopeial requirements to guarantee appropriate dosage. Applied to microneedle systems, the content uniformity should be ensured not only between patches, but also between and within individual microneedles.\textsuperscript{[43]} For coated microneedles, a critical step is the coating process. A range of protocols have been proposed aiming to achieve a uniform coating around the projections, including dip coating, spray coating, inkjet printing and gas-jet drying.\textsuperscript{[44]} The liquid-solid interaction needs to be controlled for the formulation to adhere to the microneedle surface. The viscosity and the surface tension of the formulation and the microneedle morphology, chemistry and roughness are key parameters that determine the coating morphology and uniformity. Likewise, the localization of the coating is also critical. Formulations should be located onto the microneedle projections and more precisely, on the tip of the microneedle or the part which will be in contact with skin fluids upon application.\textsuperscript{[45]} Any drug on the base substrate will not enter the skin. For dissolving microneedles, the drug needs to be uniformly distributed on individual needles and between needles.\textsuperscript{[43]} The formulation must be soluble in the matrix material, fillable into the mold and exhibit a rapid dissolution within the skin. The stability of the microneedle arrays, the drug and the system as a whole need to be guaranteed under a range of environmental conditions. Long-term morphological integrity, durability and the impact of humidity and temperature must be assessed for individual needles and microneedle arrays. Drug formulations need to retain their physicochemical and therapeutic properties. The long-term adhesion between drug coatings and microneedle arrays should be considered, as delamination of the coating is a recurrent challenge, particularly during insertion. Many traditional formulations typically require refrigerated storage and transportation (cold chain), which is expensive and complicates logistics, especially in developing countries. In this context, microneedle technology has accelerated the development of solid-state formulations that do not require cold chain and exhibit extraordinary stability at room temperature. For example, a dissolving microneedle patch loaded with trivalent influenza vaccine was demonstrated to be stable at 25 °C for over 24
months. As medical devices, microneedle arrays need high-quality manufacturing under Good Manufacturing Practices (GMP) conditions. Reducing the number of steps in the manufacturing process is important to minimize the chances of error. The need for microneedles array to be sterile will be determined by the target tissue and depth of penetration, the duration of application and the intended patient population (e.g. immunocompromised, young, elderly).

2.2. Clinical and commercial translation of microneedle products

Apart from design considerations that ensure the correct performance of microneedle systems, the acceptability to patients, clinicians, and regulator needs to be carefully considered in the development process for their successful clinical and commercial translation. Some of the benefits that microneedle systems offer to patients are reduced pain, potential self-administration, reduced risk of injury. For people suffering from needle phobia (estimated to be at least 10% of the population) microneedle systems have an increased acceptance. Indeed, it has been demonstrated that most subjects prefer the microneedle patch to needle and syringe. 24% of patients preferred needle and syringe, pointing to familiarity reasons to choose that over microneedle patches.

Benefits for healthcare professionals include reduced risk of injury, increased access to population. But for the prescriber to be willing to recommend the product, microneedle products need to be proven efficient and reliable even if lesser trained personnel or patients themselves apply the microneedle patches. Although manual insertion with thumb pressure is a convenient way for patients to insert microneedle patches on the skin, the lack of control and the increased chance of error have encouraged the spread use of applicators. A range of applicator designs have been proposed, with some of them being commercially available. For the “poke and patch” approach which involve skin puncture with microneedles that do not contain any drug, a rolling design is most common. For drug-loaded patches, applicators based on accelerating the patch to a high-speed perpendicular to the surface of the skin are most common. One of the simplest designs involves the
incorporation of a spring-loaded piston, such as those commercialized by Zosano (Figure 1B)\textsuperscript{[48]} and 3M (Figure 1C).\textsuperscript{[49]} But these may be perceived as painful by the patients. Applicators activated by thumb pressure may have a better patient acceptance. Following this assumption, Vaxxas (Figure 1D)\textsuperscript{[14a]} and Corium have designed applicators that only required thumb pressure once they are in contact with the skin.

The possibility of self-application of vital importance in cases of global pandemics, especially in developing countries where trained clinicians are in short supply. It has been shown that the training provided by a healthcare professional alongside an information leaflet resulted in a correct application of the microneedle patch. Delivery feedback is another desired feature of microneedle devices to notify patients that the patch has been applied correctly and that the microneedles have successfully delivered their payload. Nonetheless, end-user acceptance is a complex and multifaceted matter that needs to be investigated in qualitative studies.

From a regulatory standpoint, the classification of the microneedle device as a drug delivery system, consumer product or medical device is determined by its intended use. This will establish the requirements from the regulatory authority. If a microneedle system is used “in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease”, it is regarded as a medical device. If the use of microneedles involves “penetration into living layers of skin”, sterility is likely to be a mandatory requirement. Aseptic manufacturing or terminal sterilization approaches will bring additional costs and make large-scale manufacturing more challenging. Additionally, sterilization methods may damage the microneedle structure or the loaded drug.\textsuperscript{[50]} However, non-sterile products may be acceptable if microneedles are proven to have low bioburden and not increase the infection risk. It is therefore recommended that manufacturers evaluate the need for a sterile product early in development. For microneedles to be accepted for clinical use, manufacturers must ensure that they do not harm the patient. Basic quality control guidelines should include mechanical tests that ensure that microneedles adequately pierce
and penetrate the skin and do not fracture under shear representative of patient use. In the case of solid needles, they should be able to be removed without leaving sharps behind. Hollow microneedles should remain open to ensure their functionality. For dissolving microneedles, their cargo should be release within a reasonable timeframe. Other potential requirements from a regulatory body that should be addressed during the manufacturing process include uniformity of dosage, effect of humidity and temperature, microbial limits and recovery of the skin function. The packaging, re-use, disposal and deposition are also likely considerations from a regulatory perspective. The interest of regulators in microneedle technology has been recently exemplified with the release of a draft guidance on “microneedling” by the US FDA.\(^{[43]}\)

2.3. Commercial applications of microneedle technology

The field of microneedle technology has grown dramatically in the past 10 years. The research advances that have occurred in academic settings have fostered significant industrial development and investment. There are now tens of companies around the globe actively seeking commercialization and studying their microneedle products in clinical trials. Some examples of microneedle systems have already succeeded in entering the market, and some others are rapidly progressing to become commercially available (see Table 1). In this section, we discuss the main applications of microneedle products, their journey to market and the key players in the field.

2.3.1. Vaccine delivery

Vaccine delivery is the one of the most prominent applications of microneedles due to their improved immunogenicity and logistical and delivery advantages. The skin is rich in antigen presenting cells; therefore, microneedles can significantly enhance the immune response with a reduced vaccine dose. Numerous animal studies have demonstrated that microneedle vaccination had an enhanced immune response, with as little as 1/100th dose compared to conventional intramuscular vaccine injection.\(^{[14b]}\) Microneedle patches store vaccine in solid form, which is much
more stable than liquid form and holds great potential for the elimination of the cold-chain storage and supply. This is highly desirable because not only it reduces the end-to-end costs of vaccination but also makes vaccine much more accessible to mid- and low-income countries, where a cold-chain supply is often challenging. Vaccination via microneedle patches will create access to new markets that needle/syringe cannot reach. Other advantages include longer duration of immunity and stronger cellular immune response. A wide range of solid and dissolvable microneedle arrays for vaccine delivery has been demonstrated in laboratory settings.\cite{3d} A small proportion of them are already commercially available, and others are being investigated in clinical trials and progressing to enter the market.

The MicronJet600® (NanoPass, Israel) microneedle system gained FDA approval in 2017 as a medical device. It was accepted to be used for intradermal delivery of already approved drugs. The MicronJet600® consists of pyramidal-shaped hollow microneedles made of silicon and with a length of 600 µm integrated in a plastic backing.\cite{51} However, they cannot be considered microneedles arrays, but very short needles attached to standard syringes. The MicronJet600® system has been investigated in several clinical trials for vaccine delivery including H1N1 and H5N1 influenza, varicella-zoster, poliomyelitis, and seasonal influenza vaccines.\cite{52} Vaxxas (Australia) is working towards commercializing the high-density microneedles Nanopatch® developed at the University of Queensland. Due to the high density of projections the patch needs to be applied using an applicator.\cite{53} The technology has been recently tested in two clinical trials using microneedle arrays coated with monovalent influenza vaccine and manufactured by means of an aseptically validated process.\cite{47, 54} Microneedle arrays without a vaccine have been tested for safety and tolerability in humans, and data on the acceptability of the delivery system have been generated.\cite{54} Vaccination using high-density microneedles resulted in immune responses that were similar or significantly enhanced when compared to intramuscular injection. In 2020, Vaxxas announced to be the recipient of a $22m US Government award to conduct a phase 1 clinical trial.
for a pandemic influenza vaccine. Micron Biomedical (USA), a spin-off company from Mark Prausnitz’s research at Georgia Tech, developed a dissolvable microneedle patch containing 100 microneedles of 650 µm length attached to an adhesive backing (Figure 1E). The system was proved safe and immunologically effective in a phase 1 clinical trial on influenza vaccination, with good patient acceptability.\(^ {55}\)

2.3.2. Cosmetics

The first example of microneedle commercialization was for cosmetic applications and dates back from the early 2000s.\(^ {19}\) Microneedles rolled across the skin create transient micropunctures that were demonstrated to improve skin appearance and minimize skin imperfections, such as scars, hyperpigmentation, stretch marks, cellulite and wrinkles.\(^ {56}\) Dermaroller\(^ \text{®} \) (Wolfenbüttel, Germany) was launched in 1999 as a cylindrical roller covered with solid needles. The initial purpose of such devices was to stimulate collagen production and improve skin texture by reducing fine wrinkles and lines.\(^ {19}\) Dermaroller\(^ \text{®} \) has also been used in combination with topical cosmetic products for their improved skin penetration.\(^ {57}\) Similar products have been developed by other companies such as Genosys\(^ \text{®} \) (Hansderma, Downey, CA) and Dermapen\(^ \text{®} \) (USA).\(^ {58}\) Dissolving needles have also been marketed for cosmetic purposes. MicroHyala\(^ \text{®} \) (CosMED, Kyoto, Japan) consists of dissolving microneedle arrays made of hyaluronic acid that dissolve inside the skin upon exposure to its aqueous environment, reducing the effects of skin aging.\(^ {59}\) Microneedles for cosmetic applications are now well-established marketed products that have great consumer acceptance and increasing popularity.

2.3.3. Biopharmaceuticals delivery

Beyond vaccines, microneedles have the ability to deliver other biologics. Insulin,\(^ {29b, 60}\) glucagon,\(^ {61}\) PD-1 antibodies,\(^ {62}\) parathyroid hormones\(^ {63}\) and growth hormones\(^ {64}\) are some examples of biopharmaceuticals that have been transdermally delivered using microneedle systems. Of all biologics, the transdermal delivery of insulin has arguably focused the greatest research efforts.
Non-invasive delivery of insulin will be a greatly beneficial alternative to subcutaneous injection – currently the only method of insulin administration. Clinical trials in patients with type 2 diabetes have been conducted to assess the safety and efficiency of MicronJet600® for insulin delivery.\textsuperscript{[65]} These studies demonstrated a safe profile, efficient delivery and improved pharmacokinetics compared to subcutaneous administration. Three different microneedle systems (developed by Zosano, Corium, and 3M) have been employed in clinical trials for the administration of hormone-related therapies for post-menopausal osteoporosis. Zosano created a microneedle patch made of titanium projections and coated with parathyroid hormone.\textsuperscript{[63]} Results demonstrated that the system could delivery consistent and therapeutic effective doses of the hormone with a favorable pharmacokinetic profile. Zosano microneedle technology has also been investigated for the delivery of Norditropin (a recombinant human growth hormone) on the treatment of growth-related disorders.\textsuperscript{[64a]} Preclinical studies demonstrated high delivery efficiency and linear dose response, with a bioavailability comparable to that after subcutaneous injection.

2.3.4. Drug delivery

A variety of studies have demonstrated the significant enhancement of small drug permeation through the skin using microneedle systems. Zosano conducted a double-blind placebo controlled clinical trial on their microneedle-based Qtrypta® patch for the delivery of zolmitriptan in the treatment of acute migraine.\textsuperscript{[66]} The FDA accepted the new drug application for Qtrypta® in early 2020, which is currently under review. Clinical trials have been conducted for the delivery of 5-aminolevulinic acid after pre-treatment with microneedle rollers for photodynamic therapy to treat skin conditions such as actinic keratose.\textsuperscript{[67]} Pre-treatment with microneedles before a 20-minute incubation of aminolevulinic acid produced results comparable to conventional photodynamic therapy with 1 h aminolevulinic acid incubation time. Delivery of doxorubicin to treat cutaneous T cell lymphoma and basal cell carcinoma,\textsuperscript{[54]} triamcinolone acetonide for the treatment of keloids,\textsuperscript{[68]} and lidocaine for local anesthesia\textsuperscript{[69]} have also been investigated in human studies.
3. Vertical nanoneedles in clinical applications

Vertically configured nanoneedles such as vertical silicon nanowire (SiNW) arrays have been widely used for biophysical and biomedical studies due to their unique and tunable nanoscale topography, their ability in mediating an efficient intracellular delivery, their highly tunable electrical and chemical properties, biocompatibility, and antibacterial properties. Aside from these features, there has been a significant push toward development of new, cost-efficient, and easily implemented nanofabrication routes that are essential to seamlessly integrating NWs with biomedical research.

Exploration of nanoneedles in clinical applications has significantly increased, with a lot of efforts put in fine-tuning nanoneedles for monitoring, diagnoses, and treatment of diseases (such as Parkinson’s disease, obsessive compulsive disorder, and depression), as well as stem cell therapy and regenerative medicine. Although understanding of molecular mechanisms and relevant biomarkers for most diseases is still in its infancy, where there is sound understanding or established biomarkers, nanoneedle technologies have shown great potential in improving patient diagnoses and treatment outcomes.

3.1. Nanoneedle-mediated intracellular delivery and cytosolic extraction in disease monitoring

Recent studies have shown advances of one-dimensional (1D) nanoneedle arrays in mediating the delivery of drugs and bioactive cargoes, immunomodulation, electrical recording, and biochemical detection. The low toxicity and minimal invasiveness make nanoneedles a promising candidate for the sustained non-immunogenic delivery of payloads into both isolated cells in vitro and whole tissues in vivo. For instance, nanoneedles have been employed for ocular implants in vivo as drug delivery vector particulates for intravenous injection, and have been tested as a brachytherapy device in humans.
To achieve *in vivo* localized nanoinjection into a specific set of cells with minimal involvement of surrounding tissue, Chiappini et al. have developed a mesoporous Si nanoneedle (pSi-nN) array which can negotiate rapidly with local biological barriers to grant temporary cytosolic access without compromising cell viability.[70a] The tight cell–nN interface enabled live intracellular pH (pHi) sensing to differentiate cancer (OE33) from healthy (Het-1A) cells, with pHi measured as 6.7 and 7.4, respectively (Figure 2A i,ii). The pSi-nN platform also demonstrated the ability to deliver cell-impermeant nanoparticles (6nm hydrophilic quantum dots, QDs) both *in vitro* into HeLa cells, and *in vivo* to the surface of tissues with different architectures of male athymic nude mice (Figure 2A iii). Dissecting the cell–nN interface over time by confocal and FIB-SEM imaging elucidated the dynamics of cell association and nN biodegradation, indicating that the rapid interfacing led to cytosolic QD delivery within less than 30 min *in vitro*. In addition, by simple application of QD-bearing nN chips to exposed muscle and skin (back and ear) sites in mice, the researchers managed to deliver QDs to the superficial layers of architecturally different mammalian tissues, confirmed by transmission electron micrograph (TEM) imaging of cross sections of the nN-treated tissues (Figure 2B i,ii). Moreover, the nanoinjected QDs can be retained around the nN injection sites for up to 100 h (Figure 2B iii,iv), which was significantly longer than the detectable period of QDs delivered by control flat Si (< 24 h). Such prolonged retention of QDs in specific localized regions, therefore, allowed long-term monitoring of disease progression in animal models and potentially in patients for clinical trials.

In addition to one-way delivery of bioactive payloads into cells, nanoneedles can be specially designed as a powerful platform to simultaneously extract cytosolic contents, when combined with synergetic strategies such as electroporation.[82] For instance, He et al. have fabricated an array of multifunctional branched nanostraws (BNSs), which was integrated with a low-voltage nanoelectroporation system to achieve cell capture, drug delivery, and sensing of intracellular enzymes in circulating tumor cells (CTCs, Figure 2C).[77c] Using MCF7 cells as a cancer cell model,
they observed via SEM a close interface with deformed cells around BNSs. The live/dead (calcein-AM/propidium iodide, PI) cell staining assay showed that MCF7 cells were highly viable (>95%) over 24 h incubation, indicating the biocompatibility of BNSs and the feasibility of downstream regulation and analysis of captured CTCs. To facilitate the effective capture of CTCs, BNSs were conjugated with tumor-specific antibodies (anti-EpCAM) on the numerous nanobranches on the outer sidewall. It was reported that the capture efficiency of MCF7 cells by anti-EpCAM-coated BNSs was significantly higher (~93%) than that on anti-EpCAM-coated coated NSs (without nanobranches, ~70%) and PC membrane (~5%); this suggests that not only BNSs enlarged the surface area like NSs, but their unique nanobranches provided better CTC contact interfaces, which might be more analogous to the natural morphology of the extracellular matrix than nonbranched NSs. Moreover, by spiking prestained MCF7 cells into healthy human whole blood to form artificial CTC samples, anti-EpCAM-coated BNSs were able to separate ~80% of the spiked MCF7 cells from blood samples.

After validating high CTC capture efficiency, nanoelectroporation was applied through the BNSs to nondestructively porate the membranes of the captured cells at a low voltage (~15 V), which allowed the delivery of exogenous biomolecules into the cytosol and the extraction of cytosolic contents through the BNSs, without affecting cell viability. Small molecule dyes (PI) and DNA (GFP reporter) plasmids were delivered efficiently, ~84% and ~64%, respectively, into MCF7 cell via BNS + Electroporation; but BNS alone, without application of electroporation, failed to deliver biomolecules into any cells. The time-resolved, sequential delivery of PI dye and GFP plasmid was also achieved into the same set of captured cells at different time points using the BNS device. Conversely, the researchers performed repeated extraction of intracellular enzymes (caspase-3) from MCF7 cells after the treatment of 1 μM of staurosporine to induce apoptosis; this allowed
quantitative analysis of the extracted caspase-3 concentrations every 3 h, therefore real-time monitoring of the execution-phase of cell apoptosis. Together, the research findings demonstrated the capability of BNS device for selective intracellular delivery and cytosolic extraction, with spatial and temporal control, into and from captured cells, allowing real-time regulation and longitudinal monitoring of intracellular CTC activities in situ. Such technology can provide new opportunities for the comprehensive understanding of CTC pathogenesis and progression, which will in turn facilitate cancer diagnosis and treatment.

3.2. Nanoneedle-based sensors in disease diagnoses

Disease markers are crucial indicators for patient diagnosis and treatment outcome; these are referred to as diagnostic and predictive markers, respectively. The development of noninvasive tests that are rapid, sensitive, specific, and simple to detect these markers would allow preventing patient discomfort, delay in diagnosis, and the follow-up of the disease status. Advanced nanotechnology has been lauded as a promising noninvasive avenue for detecting the early signs of a disease.\cite{76a,76b,77c,83} For example, nanoparticle-based bio-barcode has been used to design tests that can spot minuscule amounts of prostate-specific antigen\cite{84} and anthrax DNA,\cite{85} and various investigators are using similar bio-barcode techniques to look for protein markers in the brain that may warn of Alzheimer disease.\cite{86} Fundamental and technological innovations in nanomaterials, such as NWs and their electroactive analogues, carbon nanotubes, and graphene, have opened clear avenues for sensing from proof-of-concept, leading to patenting technologies\cite{87} and spinoffs\cite{88} in an effort to fulfill the requirements of rapid, ultra-sensitivity and high-throughput biochemical electronic sensing for applications in life sciences and biomedical research.\cite{2c-e}

In early 2000s, Lieber’s group pioneered the field of SiNW sensors to detect a single virus, specific genetic mutations that cause a disease, and proteins associated with certain cancers.\cite{89} Due to SiNWs’ unique structural and chemical characteristics – including nanoscale dimensions, high
surface-to-volume ratios, well-defined and tailorabile surface chemistry, SiNW-based sensors have since been further modified and developed for ultra-sensitive detection of biological macromolecules. In particular, molecular-based transistors and NW-based field-effect transistors (FETs) have shown high performance in label-free, real-time, and sensitive detection of cancer-associated analytes from peripheral blood, tumor biopsy, and exhaled breath of cancer patients.

The mechanism behind NW sensing is the transduction of a molecular interaction through a NW into an electrochemical signal, such as ion-sensitive FET or memristor signal. FET nanosensors are three-electrode systems comprising source (S), drain (D), and gate (G) electrodes (Figure 2D i), whereas memristor nanosensors are two-electrode (S and D) systems. The S and D electrodes bridge the NW channel, while the G electrode serves as a reference electrode to modulate NW electric properties that can be doped with positive or negative (p- or n- type NW) dopant elements. When the soluble target molecules bind to the NW-immobilized receptor molecules, charges can be transferred to the NW, which can be quantified based on a change in conductance in an FET sensor (Figure 2D ii) or voltage gap in a memristor sensor. The sensed interaction is label-free, real-time, and can involve a large variety of biomolecules, including DNAs, RNAs, proteins, and small drugs (Figure 2D iii). By functionalization with specific antibodies to a target antigen, NW sensors enable direct detection of tumor markers, and outperform conventional methods by operating in the aM to nM range.

For example, DNA-aptamer functionalized memristor NW sensors were developed to detect prostate-specific antigen (PSA), an important marker of prostate cancer. The ultrasensitive biodetection was demonstrated for PSA with a limit of detection (LoD) down to 23 aM, the lowest value achieved by electrochemical biosensors in PSA measurement. Clearly, such NW-based memristor sensors can be proposed to detect a wide range of cancer markers with unprecedented ultrasensitivity, which has important clinical implications for early diagnosis and management of cancer.
Tseng’s group first introduced the unique “NanoVelcro” cell-affinity assay, in which capture agent-coated nanostructured substrates were utilized to immobilize CTCs with high efficiency. Vertically oriented SiNWs on the NanoVelcro chip were functionalized with anti-EpCAM antibodies and exhibited high-yield isolation of CTCs (>70%) from the peripheral blood of prostate cancer patients;[95] this allowed longitudinal monitoring during the timecourse of docetaxel therapy, and revealed low CTC counts despite PSA progression, while bone scan confirmed stable disease.[96] Further, aptamer EpCAM was used to coat the nanofluidic NanoVelcro chip, to realize the control of capture and release of CTCs from the peripheral blood of non-small cell lung cancer patients; surface-grafted aptamer EpCAM can be specifically cleaved by enzymatic treatment, resulting in the release of immobilized CTCs.[97] Coating nanostructure surfaces with thermoresponsive polymers allowed controlled release of viable and functional CTC with high efficiency.[98] When coupled with magnetic upconversion nanoparticles coated with anti-EpCAM antibodies, the CTC capture efficiency (80%) and recovery by NanoVelcro chips were dramatically increased in the presence of a magnetic field.[99] A separate study reported that with the functionalization of anti-EpCAM antibody following the deposition of a high density of gold nanoclusters (AuNCs), SiNWs can facilitate efficient capture and photothermal therapy of tumor cells.[100] Breast cancer cells were captured efficiently (88%) and were killed massively by near-IR light due to the strong absorption by AuNCs; the results, therefore, suggest the potential of simultaneous capture and plasmonic photothermal therapy for CTCs.

Compared with the relatively low level of CTCs (1–100 cells/mL) in blood, exosomes are released from cancer cells in much greater numbers (≥10^9 vesicles/mL blood) and have recently been recognized as promising tumor surrogates due to their delivery of enriched biomarkers, such as proteins, RNAs, and DNA.[101] But purification of exosomes remains technically challenging in clinical settings.[76c] To this end, a microfluidic device embedded with ciliated micropillars has been fabricated, and used for multiscale filtration of biological fluids and the isolation of exosomes.[102]
The inter-NW spacing can be tuned within 30–200 nm, creating a high density of interstitial sites that enabled the physical trapping of exosomes, while simultaneously excluding larger components such as cells; proteins, small molecules, and cell debris, on the other hand, flew through the spacing between NWs without being captured. The exosomes were trapped with high efficiency (45–60%) and can be released at high purity by dissolving the porous NWs with PBS.

In addition to exosomes, nucleic acids such as circulating DNA, RNA, and microRNA (ctDNA, ctRNA, and miRNA, respectively) are also released from tumors into patient peripheral blood, thus can be analysed as liquid biopsies. NW-based FET has demonstrated sensitive label-free detection of DNAs and of miRNAs through hybridization. In particular, two-terminal SiNWs can function as ultrasensitive and selective real-time DNA sensors at concentrations down to tens of fM range. The peptide nucleic acid (PNA) receptors coated on the NW sensor could distinguish the wildtype versus mutant DNA sequences associated with the ΔF508 mutation site in the cystic fibrosis transmembrane receptor gene, which was responsible for ∼75% of the cases of cystic fibrosis – one of the most common fatal genetic diseases among populations of European origin.

As far as miRNA is concerned, a complementary metal oxide semiconductor (CMOS)- compatible SiNW- FET biosensor was fabricated to provide low manufacturing cost but ultrahigh sensitivity for miRNA detection. By coating with carboxyl- modified DNA capture probes, the nanosensor achieved a rapid (<1 min) detection of two cancer-associated miRNAs, miR-21 and miR-205 (down-regulating the expression of tumor-suppressor genes and associated with lung tumor growth, respectively), in total RNA extracted from lung cancer cells and serum. The ultrahigh sensitivity of the nanosensor was demonstrated by the LoD of 1 zeptomole (ca. 600 copies), while the specificity was revealed by the excellent discrimination for single-nucleotide mismatched sequences of tumor-associated miRNAs. Following this work, the same research team developed a real-time
assay for multiplexed electrical detection of lung cancer biomarkers (miR-126 and carcinoembryonic antigen, CEA) by integrating SiNW-FET device into PDMS microfluidic chip, which contained two macro-scale solution chambers to allow simultaneous detection (Figure 2E i,ii).\textsuperscript{[103]} SiNWs in each chamber were functionalized with probe DNA and antibody of CEA (anti-CEA), for the specific binding and detection of miR-126 and CEA, respectively (Figure 2E iii). The nanosensor could reliably detect miR-126 and CEA with LoD of 0.1 fM and 1 fg/ml, respectively, implying the ultrasensitivity of the nanosensor. In addition, the specificity was investigated using miR-125, which has similar structure to miR-126. The introduction of miR-126 (100fM) onto unmodified SiNW-FET and miR-125 (100fM) onto DNA probe coated SiNW-FET both generated no significant change of the electrical current, suggesting the absence of nonspecific miRNA binding to the SiNW surface as well as the high specificity of DNA probe to miR-126 alone. Similar results were also achieved when adding CEA on unmodified SiNWs or BSA and CYFRA21-1 (structurally closely related to CEA) onto anti-CEA coated SiNWs, confirming the specificity for CEA detection. Due to high surface-to-volume ratio of SiNWs, the direct and highly sensitive detection of CEA was achieved with a signal-to-noise ratio (SNR)>3 for concentration down to 1 fg/ml or 5.5 aM, implying the detection floor of a single molecule. Importantly, the performance of SiNW-FET device was verified by the detection of miR-126 and CEA from lung cancer patient serum, which allowed the successful differentiation of lung cancer patients from normal person.

Apart from inorganic SiNWs, recent development in nanofabrication techniques has enabled the transfer or replication of vertically configured nanostructures from one substrate to a recipient polymeric substrate such as polystyrene, PDMS, and SU8.\textsuperscript{[75c, 105]} Such polymeric nanoneedles have the additional benefits of cost-effectiveness, optical transparency, and flexibility in physico-chemical property.\textsuperscript{[106]} They are now being used in a variety of cellular manipulations and interrogations, including intracellular signaling study,\textsuperscript{[107]} biomedical sensing,\textsuperscript{[108]} cellular
probing,\textsuperscript{[109]} and intracellular and intratissue drug delivery\textsuperscript{[106]}; this makes them a versatile and promising platform for disease diagnoses and other clinical applications.

As diseases and cancers progress differently at different stages, many clinical decisions rely on the diagnosis of specific disease markers or tumor-derived components.\textsuperscript{[110]} Nanoneedle-based nanosensors meet the demands of rapid, noninvasive, label-free, and ultrasensitive detection of biomarkers, even with exceedingly small samples. Multiplexed detection of cancer-associated analytes via nanoneedle-sensors can further provide complementary information to aid effective treatment to improve cancer survival rate.

3.3. Early success of nanoneedle applications in disease treatment

While NW-sensor diagnosis systems are at their early stages of development, nanoneedle-based approaches to disease and cancer treatment are still at the experimental stage. Nevertheless, promising results have been reported using nanoneedles for primary neuron and neurological applications,\textsuperscript{[11, 76d, 111]} treatment of retinal disorders,\textsuperscript{[112]} and regeneration of damaged tissues.\textsuperscript{[113]}

Electrogenic neurons are the control units of most biological living beings; they can sense diverse stimuli (mechanical, chemical, and thermal), and deliver accurate commands through neuromuscular junctions.\textsuperscript{[11]} With the great potential in life technologies and artificial intelligence, it has long been a major pursuit in neuroscience, bioengineering, and electrical engineering to develop seamless neural interfaces for probing, understanding, and modulating neural activities.

Recent advances in NW technology have brought about a promising solution to achieve precise and localized interrogations in neuronal electrophysiology.\textsuperscript{[76d, 114]} NWs with programmable geometries (e.g., diameter of 10–100 nm) are ideal for interfacing with neurons and measuring their intracellular potentials with minimal invasiveness.\textsuperscript{[2a, 115]}

To measure minute potential changes in individual cells at high spatial resolution, Liu et al. developed a hybrid integration scheme based on vertical NW arrays (Figure 2F i) that enabled
independent electrical addressability – which was important for localizing the origin of action potentials in neuronal networks.\textsuperscript{[116]} The novel all solid-state wafer bonding scheme on patterned Ni electrical contacts and leads resulted in a superior high-density NW–neuron interface. The arrays were used to perform electrophysiological recordings from mouse and rat primary hippocampal neurons, as well as human induced pluripotent stem cell (hiPSC)-derived neurons, which revealed high SNR and sensitivity to subthreshold postsynaptic potentials (PSPs). Physiological measurements on mouse hippocampal neurons cultured for 10–13 days \textit{in vitro} (DIV) on NWs displayed small potential fluctuations prior to both positive and negative firing events (Figure 2F ii), with the largest action potentials (99 mV) measured 10 DIV. They also measured electrical activity from rodent neurons from 8–14 DIV and from hiPSC-derived neurons at 6 weeks \textit{in vitro} post culture, and observed intimate NW–neuron interactions by TEM. The results demonstrated for the first time that vertical NW arrays can measure intracellular potentials with similar magnitudes to that of patch-clamp.\textsuperscript{[117]} But unlike the destructive and unscalable patch-clamp technique that can only measure extracellular potentials, the individually addressable SiNW probes enabled precise and simultaneous measurements of intracellular action potentials, opening new prospects on mapping neuronal activity in large networks. The sensitivity to subthreshold PSPs from multiple neurons also demonstrated the ability to detect miniature release of neurotransmitters, critical for understanding the synaptic transmission mechanisms and plasticity in neurological diseases.\textsuperscript{[118]}

In a separate study, Zhao et al. devised an ultrasmall 3D U-shaped NW FET (U-NWFET) probe for recording intracellular action potentials from primary neurons and human cardiomyocytes.\textsuperscript{[76d]} By combining deterministic shape-controlled NW transfer with spatially defined semiconductor-to-metal transformation, they addressed the challenges of NW-FET scalability (controllable tip geometry and sensor size) as well as recording amplitude (up to 100 mV intracellular action potentials). Despite some limitations on long-term stability and the number of recording channels compared with high-density multi-electrode arrays,\textsuperscript{[119]} the U-NWFET demonstrated capability of
multiplexed recording and precise targeting of individual cells and cell networks, and could foster future investigations for in vivo measurements of electrophysiological dynamics in the brain and other tissues.

Retinal degeneration is one kind of neurological disorders caused by genetic mutations and/or environmental damage to the retina, an important light-sensitive tissue consisting of multi-layers of neuronal cells, which can transduce light information into neural activities. The irreversible damage or even loss of photoreceptors can lead to severe impairment of vision and eventually blindness, which is incurable. The restoration of light response with complex spatiotemporal features using retinal prosthesis has been a considerable challenge over the past decades. Nevertheless, recent studies have shown exciting results in developing photoresponsive materials (such as semiconductor Si photodiodes and metal electrode arrays) as artificial photoreceptors for interfacing with blind retinas, as a promising alternative for retinal prosthesis; but these devices require additional microelectronic processing for signal generation, transduction, and processing, which largely limited in vivo applications. To break this bottleneck, Tang et al. developed artificial photoreceptors based on gold nanoparticle-decorated titania (Au-TiO$_2$) NW arrays (Figure 2G i–iii), for restoration of visual responses in the blind mice with degenerated photoreceptors. The decoration of Au nanoparticles enhanced the photoconversion efficiency of TiO$_2$ NW arrays into visible range, with the peak position centered around 550 nm; this is due to the electrical field amplification and the injection of surface plasmon resonance generated hot electrons into TiO$_2$ conduction band. Au-TiO$_2$ NW arrays were placed underneath the retinal degenerated 1/cone diphtheria toxin subunit-A (rd1/cDTA) blind mouse retina, with the inner nuclear layer in contact with NW arrays, and the spiking activities of retinal ganglion cells (RGCs) were recorded using patch clamp pipettes (Figure 2G iv). It was clear that the green, blue, and near UV light responses in the NW-interfaced blind mouse retinas were restored with a spatial resolution better than 100 μm. Light-response inhibition in RGCs by glutamatergic antagonists suggested that NW-interfaced...
Retinas were able to process visual information through the remaining innate retinal circuits. In addition, neurons in the primary visual cortex were responsive to light after subretinal implant of NW arrays into blind mice; pupillary light reflex was also improved in awake-behaving mice 4–8 weeks after the implant surgery, indicating the recovery of light sensitivity and visual function. The development of such NW arrays will open new opportunities in the generation of subretinal prosthetic devices for treating retinal degeneration diseases.

Despite the strong potential of vertical NW platforms – thanks to their biocompatibility and optoelectronic properties – in neurological and other biomedical applications, their rigid mechanical properties and complex fabrication processes hinder their integration onto flexible, tissue-adaptable, and large-area scaffolds; this in turn limits their practical applications. To address this issue, Park et al. generated a highly flexible patch comprising vertically aligned hydrogel nanospike arrays (hSPIKE, Figure 2H i–iii) as a transplantable platform to enhance the growth and differentiation of stem cells while suppressing biofilm formation (Figure 2H iv). The hSPIKE was based on the biocompatible poly(ethylene glycol) dimethacrylate polymer; individual nanospikes had a top diameter of 50 nm, height of 300 nm, and pitches of 500 nm, 1 μm, and 4 μm. To investigate the effects of hSPIKEs on stem cell adhesion and morphology, Dental pulp stem cells (DPSCs) were cultured on the arrays for 12 h. The optical and SEM imaging results showed that the morphology of DPSCs (nucleus and cell body) were susceptible to the different nanotopographies and could be regulated by modulating the geometry of the hSPIKEs. To further investigate the effects on stem cell proliferation and differentiation, the DPSCs were cultured on the hSPIKE arrays for 3–21 days. After 3 days of culture, DPSCs on the hSPIKEs showed slightly higher proliferation than that of those grown on the flat substrate; and higher cell proliferation was found on hSPIKEs with a smaller pitch. After 7–21 days of culture, the differentiation of DPSCs was evaluated into multiple lineages including osteogenic, chondrogenic, and adipogenic lineages. Osteogenesis of DPSCs was enhanced on the hSPIKEs with a 500 nm pitch (Figure 2H v) but decreased on the hSPIKEs with 1
and 4 μm pitches, indicating that the stem cells sensitively respond to the density of the hSPIKEs. In addition, the physical stimulus by the hSPIKEs enhanced the cell membrane penetration and facilitated the delivery of near-infrared (NIR) fluorophores into DPSCs. The nanoscale physical stimulation also promoted the secretion of higher levels of growth factors, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF)-1, than did cells on flat control; these growth factors can regulate the cellular and tissue environments through the repair or regeneration of damaged tissue. Meanwhile, the hSPIKE arrays possessed effective bactericidal and antibiofouling activities, most likely through physically rupturing the bacterial cell membrane. Finally, in vivo studies demonstrated that compared with control flat patch, the flexible hSPIKE significantly promoted the regeneration of damaged cranial bone tissues (Figure 2H vi) while suppressing pathogenic bacterial infections in mouse models. With the unique pro-stem cell but anti-bacterial feature, such flexible hSPIKE material will have wide applications as a bioactive and transplantable stem cell scaffold.

4. Nanoparticles in the clinic

The world has witnessed the important role of nanoparticles in the development of messenger RNA (mRNA)-based vaccines against the SARS-CoV-2 infection. Nanoparticles aim to serve as a vehicle that both protects the cargo and enhances the therapeutic indices of various agents (e.g. small molecule chemotherapy drugs, gene delivery, biomolecules), predominantly through alteration of their pharmacokinetics and pharmacodynamics. To date, there are currently 29 FDA-approved nanoparticle formulations on the market (Figure 3A). Of the approved nanoparticle formulations the two main compositions are liposomal (13 formulations) or inorganic nanoparticles (12 formulations) which are used as iron replacements or MRI contrast agents. Of these, liposomes are one of the oldest forms of nanomedicine, having been extensively studied since the 1960’s. Thus the accrual of liposomal knowledge in their safety and activity has been well...
documented for decades.\textsuperscript{[126a, 127]} Thus, it is of little surprise that most therapeutic nanomedicine that has been translated onto the market comprise of liposomes encapsulating small molecule drugs in order to enhance safety and efficacy. Although nanomedicine has been heavily targeted towards cancer over the last few decades – for example, 8 out of the 13 liposomal formulations are against cancer\textsuperscript{[4b]} – there has been an increase in clinical trials of nanoparticles to treat various other diseases such as autoimmune diseases and macular degeneration.\textsuperscript{[4b]}

The two most recent FDA approved nanoparticle formulations via systemic administration, VYXEOS and Onpattro, currently highlight some of the key advantages of utilizing nanoparticle formulations for improved therapeutical outcomes.

4.1. Recently approved nanoparticles

4.1.1. VYXEOS (combinatorial therapy)

VYXEOS is an FDA approved (August 2017) liposomal formulation for the treatment of adults with certain types of acute myeloid leukemia marketed by Jazz Pharmaceuticals. In the landmark Phase III efficacy study (NCT01696084), VYXEOS provided a significant improvement in overall median patient survival of 9.6 months as compared to 5.9 months when both free chemotherapeutic drugs were administered (Figure 3B).\textsuperscript{[128]} The formulation encapsulates a combinatorial ratio of cytarabine to daunorubicin at a 5:1 ratio in 100 nm bilamellar liposomes where the lipid membrane consists of desaturated phosphatidylcholine:distearylphosphatidylglycerol:cholesterol at a 7:2:1 molar ratio.\textsuperscript{[129]} Delivery of cytarabine and daunorubicin without the liposomal formulation would lead to each drug exhibiting distinct pharmacokinetic profiles that would be metabolized at different rates. In contrast, by encapsulating the two drugs in the liposomal formulation, the two different pharmacokinetic profiles are unified into an individual profile. VYXEOS is the first FDA approved nanoparticle for the co-delivery of two therapeutic agents. VYXEOS is a crucial example
demonstrating that by encapsulating two small molecules drugs with distinct pharmacokinetic profiles, at an optimal ratio, an increase in measured efficacy (median survival) can be achieved.

4.1.2. ONPATTRO and siRNA delivery

ONPATTRO is a first of its kind, FDA approved (August 2018), siRNA-delivering lipid-based nanoparticle for the treatment of peripheral nerve disease (polyneuropathy) caused by hereditary transthyretin-mediated amyloidosis (hATTR) marketed by Alnylam Pharmaceuticals. It is the first FDA approval for delivering small interfering RNA (siRNA), a new modality and class of drug, in which ONPATTRO is responsible for silencing a specific gene responsible for the expression of transthyretin. In the key Phase III efficacy study (NCT01960348), 56% of patients who received ONPATTRO evinced an improvement in measured efficacy (modified Neuropathy Impairment Score+7 (mNIS+7)) compared to the 4% of patients who received the placebo. Furthermore, serum transthyretin decreased by over 70% in patients receiving ONPATTRO as compared to less than 20% who had received the placebo.\(^{[130]}\)

ONPATTRO encapsulates siRNA molecules in a lipid nanoparticle consisting of four lipid excipients, in which two were FDA approved and two were novel at the time lipid components. DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and cholesterol were incorporated into the lipid nanoparticle to provide and improve the physicochemical stability of the nanoparticles.\(^{[131]}\) The two novel lipid components consisted of DLin-MC3-DMA ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate) and PEG\(_{2000}\)-C-DMG (α-(3-((1,2-di(myristyloxy)propanoxy)carbonylamino)propyl)-ω-methoxy, polyoxyethylene). DLin-MC3-DMA aided in particle formation, fusogenicity, cellular uptake and the endosomal release of the siRNA payload, while PEG\(_{2000}\)-C-DMG aided in improving the stability and optimal circulation time of the lipid nanoparticle.\(^{[132]}\) The mechanism of action is of great interest, as when administered intravenously, the lipid nanoparticles are opsonized by apolipoprotein E (ApoE),
where the particles are cleared to the liver and bind to ApoE receptors on the surface of hepatocytes (Figure 3C). \[^{133}\]

ONPATTRO has paved the way for new RNA interference (RNAi) therapeutics from Alnylam Pharmaceuticals where many more siRNA candidates have been spearheaded into clinical trials. Givosiran (GIVLAARI) was approved by the FDA in November 2019 for the treatment of acute hepatic porphyria in adults, a genetic disorder resulting in the buildup of toxic porphyrin molecules which are formed during the production of heme (precursor of haemoglobin). Unlike ONPATTRO, the formulation does not utilize lipid nanoparticles but uses GalNAc (N-Acetylgalactosamine) siRNA conjugates. The GalNAc sugar derivative is used as a targeting ligand, binding to asialoglycoprotein receptors on hepatocytes. \[^{134}\] Following Givosiran, Lumisiran targeting glycolate oxidase in development for the treatment of primary hyperoxaluria type 1 is currently in late Phase III trials (NCT04152200), while seeking FDA approval. Furthermore, on top of Alnylam Pharmaceuticals, both Arrowhead Pharmaceuticals and Dicerna Pharmaceuticals are also pursuing various biological targets using GalNAc-siRNA conjugates.

Therefore, with plenty of GalNAc-siRNA conjugates currently in clinical trials and with the two FDA approvals of ONPATTRO and GIVLAARI, their use in targeting and efficaciously treating numerous liver diseases appears like a promising strategy. Interestingly, both lipid nanoparticles (ONPATTRO) and GalNAc-siRNA conjugates (GIVLAARI) utilize receptor recognition on hepatocytes via different approaches. While ONPATTRO employs natural biological opsonization of native ApoE protein for receptor specificity, GIVLAARI incorporates an active targeting ligand (GalNAc). Both approaches highlight two of the currently more debated topics in the nanomedicine/nanoparticle research community: (i) exploiting natural biological processes and (ii) the incorporation of active targeting ligands onto the surface of nanoparticles to maximize tissue and/or cell selectivity. \[^{125a, 135}\]
Examples in the literature have exploited natural biological processes in order to improve therapeutic outcome of nanoparticles such as mimicking natural biological processes. For example, several biomimetic approaches have been investigated including (i) camouflaging therapeutic nanoparticles in extruded human cellular membranes, and (ii) hitching onto red blood cells in order to evade renal or hepatic clearance.\(^{[136]}\)

Alternatively, the incorporation of active targeting ligands on the surface of therapeutic nanoparticles has garnered interest for the delivery of increased therapeutic dosage to the target site while limiting unwanted off-target effects.\(^{[135]}\) This is especially prevalent in targeted nanoparticles for the treatment of cancer, where cytotoxic small molecule drugs like doxorubicin are encapsulated in targeted nanoparticles in order to limit off-site toxicity after systemic administration.\(^{[137]}\) However, the translation of novel targeted nanoparticles very rarely progresses towards clinical evaluation despite the plethora of reported preclinical success.\(^{[138]}\) Novel formulations are potent in preclinical models; however, those that do reach the clinic tend to fail due to poor efficacy in Phase II and III trials.\(^{[138]}\)

4.2. Lessons learnt and future directions

It begs the question, should we be innovating or learning from past success? Currently FDA approved nanoparticles like VYXEOS and ONPATTRO are simple liposomes with specific molar ratios of lipid excipients. Both nanoformulations, as well as the BioNtech-Pfizer and Moderna mRNA vaccines, have a diameter of around 100 nm and low surface charge.\(^{[21, 2m]}\) This ensures that the simplicity and scalability of said liposomes can be manufactured to meet clinical needs with utmost reproducibility and quality control. Successful nanomedicine such as ONPATTRO and GIVLAARI have harnessed the body’s hepatic clearance of foreign material,\(^{[139]}\) utilizing this process to target different liver diseases. However, with diseases outside the liver, targeted
nanoparticle therapy has yet to succeed clinically, despite reported preclinical efficacy. This is because we face several underlying issues:

4.2.1. Scalability of active and passive targeting strategies

Researchers have tried to enhance tissue and cell targeting capabilities by either i) manipulating the nanoparticle physicochemical properties (passive targeting) and/or ii) attaching targeting moieties against specific receptors to the nanoparticle surface (active targeting).\cite{1a, 140} We have seen how virus-like nanoparticles (sizes around 100 nm) are highly effective at both targeting the liver following intravenous administration and as next-generation mRNA vaccines.\cite{2l, 2m} While it is apparent that the biodistribution and therapeutic effect of nanoparticles with different sizes, shapes, surface chemistries and rigidity can be fine-tuned, it is challenging to draw ultimate conclusions from current studies as the experimental conditions are intrinsically different and hard to compare.\cite{1a, 140a, 141} What is clear is that all parameters (size, shape, surface charge and rigidity) should be taken into account when engineering nanomedicines to maximize accumulation to the target tissue.\cite{140b, 140c} Active targeting – the incorporation of a targeting agents such as antibodies, aptamers or DNA onto the nanoparticle surface – has become the focus of nanomedicine research for the past decade, particularly in the development of anticancer nanomedicines.\cite{4a, 141} Preclinical studies have shown that actively targeted nanoparticles not only have increased specificity but also facilitates cell internalization, leading to an enhanced therapeutic efficacy and reduced side effects.\cite{141} While these are all exciting discoveries, we are yet to see nanoparticles that are actively targeted or not spherical in the clinic.

These successful targeted nanoparticles in preclinical models generally aim to tackle different facets of drug delivery with a solution.\cite{142} When these solutions are combined, the complexity and feasibility of scalable nanoparticle production substantially increases – and while novel and efficacious, they would be increasingly difficult to reproduce bench-side formulations at a larger scale.\cite{143} On top of reproducibility, a complex system with multilayered steps leads to increased
chances of failure, with many quality control hurdles. Thus, complex nanoparticle production at a large scale for clinical trials has not been successfully explored to date. Additionally, these production issues have yet to factor the large quantities and costs of costly raw materials.\[143\] This is probably why nanoparticle formulations that have been approved for therapeutic use have been centered around liposomal formulations as they are well studied and simple to produce at a large scale.\[144\] The incorporation of a targeting ligand on a nanoparticle surface adds an extra layer of complexity, where a successful nanoparticle formulation has yet to be show promising clinical efficacy.

4.2.2. Bottlenecks in preclinical research

Research is forever ongoing within the nanomedicine community both in fundamental and translational nanomedicine to further our understanding towards effective nanoparticle delivery towards various diseases. However, the nano–bio interface, particularly in cancer research, is extremely complex and unfortunately still poorly understood – leading to disappointing results in clinical translation.\[124\] This is because there is a big gap between animal and human studies, as preclinical models typically used in nanoparticle assessment do not fully recapitulate the physiology of human diseases.\[4a, 7a\] With the aim to bridge this gap between animal and human biology, we have recently used, for the first time, tumor tissue sections directly resected from cancer patients – so called patient-derived explants – to assess nanoparticle performance \textit{ex vivo}.\[3e\] These explants maintain the most important physiological components such as stromal and tumor-associated immune cells and cell-to-cell signaling, providing a more clinically-relevant and predictable platform to probe the nano–bio interactions at a tissue level.\[145\]

But before they reach the disease site, nanoparticles face many barriers that will remove them from their journey such as the formation of a protein corona which causes aggregation,\[140c, 146\] alters their biodistribution and induces immune cell sequestration and fast clearance.\[147\] A highly cited paper analyzed 117 manuscripts in the literature between 2005 and 2015 to find out that only 0.7%
(median) of injected nanoparticles actually reach the tumor site.\textsupERSsup{148} After this report, there has been a concentrated effort from the field to overcome the biological barriers blocking nanoparticle delivery. An interesting concept towards solid tumor delivery has recently been reported and discussed by Ouyang et al.\textsupERSsup{149} They discovered that 1 trillion nanoparticles are required to be injected to achieve a 12-fold increase in nanoparticle tumor accumulation in mice. This exuberant dose threshold needs to be breached to exhaust and overwhelm Kupffer cellular uptake rates; decreasing hepatic clearance and prolonging circulation time for increased solid tumor delivery. A further literature review of current nanoparticle formulations that had been FDA approved found this to be the case, where when extrapolated to an average 70 kg weighing human, a dose threshold of 1.5 quadrillion nanoparticles was required to overwhelm the human liver and enable effective dosage at the solid tumor.\textsupERSsup{149} A survey of the literature found that successful clinical nanoparticles such as Caelyx (liposomal doxorubicin) and Onivyde (liposomal irinotecan) exceeded the dose threshold at 8.6 and 1.9 quadrillion nanoparticles, respectively. However, examples of failed nanoparticles such as BIND-104 and NK105 (1 and 0.9 quadrillion, respectively) during clinical trials fell below the dosage threshold, a possible reason for failure due to insufficient nanoparticle dosage.\textsupERSsup{150}

5. Microfluidic-based organ on chip system in drug development

Microfluidics technology manipulates miniscule volume of fluids (nanoliters to microliters) in a system that enables high throughput, automation and in system integration and detection. With a requirement of small volume for handling, valuable or limited reagents can be utilized in a cost-effective manner. Originated its root from the microelectronic industry, microfluidic technology in the past 30 years has evolved and integrated into a very diverse field today, such as in chemical syntheses, biological assays, cancer research, proteomics, point-of-care diagnostics, toxicology, to name just a few.\textsupERSsup{[2f-j]}
Especially in biological sciences, microfluidic technology plays a vital role for its capability to finely control and manipulate cellular microenvironment that simulates what cells would experience in the body. To reconstruct those cellular ensembles (mini-“organ”) on a chip platform has stemmed the organ-on-chip (OOC) system. Human OOCs are miniaturized versions of vital human organs embedded in microfluidic chip devices: heart, lung, liver, kidney, intestine, bone, skin, blood vessel, etc.\(^{[12a]}\) OOC is also referred to as “microphysiological system” that can accurately reproduce the key functions of cellular architecture and physiology.\(^{[12b]}\) The ultimate goal for OOC system is to recapitulate accurately of the most basic functional unit of an organ or tissue, and not the whole organ.\(^{[12a]}\) OOC expands the conventional cell culture platforms, such as cell culture flasks or well plates, to customizable cell microenvironments integrated with precise structural, mechanical and fluidic controls (Figure 4A).

One important adoption of OOC technology is in drug development, especially in the preclinical application stage. Traditionally animal models are often used for initial screening of new drug compound pharmacology, efficacy, and toxicity. Although valuable information could be extracted from those animal testing models, such as pharmacokinetic/pharmacodynamic (PK/PD) profile, there is still major concern over the extrapolation of animal model data to human. Numerous reports have indicated that some drugs exhibited some initial success in animal testing, however, failed at clinical trials. Due to the vast differences in the genome between rodents (and other testing animals) and human, extrapolation of the drug molecule efficacy and toxicity from one specie to another can undoubtedly introduce mistranslation and often can become fatal. Traditional simple culture method (e.g., cell culture flasks and well plate vessels) of using human derived cells can provide some broad information regarding drug toxicity and efficacy. However, those assays have failed to recapitulate critical cell function and physiology or tissue-tissue interactions. Current approaches using such \textit{in vitro} 2D culture and \textit{in vivo} preclinical models thus yield limited predictive capability for translation to the clinical setting.
This newly emerged OOC technology, on the other hand, provides a close mimicry to the human physiology via unique combination of 3D culturing of human-derived cells with microfluidic techniques. It can better predict the drug safety and efficacy profile and could potentially lead to a reduction or replacement of animal testing at preclinical trials. The commercial interest for OOC is quite strong and increasing. The OOC market is estimated to exceed $6 billion by 2025. However, some hurdles and workflow associated with this new technology should be clearly examined, for example, the need for specialized training, labor intensive nature of working with OOC, and cost/benefit ratio of OOC comparison to current biochemistry/cellular testing.

5.1. Organ on chip: in the laboratory

The first attempt of recreating organ-level function using cells inside a microfluidic chip was reported back in 2004 where a cell culture analogue was designed to study the systemic interaction between lung cells and liver cells. And in 2010, Donald Ingber invented the term “organ-on-a-chip” based on a research to capture lung organ-level function in a microfluidic device. Various microfluidic system-based OOCs have been extensively explored in the past 10 years to mimic vital human organs embedded in a chip, such as liver, kidney, heart, blood-brain barrier, intestine and others (Figure 4B).

The first lung-on-a-chip microsystem was designed and developed by Huh and colleagues which resembled human breathing mechanism. This microdevice consists of a porous membrane separating human alveolar epithelial cells and human pulmonary microvascular endothelial cells (forming an alveolar-capillary barrier). Vacuum induced mechanical stretching was applied in the side chambers to recreate human breathing motion. Pulmonary inflammation and bacterial infection events were tested on chip via the stimulation with TNF-α and challenging with E. coli, respectively. Silica nanoparticles were tested in this lung OOC system and were found to induce oxidative response. This oxidative response was further enhanced by mechanical strain comparing to non-stretched condition. Moreover, the mechanical strain enhanced cellular uptake of silica nanoparticles.
nanoparticles. The device was further used to study the pathological condition of pulmonary edema where interleukin-2 was used to induce pulmonary leakage.\cite{158} Cyclic mechanical strain was applied to the chip system and demonstrated the stretching force further comprised the pulmonary barrier. Those findings could not otherwise be obtained using traditional culture methods. A novel pharmacological drug was tested in the lung OOC and demonstrated a stabilization effect towards epithelial barrier leakage highlighting the capability of this system.

The liver is the principal organ for regulating drug metabolism. In fact, liver and heart toxicities have been a major cause for drug recalls. Liver OOCs have been one of the highly investigated microsystems for testing pharmaceutical drugs. For example, Chao and coworkers constructed a microfluidic-based continuous perfusion culture for primary human hepatocytes to predict hepatic clearance.\cite{159} Six marketed model pharmaceuticals drugs (carbamazepine, caffeine, timolol, sildenafil, imipramine, and buspirone) were tested on liver-on-a-chip system for comparing the intrinsic hepatic clearance rates with static cell culture and \textit{in vivo} model. Further to couple with coculturing with nonparenchymal cells, the liver chip system is capable of hepatic clearing, with improved resolution and predictive value than static and monoculture.\cite{160}

Those organ-on-chip devices have drastically transformed the traditional way of testing drug compounds in a setting that is more physiologically relevant. However, to obtain information regarding the PK/PD profiles or the interactions/communications among different organs, drug molecules should be tested in a multi-organ setting, the “whole body” response. Integrated systems with multiple microscale cellular environments can be designed to simulate the systematic function of the human body and to predict the pharmacokinetics of new drugs. Coupled with PK models, some interconnected multi-organs on chip can mimic the physiological complexity of inter organ interactions. Such system could be beneficial towards the assessment of how the human body absorbs, distributes, metabolizes and eliminates (ADME) drugs. Ensuring accurate ADME properties are important to determine if a drug can remain therapeutically effective when reaches its
targeting organ and with no toxicity. Several microfluidic perfusion systems have thus been developed for co-cultures of multiple tissue types for determining the pharmacokinetic ADME process of testing pharmaceutical compounds.

One of the earliest multi-organ system was developed by the Schuler group to assess potential toxicity of naphthalene. A microscale cell culture device consists of a fluidic network of four chamber compartments to mimic the circulatory system (lung, liver, fat, and other tissue). They were able to examine how the reactive metabolites produced by the “liver” compartment and circulated to the “lung” compartment and subsequent effects on the “lung” cells. The other compartments do not actively react or absorb drugs but contribute significantly to the fluid flow and residence times. Further modification of such system has been used to test metabolism-dependent toxicity of several drugs, such as cytotoxic effect of anticancer drug, Tegafur.

Another multi-organ chip system developed by Maschmeyer and colleagues constituted interconnected human intestine, liver, skin and kidney equivalents were able to maintain functionality over 4 weeks coculture. Comparing to human counterpart organs, their intestine and skin models are 100,000 times smaller. 3D spheroid construct equivalent to ten liver lobules was used for liver model. A monolayer barrier of human proximal tubule epithelial cells was also incorporated as kidney mimic. This system design has well taken consideration of physiological fluid-to-tissue ratios. In depth metabolic (e.g. glucose concentration and LDH activities) and gene expression were examined for the four “organs” and demonstrated a reproducible homeostasis between the tissues. A separate medium reservoir was designed to be located at the apical surface of the intestinal barrier which can mimic the “oral” administration route of a drug candidate. This four-organ-chip is well suited to support the ADME profiling of drugs and testing for the drug dose systemic toxicity.

A more sophisticated system consisting a 14 chamber (representing 13 organs) microfluidic cell culture device has recently been reported which could be used to emulate drug distribution,
metabolism, and action in the body.[13a] Chemical or biological reagents could enter the barrier tissue compartment (e.g., skin, lung, and gastrointestinal tract) before reaching the none-barrier tissue chambers (e.g., brain, kidney, heart, liver, spleen) and fluid circulation. Due to the complexity of such a system, only five cell lines were initially tested on such device, and it was demonstrated that cell viability and functionality were maintained for over 7 days. The dimension and flow rate of each chamber/channel were designed by scaling from the physiological human organs.

5.2. Organ on chip: out in the market

OOC systems have attracted many international attentions aiming at its development and maturation. Major organizations in the United States, such as the Food and Drug Administration, the National Institute of Health, and the Defense Advanced Research Projects Agency have funded and launched several programs to support the OOC research.[163] Various not-for-profit organisations, such as the People for Ethical Treatment of Animals (PETA) have strongly supported for such system development. The European Union has also funded multi-million dollars in grants to support early-stage OOC research projects. In less than 10 years of the emergence of the OOC concept, some of the systems have materialized and OOC start-up companies (mostly spinoffs from universities) have appeared in the market.

The lung organ-on-chip system developed by Huh and colleagues, after the initially successful laboratory validations, has begun to be commercialized a couple years afterwards under the company trade name of Emulate Inc. Emulate is one of the earliest OOC companies. It was founded in 2014 and was spun out from Wyss Institute at Harvard University. The main product line of the company are the stretchable devices composed of two microfluidic chambers separated by a thin polymeric porous membrane that can underdo cyclic stretch, mimicking the lung breath motion. Aside from the product being used as a model for lung physiology, this device setup can also be configured to simulate other organs and barriers, such as liver, kidney, and intestine models by
seeding with respective cells. More recently, Emulate donated devices to the United Kingdom Organ-on-a-chip Technologies Network to fast track the development of therapeutics for combating the global pandemic caused by a novel coronavirus, SARS-CoV-2. Another start-up OOC company, Alveolix, founded in 2015, commercializes exclusively the lung-on-chip system. AlveoliX’ organ-on-chip recreates the micro-environment of the lung alveoli, particularly of the air-blood barrier. Their device is based on a 96-well plate format and enables the seeding of the cells on either side of an ultrathin stretchable membrane. Although this device is capable of stretching motion by external electro-pneumatic controller, it does permit for vascular perfusion. Xona microfluidics, commercializes silicone devices fabricated from polydimethylsiloxane (PDMS) which is commonly used in research setting. This product contains multiple parallel perfusion channels (2-3) interconnected by micro-grooves for neuronal cell cultures. The main objective for this technique is to isolate axons from neuronal cell body for studies in axonal transport and regeneration. SynVivo developed silicon-based microfluidic chips having channel networks resembling the architecture of a microvascular network by using actual images of tissue microvasculature. Their success builds upon the coupling of digitized tissue imaging with silicon etching technologies. Currently the developed 3D tissue models from the company extended their applications with specific tailoring in the chip design in blood brain barrier model, cancer models, inflammation model, toxicology and lung model.

Other than commercializing devices containing single “organ” unit, some companies have focused on the interconnected organs. Especially important in the drug discovery application, to test how the human body ADME drugs, the interconnected organ platform representing key “organs” is arguably a more powerful approach. For example, Hesperos, Inc. is a leading company to develop fully functional, interconnected multi-organ systems. Their core technology are the pumpless multi organ platforms (e.g., heart-liver-muscle-neuron) that uses gravitational flow with a goal to transform toxicology testing and efficacy evaluation for drug discovery. Circulation of a common serum-
free medium between different compartment allows multi-organ system communication with integrated computational PK/PD modeling. Functional readouts such as contractile force generation or neuronal spontaneous action potential have been successfully integrated for a real time and non-invasive monitoring. Hesperos also commercializes other systemic toxicology models with interlinked organs, including: two organ models (e.g., neuron-muscle), three organ model (i.e., heart-liver-cancer), barrier tissue modules (e.g., blood-brain barrier and gastrointestinal tract).

TissUse GmbH is a European startup company commercializes microfluidic devices composed of two, three or four organ models. Their technology incorporates a built-in micropump controlled by an external pneumatic controller.[165] Furthermore, the device itself is made from thermoplastic material, other than PDMS material, which may absorb certain hydrophobic molecules. CN Bio Innovations provides “PhysioMimix Organ-on-Chip” platform which is a perfusion-based multi-well plate system.[166] This device has a similar setup to the traditional Transwell plates that are commonly used in the laboratory and the perfusion is achieved by built-in micropumps. This is rather a simple setup and does not require the end users to have prior experiences/trainings in fluidic handleings.

While the OOC companies are slowly becoming more mature, lab-on-chip manufacturing companies are partnering up with OOC companies to develop hardware to standardize and scale up the chip production. For example, Micronit Microfluidics focused initially on manufacturing miniaturization of devices has shifted its attention to life science applications. Micronit owns ISO-9001 and ISO-13485 certified advanced cleanroom production facilities and capable of a wide range of materials for chip production. Microfluidic ChipShop, based in Germany, also offers a range of product development and fabrication services. Elveflow and Fluigent commercialize cutting-edge pressure pumps for precise and automation of fluid manipulations.

5.3. Organ on chip: current challenges
Nevertheless, some technical and engineering challenges facing the OOC development still need to be carefully addressed. For example, each “organ” within the multi-organ chip system requires a particular type of media for cells to grow, differentiate and maintain specific functionality. A new formulation of a universal media that is suitable for every organ should be investigated. Some engineering aspects, such as how the media or testing pharmaceutics are delivered into the system should be consider: using gravitational driven flow, external pump system (syringe, pressure, peristaltic pump), or built-in micropump? A proper scaling of the dimension of the “organs” in relative to physiological conditions (such as fluid flow rate to volume ratio) should be factored into the chip design. Furthermore, what are the suitable assay readouts can be integrated into the system? Will it be a real time monitoring or end point readouts? Other important key designing factors should be considered as well: how to minimize testing drug none-specific adsorption to device material, tubing and membranes; how to prevent air bubbles from entering the system during perfusion; should the device be made disposable or reusable?

Immortalized cell lines and primary cells are still predominately used in OOC systems due to the ease of handling and their cost. Stem cell derived cells for culturing in organ-on-chip is starting to become more popular and promising.\cite{13b} For example, human induced pluripotent stem cells (iPSCs)-derived cardiac tissue has been shown to be cultured in a microfluidic system to be viable and functional over multiple weeks, and was used for testing cardiotoxicity.\cite{155a} Human iPSCs derived mature, post-mitotic kidney glomerular podocytes were cultured within an OOC device to build a human kidney glomerulus chip that mimics the structure and function of the kidney glomerular capillary wall \textit{in vitro} over 35 days.\cite{154c} A human blood-brain barrier chip constituted entirely with iPSCs-derived brain microvascular endothelial cells, astrocytes, and neurons was reported to create a neurovascular unit that recapitulates the complexity of human BBB functions.\cite{156b} Combining OOC technology and human iPSC-derived tissue generates a platform that not only accurately models various disease types, but also advances high throughput drug
screening for achieving personalized medicine and to yield a better and more predictive clinical outcomes.

To achieve a successfully transition of OOC technology into the global market, OOC products should outperform existing market products in terms of capabilities and price. The current OOC technology is still too expensive for wide-scale adoption. The academic force should be in full front dialogue with the industry partner to address the potential success of the technology in the market, the requirement and expectation from end users, delivering a fully integrated product. Since microfluidic technology is relatively new to the market, the OOC chip system should be relatively easy to use and can be integrated into exiting work flow in end user infrastructure. Thus far, each company specialized in a specific “organ”/technology, and to achieve the fully functional “whole body” on a chip, multiple companies need to join forces to integrate their techniques and patents to revolutionize advanced products for drug development and toxicity testing.

6. Nanomaterial-enhanced mass spectrometry in drug discovery and development

Mass spectrometry is a mature technology in the drug discovery and development (DDD) space, well considered the ‘gold-standard’ technique due to the requirement for accurate and precise quantifiable measurements of molecular components in pharmaceutical research. While MS is unlikely to shift as a robust technology in DDD, from a regulatory and application point of view, combining nanomaterials into MS approaches shows great promise in enhancing the sensitivity, selectivity and spatial information that can be achieved in all stages of applied DDD. Nanomaterials are being fabricated to specifically capture low abundant bio-molecules in complex mixtures for selective extraction, enrichment and improved analysis. Their use in sample preparation can remove many of the error prone steps or contaminating species prior to sample introduction into an analytical instrument. Advanced nanomaterials are being utilized in the ‘omics’ analysis of DDD related biomarkers, infectious agents and translational studies. However, most excitingly,
nanomaterial substrates are being generated to enhance the application of a powerful DDD technique, mass spectrometry imaging (MSI); where nanofabricated substrates enhance the chemical detection of drugs, drug metabolites, drug carriers and endogenous metabolites to map drug metabolism *in situ* within tissues and organs derived from drug models and clinical samples.\(^{15a-g}\)

### 6.1. Nanoparticles improve the mass spectrometry DDD workflow

Elucidating the mechanism of action is a crucial step in drug development. The detection of signaling pathways associated with a drug's action requires sensitive and selective analysis of the changing proteome and metabolome associated with a dose response, often termed ‘systems pharmacology’.\(^{170}\) In many cases, drug-dose related changes in protein and metabolite levels are subtle shifts which can be extremely difficult to discern in complex biological matrices (e.g. blood serum). Innovations in nanoparticle design and conjugation strategies are allowing the multi-selective capture of drug related targets in complex mixtures,\(^{171}\) or improving sensitivity through selective extraction. This approach to MS analysis has been commercially valuable with the uptake of Dynabeads® magnetic separation technology, where miniaturization of selective capture is done with smaller nanoscale materials that offer vastly larger surface areas for material-analyte interaction. Alternatively, materials that play the same role as ‘in solution’ particles have been adopted, where biofluids are applied to a material and directly interfaced with a MS.

### 6.2. Omics analysis by size exclusion nanomaterials

Nanomaterials offer various advantages in MS when applied in the sample pre-treatment phase of the analytical pipeline. In the -omics workspace, biological samples contain a variety of different high and low molecular weight compounds, which can obscure low abundant targets.\(^{172}\) The development of nanomaterials in this space has focused on ensuring low abundance components can be effectively detected by tuning the nanomaterial to trap the specific analyte via size exclusion.\(^{15h}\) For example, Liu et al.\(^{173}\) created a pSi NanoDisk-MS assay for detecting circulating...
peptides of *Mycobacterium tuberculosis* from infected or treatment resistant patients, demonstrating that size exclusion to trap tuberculosis peptides prior to antibody recognition greatly enhanced assay sensitivity. This enrichment effect was also observed during the synthesis of core-shell magnetic covalent organic frameworks which enabled high absorbance of peptides and simultaneous exclusion of proteins from complex biological samples, which are then purified through magnetic collection. Selective molecular trapping is also possible by fabricated nanomaterials with various pore sizes that allow more than one molecule to be absorbed and subsequently analyzed, a useful approach to illicit drug and peptide detection in biofluids. The use of these fine-tuned nanomaterials can also promote an enhancement in observed signal not only from the trapping of the analyte but also from the properties of the nanomaterial when coupled with the MS instrument of choice.

In the field of proteomics, nanomaterials have been studied with great interest to further contribute to MS-based analysis for in-depth profiling. The nanomaterials used are comprised of the substrate and the functionalized moiety, with both factors required to work in tandem with each other for effective results. Common substrates include metal and semiconductor-based nanoparticles and graphene, amongst others. Their adoption and use has also been attributed to their controllable and facile fabrication, high surface area, and ease of surface functionalization. A key component in proteomics is the research of post-translational modification (PTM) which has significantly improved our understanding of cellular processes. One such PTM mechanism in eukaryotes is phosphorylation, critical in enzyme activity regulation. As phosphorylation has a direct effect on the protein function and its respective cellular signaling pathways, it is important that sensitive and selective detection is achieved. Exhaustive research has thus been performed to fabricate MS-appropriate nanomaterials that can act as capture devices for phosphorylation, increasing the obtained signal from low abundance. Immobilized metal affinity chromatography (IMAC) and metal oxide affinity chromatography (MOAC) are primary techniques that allow the retention of
proteins with a specific affinity for metal ions.\textsuperscript{2k, 176} IMAC-based nanomaterials include polydopamine and phosphate linkers for metal ions including Ti and Fe.\textsuperscript{2k} MOAC-based nanomaterials include TiO\textsubscript{2} and ZrO\textsubscript{2} and the integration of different metal oxides in a single device that could take advantage of differing protein affinities.\textsuperscript{2k} TiO\textsubscript{2} has shown a selective enrichment for multi-phosphorylated peptides while ZrO\textsubscript{2} enriches mono-phosphorylated peptides.\textsuperscript{179} Research has also been performed on a combination of IMAC and MOAC nanomaterials, such as TiO\textsubscript{2} and Ti-IMAC, that further enhance the efficacy of phosphorylation-based proteomics.\textsuperscript{180} Furthermore, protein tags can be added to the protein of interest to promote metal ion affinity, if not previously present.\textsuperscript{181} The use of nanomaterials and PTM targeting is being applied to a range of central nervous system (CNS) diseases, including dysregulated kinases in Parkinson’s disease (e.g. LRRK2).\textsuperscript{182}

6.3. Biomarker detection through nanomaterial enrichment

Along with phosphorylation, glycosylation is another important PTM parameter. Glycosylation involves the attachment of a glycosyl donor to an organic molecule and plays a crucial role in cell signaling and immunological recognition.\textsuperscript{183} Several glycosylated proteins have been noted for their role in physiological conditions and defects in such proteins have been observed during disease onset and progression, particularly autoimmune diseases resulting from T-cell proliferation.\textsuperscript{184} Early-stage identification of these biomarkers is therefore critical in treatment decision-making.\textsuperscript{185} Accurate and sensitive detection is required using gold-standard MS, which can be further improved by pre-treatment of the required sample.

Sample pre-treatment allows removal of high-abundant, interfering analytes and concentration of the glycosylated analyte of interest for sensitive detection.\textsuperscript{15h} A prominent pre-treatment approach for glycoproteins and glycopeptides is the use of lectins which are able to bind to the glycan molecule with high specificity and allow enrichment of specific glycosylated species.\textsuperscript{186} This enrichment allows biomarker monitoring for diseases, including various cancers, to be followed in-
depth to track stages of disease progression.\textsuperscript{[185]} Nanomaterials have entered this research environment due to their high surface area and surface functionalization capabilities, enabling the detection of glycovariants of cancer antigens, for example, CA15-3 monitoring for metastatic breast cancer.\textsuperscript{[15h-k]}

6.4. Exosome detection through nanopurification

In recent times, extracellular vesicles have become known as prominent facilitators of intracellular communication due to their ability to carry cellular cargoes including proteins, lipids and nucleic acids.\textsuperscript{[187]} Exosomes, one type of extracellular vesicle, have been studied extensively for their role in influencing biological processes, namely immune responses and suppression for pathogens and tumors, designating them as attractive candidates in the study of disease diagnostics and management.\textsuperscript{[188]} Exosomes are secreted from most eukaryotic cells and their release occurs continuously in cancer cells, contributing to tumor formation and metastasis.\textsuperscript{[189]} The use of exosomes as cancer biomarkers has been studied in great depth in order to initiate early disease detection and monitoring, performed in a rapid and non-invasive manner using MS techniques and as potential therapeutic targets for cancer-specific drug models.\textsuperscript{[187, 190]} Current isolation, purification and characterization techniques are time-consuming and ineffective, resulting in low exosome recovery and prevalence of analyte contamination.\textsuperscript{[191]} Nanomaterials have increased in popularity, again due to their functional properties, as a means to effectively isolate and recover exosomes.\textsuperscript{[192]} Exosome isolation and analysis was performed from complex biological samples by Fang et al. who used an integration of graphene foam and periodic mesoporous organosilica.\textsuperscript{[191]} Using the nanomaterial-based approach, 344 proteins were identified in comparison to 151 proteins from a commercial exosome isolation kit, demonstrating a higher rate of recovery. Such results further promote the use of nanomaterials as a sample pre-treatment enrichment technique in the identification of exosome biomarkers for disease monitoring.

6.5. Nanomaterials in SALDI-MS

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The application of nanomaterials in MS has become imperative in expanding the capabilities of MS instrumentation further than what was initially thought possible. This expansion has led to significant advances in the MS field, specifically with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), a soft-ionization technique which is widely used in the analysis of high molecular weight compounds including proteins and peptides. The MALDI workflow relies on mixing a target analyte with a chemical matrix and dried as a crystalline spot. This analyte-matrix co-crystallization assists in the absorption of the MALDI laser energy for vaporization of the sample into the gas phase where analytes are ionized and subsequently detected using a mass spectrometer. Traditional MALDI-MS has several disadvantages associated with its use including co-crystallization difficulty and the generation of ‘sweet spots’ from inhomogeneous co-crystallization. The detection of small molecules (<700 Da) also remains difficult due to the large abundance of matrix ions and their fragment peaks, masking the signal of analytes in the low mass range. Development of alternative techniques that avoid the use of a matrix while still facilitating sensitive LDI-MS has led to a shift in ideology termed surface assisted laser desorption/ionization (SALDI). In recent years, nanomaterial-based SALDI-MS has emerged as the premier technique for the rapid and sensitive detection of low molecular weight analytes, which as a field has employed novel nanofabrication strategies that enhance sensitive detection based on the desired target. SALDI-MS and its increasing adoption as an analytical technique can be partially attributed to the versatility of the chosen nanomaterial. During the formulation process, the selected nanomaterial can be modified for specific analyte detection, due to its highly controllable physical and chemical properties. The SALDI mechanism of action involves using the nanomaterial substrate in place of a matrix, allowing thermal driven desorption initiated through laser-induced surface heating and heat confinement within the substrate’s nanopore structure. The nanomaterial substrate is able to
shield the analyte from the direct UV laser, allowing ionization to occur with minimal molecular fragmentation.

The first instance of SALDI-MS was developed by Tanaka et al. in 1988, who used 30 nm sized cobalt powder to detect high molecular weight proteins using time-of-flight mass spectrometry (TOF-MS). Following this work, Sunner et al. was able to use 2-150 µm sized graphite particles suspended in glycerol for the analysis of peptides and proteins. In the decades since, research in SALDI-MS has led to an abundance of nanomaterial substrates explored for their analytical appropriateness. These substrates are routinely separated into three distinct groups, namely: carbon, metal and semiconductor, depending on the base used to form the nanomaterial (Figure 5A).

Selection of nanomaterial candidates is dependent on several factors. One such factor is the requirement of the substrate to have sufficient absorptivity for the required emission wavelength of the laser irradiation source, ensuring efficient energy transfer. The ideal substrate should also show minimal surface related MS background peaks as these can obscure analytes that appear in the same spectral range. Complementation of the substrate with SALDI-MS also ensures reproducibility of obtained data and greater analytical sensitivity.

Carbon-based nanomaterials, including carbon nanotubes and graphite structures, have been used as an alternative to the conventional matrix due to their functional advantages including increased heat capacity and high thermal and electrical conductivity. Their low cost and tunable surface properties have allowed the extensive detection of biomolecules, including carbohydrates, polymers and peptides in pharmaceutical applications. Tang et al. investigated six different carbon nanomaterials used in SALDI-MS and found that carbon nanotubes and buckminsterfullerene’s exhibited higher desorption efficiencies due to a reduction in internal energy transfer through the nanomaterial.

Metal-based nanomaterials, including Au and Pt nanoparticles, have also been thoroughly researched for optimum SALDI-MS performance and show promise in the detection of high-mass
proteins and peptides, due to their high conductivity and low resistivity.\cite{195} Chiang et al. investigated several metal-based nanomaterials and their applicability as SALDI-MS substrates.\cite{202} They determined that Au nanoparticles were effective for the detection of small analytes, including glutathione, while Pt nanosponges were effective for the detection of proteins. The nanomaterials also displayed lower limits of detection and less batch-to-batch variation in comparison to conventional organic matrices.

Semiconductor-based nanomaterials have arguably become the most successful substrate of choice for SALDI-MS.\cite{203} pSi,\cite{204} SiNWs,\cite{204} and mesoporous germanium,\cite{205} have been used in MS studies due to their high UV absorption and thermal conductivity along with their functionalization capabilities to ensure tailor-made analysis. Nanostructured-silicon substrates have been explored in greater detail and emerged as the frontrunner for the SALDI-MS detection of low molecular weight drugs,\cite{175b, 206} metabolites,\cite{207} and the profiling of biological fluids.\cite{208} In regards to the detection of athlete doping, nanostructured silicon offers several advantages in comparison to conventional analytical methods. Low sample volumes, rapid analysis time and high throughput analysis due to automation capabilities eliminate the difficulties currently observed in this testing environment.\cite{207b}

These advantages are also observed during illicit drug detection from oral fluid which is of major interest in the fields of law enforcement and workplace drug testing. Detection limits lower than current legal guidelines and excellent sensitivity further promote nanostructured-silicon SALDI-MS as an alternative analytical technique.\cite{175b}

### 6.6. Nano-substrates for mass spectrometry imaging MSI

The nanomaterial strategy applied to enhance the detection of target analytes in biofluids has also been elaborated to the technique used for drug mapping, MSI. In the case of MSI, the laser used for desorption/ionization to enable a sample to vaporize into the gas phase (and enter a MS instrument) is applied sequentially across a tissue section in a selected 2D area, where each geometrically aligned XY laser spot provides a mass spectrum. These mass spectra are combined into a chemical...
map related to the tissue, showing all detected ion (drugs, metabolites, peptides) by location and intensity (Figure 6). MSI effectively enables ‘chemical histology’, where drugs and drug metabolites are mapped directly from tissue sections, effectively allowing the ‘spatial translation’ of drugs/drug metabolites in disease/toxicity/drug models or clinical samples like biopsies.

A range of nanomaterial substrates have been commercialized specifically for use in MS and MSI, including a select few that have reach commercial production, e.g. Bruker’s NALDI™, DIUTHAME™, nanopillar array (NAPA) based REDIchip™, and Waters MassPREP™ DIOS-target™ (Figure 5B). MSI used in combination with these nanomaterial substrates, as a field, has been termed SALDI-MSI or by the material used, e.g. DIOS-MSI. As nanofabrication techniques are highly controlled and tunable, the resulting nanomaterial substrates contain reproducible structures including nanopore size, depth, nanopillar height, all with a high degree of uniformity. Reproducible surfaces are essential in sensitive applications including MS, and especially so with MSI, where natural biological variation in tissues does not need further amplification in technical variation. MSI experiments take long amounts of analytical time – many hours to days for imaging runs – so keeping technical variation low and focusing on finding true means in biological variation is essential to MSI results. Controllable nanofabrication is therefore a valuable advantage for SALDI-MSI.

Where the combination of nanomaterial and tissue is not sufficiently sensitive to map a target analyte, further preparative steps can be taken to increase analyte signal including the addition of a non-interfering matrix (matrix enhanced-SALDI-MSI), nanomaterial matrices, chemical derivatization of the target analyte using instrument controlled spraying, and iterative improvements in instrumentation and data workflows. The value of the resulting chemical maps is often high, where MSI is playing an important role in the way drugs are formulated, delivered and how dose responses are measured. MSI is effectively moving into a commercial space, where
pharmaceutical companies have their own infrastructure, or engage an imaging service company, e.g. ImaBiotech, or university affiliated analytical lab service, e.g. HMSTrust Analytical Laboratory of Monash University.

SALDI-MSI has been used to elucidate a range of applications in tracking the in vivo fate of drugs, where it performs well with drug metabolites that become insoluble for other MS applications (Figure 5C). For example, DIOS-MSI was able to track the oral absorption of brominated indoles that have promising anti-inflammatory,\cite{214} and chemo preventative properties;\cite{15f,215} where a proportion of metabolites became insoluble in vivo and would otherwise be unrecoverable for conventional LC-MS analysis, the technique typically used for drug analysis.\cite{15f,207a,215}

Nanostructured matrices have also been optimized for spatial analysis of well-developed drugs including paclitaxel, ortataxel, imatinib, lucitanib, trabectedin and doxorubicin.\cite{216} As MSI drug metabolism improves and moves toward high-resolution imaging, data-rich maps are finding new informatic pipelines for direct annotation from a range of metabolite databases, for example, METASPACE2020.\cite{217}

DIOS-MSI has also been utilized in the search for novel drug leads,\cite{218} where it could detect the spatial biosynthesis of isatin and indole structures in marine molluses and natural products from bacterial interactions, including the nerve growth-factor promoting fellutamides.\cite{219} Spatially characterizing microbial interactions and chemical defense from microbes offers a wealth of opportunity in the drug discovery space, where over half of current pharmaceuticals are derived from microbial sources.\cite{220} Nanostructured surfaces enable the chemical interaction between microbial colonies to be mapped unencumbered, where the interface reveals the chemical secondary metabolites used by bacteria or fungi to disarm or kill a competing colony – secondary metabolites that later often become drug candidates.\cite{221}

7. Conclusions and future perspectives

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7.1. Microneedles

The field of microneedle systems has dramatically grown in the last 25 years, fostered by the convenience of the transdermal route together with the increasing number of biological drugs. Numerous studies have proven the versatility, efficacy, safety, robustness and reliability of microneedle systems for the delivery of molecules otherwise impermeable to the skin. Cosmetics applications pioneered the commercial translation and paved the way to validate the concept, demonstrate user/patient acceptability, and gain regulatory approval. But healthcare applications are currently attracting the great majority of research efforts, with clinical studies that focus on intradermal vaccination, diabetes and anesthesia being the most common. The benefits of using microneedle devices have now been validated by patients, clinicians, and regulators. Academic research has been mostly devoted to tailoring microneedle designs for a variety of applications that depend on the nature of drug, the release kinetics, and desired dose. Challenges that relate to biocompatibility, stability, and limited loading capacity still remain, and can be addressed from the materials science standpoint, where nanotechnology has a major role to play. New materials development should be accompanied by additional investigations on the long-term toxicity and biosafety. Industrial research has prioritized safety, cost-effectiveness, reliability, and device integration. Combined academic and industrial efforts have already resulted in the successful translation of a variety of microneedle products with different designs. But to further accelerate the clinical translation, scientific innovations should be combined with strong commercially focused schemes early in the development process. Healthcare professionals and patients should be actively involved in the device design, learning from products that have failed in the past as the end-users were overlooked in the development process. Microneedle technology is currently building on strong foundations and it is highly anticipated that it will succeed in the commercialization and clinical translation, with vaccination in low- and mid-income countries predictably being the most impactful immediate application.
7.2. Nanoneedles

Smart and functional nanoneedle devices with adaptable architecture are efficient platforms for interfacing with living cells and tissues. The highly tunable mechanical, chemical, and bioelectrical properties of nanoneedle make them unprecedented tools in advanced biomedical applications from disease monitoring, diagnosis, to treatment. Despite the fast development of nanoneedle-based technology, especially NW-FET sensors, from proof of concept in a simple controlled environment to an early phase of target detection in complex patient clinical specimens, several obstacles remain and hinder their practical applications in precision medicine.

First, though substantial studies have been done to demonstrate the capability of nanoneedles in cellular manipulation and genetic modification, which are important for cell-based therapies, most of them have used cell lines or primary cells but in the *ex vivo* context. To fully address the function and usefulness of nanoneedles in clinical applications, more work needs to be done using *in vivo* models or with environment tightly controlled similar to the physiological condition. Second, to benchmark with or outperform a gold-standard technique that is commercially available, a more cost-effective, reliable, and uniform fabrication of nanoneedle devices must be demonstrated. To minimize the impact of variations by current fabrication processes, nanoneedles from different batches must be systematically evaluated through robust multiparameter performance metrics.

Third, for NW-based sensors, individual sensor calibration is required for quantitative analysis, which limits multiplex capabilities of a sensor but allowing better statistical analysis. Integrating NWs into microfluidic systems can enable automated multiplex functionalization and detection, representing a promising path towards clinical applications. However, to date, NW-based sensors have shown low multiplex detection capacity, and the fabrication of sensors with higher multiplex detection schemes is mandatory to sense a diversity of analytes. Last, for potential clinical trials, the nanoneedle devices must meet regulatory requirements to ensure their effective use; and their
performance must be tested in clinical settings and approved for use by the relevant regulatory body before launch (e.g., the FDA in the USA).

7.3. Nanoparticles

There are many lessons that current FDA approved nanomedicine have taught us. VYXEOS has taught us that nanoparticles are here to improve pharmacokinetic profiles of small molecule drugs and the successful delivery of two synergistic drugs at an optimal ratio is made possible using nanoparticles. ONPATTRO and the GalNAc-siRNA conjugates (e.g. GIVLAARI) demonstrated that when nanoparticles are hepatically cleared, delivery into cells utilize cell surface receptor recognition towards targeted cells. Thus, to replicate receptor recognition in other organs or solid tumors, nanoparticle formulations have incorporated targeting ligands into the formulation, although none have been successfully translated into the market. As such, there is still a lot to understand in the translation of nanomedicine into the market, as we are not fully sure of what is the critical framework vital to translation. Factors such as the optimal (i) size of nanoparticle,\textsuperscript{140b, 223} (ii) ligand density,\textsuperscript{224} (iii) therapeutic encapsulation and release kinetics,\textsuperscript{225} or even the (iv) number of nanoparticles dosed.\textsuperscript{149} Each of these parameters hold their own set of questions, nevertheless, only the surface of nanomedicine has been scratched. We can be optimistic that more and more nanoparticle formulations are entering clinical trials in hope of improving patient outcome.

7.4. OOC technology

Despite organ-on-chip technology has only recently emerged, their exceptional potential in drug discovery and disease modeling/research is unprecedented. OOC has not only attracted enormous research interests in academic settings, pharmaceutical industries, regulatory agencies, non-profit organizations and even national defense agencies have collaborated and supported the development of such technology.\textsuperscript{163} It is with confidence to conclude that the OOC has passed the initial proof-of-concept stage demonstrated of its value by the evidence of recapitulating important and realistic
biological functionality and validated with standard drugs. However, to fully integrate OOC into the existing drug development pipeline still requires substantial amount of effort to large scale validation, refinement, and approval from the regulatory authorities. For example, as per regulatory guideline, a novel drug should be tested for its carcinogenicity and reproductive toxicity for drug administration of at least 6 months. The OOC system developed so far has not been demonstrated to be able to maintain cells viable and functional for such long duration of time required for these tests. Nevertheless, with the spawning of OOC startup companies commercializing various types of “organ” chips, we would anticipate the fast track of drug discovery and more accurate drug testing scheme (rather than using animal models) in the near future. Furthermore, combining with the maturation of stem cell technology, personalized “organs” could be made possible and revolutionize the traditional way of drug development.

7.5. Mass spectrometry
The future of nanomaterials in drug development and mass spectrometry is promising, as increasingly there is a need for sensitive analytical approaches to spatially or selectively detect drugs from highly complex environments including tissues. The enhanced ionization and controllable nature of nanomaterials in MS platforms is meeting the demands of drug development specifically in the low molecular weight drug range. Currently, open-platform type nanostructured substrates are commercially available for sensitive MS application; however, as more targeted and sensitive demands are being made for drug and biomarker studies, tailored and simple affinity-based methods could find favour in much the same way that pre-ordered Dynabeads® can be custom-made and sent to researchers. This same customization would also be highly favorable for MSI substrates that selectively enhance analyte type (e.g. phosphorylated-proteins). Additionally, materials that enable multiplex analysis would be attractive, where MSI drug metabolism in tissue, e.g. kidneys, could be co-registered against a spatial multiplex analysis like gene expression mapping – spatial metabolism and its contextually relevant side effects.
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| Product            | Manufacturer         | Type            | Application                                                                 |
|--------------------|----------------------|-----------------|-----------------------------------------------------------------------------|
| Dermaroller®       | DermaSpark, Canada   | Solid metal     | Skin rejuvenation, scar treatment. Enhance drug and cosmetic adsorption     |
| MicroHyala®        | CosMED, Japan        | Dissolvable     | Intradermal delivery of hyaluronic acid to combat skin aging                |
| Macroflux®         | Zosano Pharma, USA   | Coated metal    | Hormonal treatment for post-menopausal osteoporosis                         |
| Qtrypta®           | Zosano Pharma, USA   | Coated metal    | Delivery of zolmitriptan for the treatment of acute migraine                |
| MicronJet600®      | NanoPass, Israel     | Hollow silicon  | Vaccination: pandemic Influenza (H1N1), seasonal influenza, polio           |
| MicroCore®         | Corium, USA          | Dissolvable     | Hormonal treatment for post-menopausal osteoporosis                         |
| Microstructured    | 3M Corp, USA         | Hollow polymeric| Hormonal treatment for post-menopausal osteoporosis                         |
| transdermal patch  |                      |                 |                                                                             |
| Nanopatch®         | Vaxxas, Australia    | Coated polymeric| Influenza vaccine delivery                                                  |
| Dissolvable microneedle patch | Micron Biomedical, USA | Dissolvable | Influenza and measles-rubella vaccination; contraceptive drug delivery       |
Figure 1. A) Methods of drug delivery to the skin using microneedles, adapted with permission.\textsuperscript{[3d]} Copyright 2012, Elsevier. B) Zonsano’s coated microneedle delivery system: applicator (left), 5 cm\textsuperscript{2} adhesive backing with microprojection arrays (3 cm\textsuperscript{2}) (middle) and micrograph of solid microneedles arrays coated with zolmitriptan (right), adapted with permission.\textsuperscript{[48]} Copyright 2020, Future Medicine Ltd. C) 3M hollow microstructured transdermal system, adapted under CC BY license.\textsuperscript{[49]} Copyright 2010, The Authors. D) Vaxxas Nanopatch\textsuperscript{®} applicator, adapted with permission.\textsuperscript{[14a]} Copyright 2019, Elsevier. E) Micron’s dissolvable microneedle patch on adhesive backing, adapted with permission.\textsuperscript{[34b]} Copyright 2019, Springer Nature.
Figure 2. Vertical nanostructures in early clinical applications. A) (i) FIB-SEM cross sections of a nanoinjected cell by pSi-nN (up) and 3D reconstruction FIB-SEM slice through segmentation (down), nanoneedles are in blue, cell membrane is in purple, nuclear envelope is in yellow; (ii) SEM image of a pSi-nN array (up) and sensing of intracellular and extracellular pH for OE33 cells (down), cells (nucleus in blue, membrane in magenta); (iii) FIB-SEM cross sections of cells nanoinjected with QDs by pSi-nN (up) and fluorescent live imaging of the nanoinjection site (down). B) (i) TEM cross section of the muscle tissue treated with pSi-nN. Red arrows indicate QD accumulations; (ii) Fluorescent live imaging of the muscle (left) and skin (right) QD nanoinjection sites; (iii) Fluorescent live animal imaging of the QD nanoinjection sites for muscle and skin at.

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different time points (before nanojection to up to 100 h); (iv) Quantification of fluorescent intensity as in (iii) showing the amount of QDs dispersed away from the delivery site as a function of time. Adapted with permission. [70a] Copyright 2015, ACS. C) Schematics of the multifunctional BNS-electroporation system for capture of CTCs, followed by in situ intracellular drug delivery and intracellular contents extraction. The BNSs were modified with specific biomolecules, anti-EpCAM, to specifically capture CTCs, followed by integration with a microfluidic nanoelectroporation system for nondestructive cell poration. Adapted with permission. [77c] Copyright 2019, ACS. D) (i–ii) FET p- and n-type NW sensors exhibit a decrease or increase in conductance upon signal transduction in response to interaction of immobilized antibody with a soluble antigen (in red); (iii) Field of application of three types of NW-based sensors. Adapted with permission. [92a] Copyright 2018, Elsevier Ltd. E) (i) SEM image of a SiNW with width ~60 nm; (ii) Schematics of a SiNW-FET integrated into PDMS chip with two macro-scale solution chambers to allow simultaneous sensing; (iii) Schematics of label-free multiplexed electrical detection of CEA and miRNA-126. Adapted with permission. [103] Copyright 2017, Elsevier B.V. F) (i) False-colored angle-view SEM image showing cell morphology and neurite outgrowth to nearby NWs; (ii) Recordings showing the positive (~20 mV p-p, up) and negative (~10 mV p-p, down) measured signal. Adapted with permission. [116] Copyright 2017, ACS. G) (i) Top-view and (ii) side-view SEM images of Au–TiO$_2$ NW arrays; (iii) SEM images of the interface between the retina and NW arrays; (iv) Schematics of an eye (left), the multilayer of neural cells of a retina (middle), and NW arrays-interfaced blind retina that lacks photoreceptors (right). The necrotic photoreceptor layer (rod and cone cells) in the blind retina is replaced by an Au–TiO$_2$ NW array as artificial photoreceptors. Adapted under CC BY 4.0 license. [112] Copyright 2018, The Authors. H) (i–iii) SEM of the hSPIKE arrays with different pitches (i, 500 nm; ii, 1 μm; iii, 4 μm); (iv) Photograph of the flexible hSPIKE patch and schematics of the transplantation of hSPIKE patch into in vivo animal models and human beings; (v) Schematic of the enhanced stem cell function by secreting growth factors; (vi) Representative fluorescence microscopy images showing DAPI (blue) and osteopontin (green) immunostaining of the regenerative cranial bone on the flat (left) and hSPIKE patches (right), indicating the enhanced osteogenesis by the patch. Adapted with permission. [113] Copyright 2019, ACS.
Figure 3. A) Number of FDA-approved nanoparticles in the market as of 22/10/2020. B) Kaplan-Meier estimates on the median overall survival comparing patients treated with VYXEOS (CPX-351) or the 7+3 study control regimen (cytarabine infused continuously for 7 days with 3 once-daily injections of daunorubicin). Adapted with permission.[128] Copyright 2018, Wolters Kluwer Health, Inc. C) Mechanism of action of ONPATTRO.[131]a Briefly, after systemic administration, the lipid nanoparticles are opsonized by apolipoprotein E (ApoE). When cleared to the liver, the lipid nanoparticles bind to ApoE-binding cell surface receptor (e.g. low-density lipoprotein receptor (LDLR). The lipid nanoparticles are endocytosed, in which the nanoparticles disrupt the endosomal membrane and release siRNA into the cytoplasm. The endogenous RNA interference (RNAi) pathway via the RNA-inducing silencing complex (RISC) causes the degradation of TTR (transthyretin) mRNA reducing the production of TTR protein.
Figure 4. A) Evolution timeline of the complexity of cell culture platforms. Adapted with permission.[226] Copyright 2016, Elsevier. B) Human organ-on-chip platforms have been explored to mimic the human organs. Adapted with permission.[227] Copyright 2018, RSC.
Figure 5. Nanomaterials used in drug development related MS and the drug imaging technique, MSI. A) Three major material bases for particle fabrication (semi-conductor, carbon and metal) with examples of well published particle types used in MS workflows. B) Range of both tailored and commercially available nano-substrates for MS, which can be integrated into the workflow for MSI in drug development applications. C) Tissue from model studies, e.g. knock-out mice (KO) vs wild-type (WT), or clinical samples can be prepared via cryo-sectioning and mounted onto substrates for surface-assisted (SA)-LDI or MALDI analysis, with or without derivatization. MSI can be achieved in imaging capable MALDI-TOFs, MALDI-HRMS or in atmospheric pressure (AP)-MALDI units couple to a variety of mass spectrometers. Drug maps are generated that can be compared to endogenous metabolites or directly to H and E stained histology sections to give spatial context. Adapted with permission. Copyright 2018, Wiley; 2015, RSC; 2013, Wiley.
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The review discusses the use of engineered nano–bio interfaces – driven by vertical micro-/nanoneedles, nanoparticles, organ-on-chip devices, and a diversity of nano-substrates for mass spectroscopy imaging, which are spurring scientific and technological progress from fundamental to transnational biomedical research. The review also highlights main rules in designing bio–nano interfaces that have successfully achieved translation into the market.

Yaping Chen gained her Ph.D. degree in immunology in 2017 from the School of Biomedical Sciences, Monash University, and has since been a research fellow at the Monash Institute of Pharmaceutical Sciences. Her expertise is in the interdisciplinary field of nano–bio technology, especially the development of novel, smart, and functional nano–bio interfaces for genetic modification and cellular immunotherapy.
Roey Elnathan gained his Ph.D. in chemistry in 2012 at Tel Aviv University. He was a Research Fellow in nanobiotechnology, UniSA (2012–2015) and a Foundation Fellow in nanobiotechnology, UniSA (2015–2017). He is now a Senior Research Fellow (DECRA Fellow) in the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University, Australia. His research interest lies in the field of engineered nano–bio cellular interfaces, especially in the fabrication and design of nanotopographies optimized to maximize bioactive cargo delivery and orchestrating specific cellular processes.

Dr Anna Cifuentes-Rius is an NHMRC Early Career Fellow at Monash University. She has established an international and multidisciplinary research program with a focus on both fundamental and applied nanomedicine. Her research aims to understand the interactions between nanomaterials and biological tissues with the ultimate goal of improving current therapy and diagnostic approaches. She has been trained at the Massachusetts Institute of Technology, University of Queensland, and University of South Australia. In 2013, she completed her PhD at Institut Quimic de Sarria (Ramon Llull University, Barcelona). Among her passions are empowering young researchers and promoting equitable leadership in science.

Prof. Nicolas Voelcker is the Scientific Director of the Melbourne Centre for Nanofabrication, Professor at the Monash Institute of Pharmaceutical Sciences at Monash University, and Science Leader at the Commonwealth Scientific and Industrial Research Organisation (CSIRO). His key research interest lies in the fabrication and surface modification of silicon nanomaterials for applications in biosensors, biochips, biomaterials, and drug delivery. A core research activity in his laboratory is the study of porous silicon–based nanostructures and their surface chemistry.