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Exploring the potential of microfluidics for next-generation drug delivery systems

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ABSTRACT

The platform of microfluidics offers a precise control and manipulation over fluids at a small scale and therefore has gained much attention in recent times. This topic is currently applied to automation and high-throughput analysis in several areas, including extraction of DNA, RNA and proteins, gene identification, gene assembly, cloning, single-cell analysis, organs grown on chips, PCR, drug screening, toxicity testing and drug delivery. Conventional methods used for drug delivery are sometimes non-targeted leading to loss of administered drugs and reduced drug effectiveness. Recent advances in microfluidics allow precise dose-dependent delivery of a drug to a targeted location. Several microfluidics designs have been implemented to improve the precision of treatment in clinics. This review highlights currently available tools in microfluidics, designs for drug carriers, delivery methods, robotics and artificial intelligence in the field of microfluidics.

1. Introduction

Microfluidics is a rapidly growing area of study and a powerful tool with a wide range of applications covering the basic sciences to biomedical and translational research. It finds applications in gene detection, cloning, diagnosis, drug discovery and high-throughput screening, toxicity testing, analysis of single cells, and drug delivery [1–5]. Microfluidics chips are usually composed of polydimethylsiloxane or dimethicone, a transparent silicon polymer that is non-reactive, non-flammable and nontoxic [6,7]. Microfluidics drug delivery systems have the potential for improving the treatment of human diseases, both infectious and non-infectious [8,9]. In a study, Zhao et al. [10] reported that microfluidics devices can easily generate monodisperse emulsions of one or more droplets with precise control. Such devices have been used to fabricate complex systems of microparticles, microgels, and microcapsules of uniform

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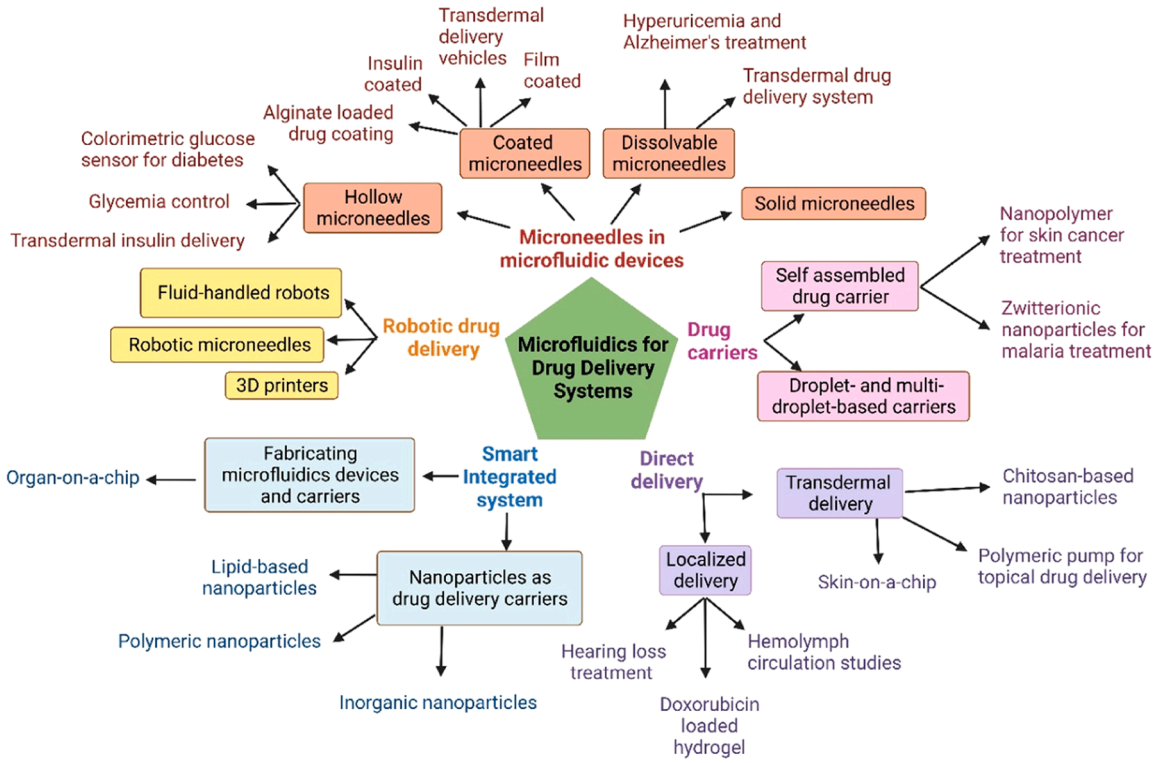

Fig. 1. An overview of the structure of the review encompassing microfluidics-driven drug delivery systems. Various types of microneedles such as solid, coated, dissolvable and hollow microneedles have been integrated into microfluidic channels for drug delivery purposes in many diseases. Drug carriers are mainly divided into self-assembled and droplet-based carriers. Microfluidics-based drug delivery systems can be used to deliver the drug localized or transdermal way. In a smart integrated system of microfluidics, the microfluidic devices can be converted into organ-on-a-chip that replicate tissue and organs for a better understanding of delivery systems. Moreover, different microfluidic nanoparticles such as lipid-based, polymeric and inorganic nanoparticles have also been developed for better drug delivery. Applications of robotics in microfluidics drug delivery include fluid-handled robots, robotic microneedles and 3D printers. The figure was created with Biorender.com.

size, precise distribution, and other desirable properties that impart great potential for use in drug delivery systems.

Given requirement of very small amounts of reagents and the ease of use, microfluidics devices have gained much research interest. These systems offer several advantages for targeted and precise drug delivery *in vitro, in situ*, and *in vivo* as compared to the conventional approaches [11,12]. It can precisely control the drug delivery to ensure that the therapeutic is released at the targeted site at the desired rate. Integrating various components of drug delivery systems into single microfluidics chips have shown to ensure a better control over drug release and ultimately disease treatment [13].

Microfluidics-based drug delivery is also used to administer therapeutics to specific cells or tissues and to tabulate a therapeutic index for use in personalized medicine [14,15]. Although *in vivo* and *in situ* microfluidics-based drug delivery systems are in the developmental stage, further development is expected to enable drug delivery to different organs or tissues [11,12,16]. This review highlights recent and breakthrough advances encompassing major aspects of microfluidics systems playing significant roles as drug carriers, delivery vehicles and efficient lab-on-chip devices. This review is categorized into five major aspects of microfluidics based on the kind of microfluidic devices and the application of these devices in point-of-care applications (Fig. 1). The review also aims at simplifying the concepts of basic operations, fabrication, manipulation, automation, packaging and portability of the microfluidics devices for the readers. The inclusion of robotics and artificial intelligence in microfluidics devices used to deliver drugs for precise treatment of infectious and non-infectious diseases and their implications in healthcare and medicine have been instrumental, and therefore, are included as separate sections in the review. These have been further evaluated to provide a viewpoint on the future of microfluidic devices and their routine integration in biomedical applications. Whether used alone or in combination with other devices, these tools serve as remarkable resources to carry out multiple biological assays and state-of-the-art platforms mimicking conditions similar to the biological systems at the microscale levels. The study concludes with suggestions for future research to maximize the use of microfluidics platforms in clinical application.

Table 1
Summarizing some case studies on Self-assembled drug carriers.

Object	Research purposes	Reference
Polymeric micelles	mPEG-hexPLA polymeric micelles for the diagnosis and treatment of skin cancer	[19]
Amphiphilic zwitterionic nanoparticles	To target *Plasmodium*-infected cells for delivering antimalarial drug orally	[20]
Peptide self-assembled nanostructures	Peptide self-assembled nanostructures for drug delivery applications	[22,24]
Lipids and lipopolymers self-assembled	Self-assembled multicompartment liquid crystalline lipid carriers for protein, peptide, and nucleic acid drug delivery	[26]
Stimulus-responsive dipeptide-based self-assembled nanoparticles	Cysteine-phenylalanine-derived self-assembled nanoparticles as stimulus(glutathione)-responsive drug-delivery systems	[28]
Nontoxic crosslinker-free drug-loaded protein nanoparticles	Self-assembled non-covalent protein-drug nanoparticles: an emerging delivery platform for anti-cancer drugs	[29]

2. Microfluidics designs for drug carriers

2.1. Self-assembled drug carriers

Self-assembled drug carriers are one of the most important and potentially useful methods currently being developed [17]. Of particular interest are nanostructured drug delivery systems, which includes the benefits of the ease of design change, allowing controlled accuracy and speed of drug release, and most importantly, the ability to carry several therapeutic agents to specific targets in the body [18]. In 2019, Lapteva and colleagues conducted research with the methoxy-polyethylene glycol (mPEG)-hexPLA nanopolymer micelles to aid the diagnosis and treatment of skin cancer, wherein, it was observed that the combination of mPEG-hexPLA and imiquimod at the rate studied was superior to that of the commercial formulation being used clinically, and they were able to find the ratio that improved the effectiveness, efficiency, and compatibility of the drug when treating a patient's epidermis and dermis [19]. Another study used zwitterionic-assembled nanoparticles primarily composed of poly butyl methacrylate-co-morpholinoethyl sulfobetaine methacrylate are created to specifically recognize and target *Plasmodium* as demonstrated on *Plasmodium*-infected cells for oral treatment of malaria [20]. Pharmaceuticals use self-assembled nanopolymers to deliver bioactive substances to the body; common forms of which include polymers, nanogels, and polyelectrolyte complexes [21]. Some case studies on self-assembled drug carriers are summarized in Table 1.

Self-assembled nanostructures composed of natural or synthetic peptides resembling different shapes and sizes are used for a wide range of biomedical applications as in biosensors, bioimaging, tissue regeneration, and drug administration. The simple structures of self-assembled peptide nanostructures aid a better understanding of complicated biological systems and underlying mechanisms [22]. To suit the purpose of drug delivery, the most sought properties expected of self-assembled peptide nanostructures are to be biocompatible, biodegradable and multifunctional [23]. Peptide self-assembled nanostructures have the upper hand in drug administration over other organic materials because of their intrinsic physical and biological characteristics. In a study supporting this fact, eight-residue cyclic peptides involving Glu-and Cys-amino acids were used to generate cyclic self-assembled peptide nanotubes to deliver drugs. According to the findings, PEG-modified nanotubes loaded with doxorubicin demonstrated a better drug encapsulation ratio as compared to their non-modified counterpart. Those modified drug-loaded nanotubes were also able to exhibit higher cytotoxicity and enhanced doxorubicin absorption in human breast cancer MCF-7/ADR cells *in vitro* as compared to the free doxorubicin. The PEG-modified doxorubicin-loaded nanotubes showed potential in multidrug resistance tumor therapy [24].

Multicompartment lipidic nanocarriers offer efficient encapsulation of biopharmaceuticals. The phospholipids nanoparticles encasing therapeutic proteins, in turn called liposomal nanomedicines, are amongst the most cutting-edge classes of drug delivery systems [25]. The hierarchical architecture of such nanochannel compartments are usually partitioned by lipid bilayer membranes which facilitates improved encapsulation of biomolecules and promotes their delayed and steady release. Based upon their nature and function, small-sized proteins can be held and protected either in the aqueous compartment (water-soluble proteins) or in the compartment separated by the lipid bilayer (membrane proteins) and released accordingly [26]. Reports from forefront study on long-circulating phospholipid nanoparticles employed for gene delivery has seen success with sterically stabilized (PEGylated) lipid/DNA lipoplex nanoparticles [27]. Development of lipid-based nanocarriers with tunable internal organization and nanochannel sizes could well direct and accelerate the projected clinical applications sooner because of the growing interest in studying particulate lipid carriers with interior multicompartment structures.

Presently, objects showcasing responsiveness to biological cues are particularly of great interest in the area of drug delivery. A subtle redox difference exists between the intra- and extracellular environment which could be rewarding to achieve site-specific cell-triggered drug delivery if harnessed properly. In an attempt to develop cancer-targeting redox-responsive nanoparticles, GSH-responsive self-assembled NPs were synthesized from oxidized cysteine-phenylalanine (CFO) dipeptides [28]. These dipeptide nanoparticles displayed GSH-responsive behavior because of the disulphide linkage between the two dipeptides (CF-CF) molecules. Besides, the availability of free amine and carboxyl groups proved advantageous in adorning tumor-targeting ligands, for example, folic acid (FA). Nanoparticles conjugated with FA precisely targeted cancer cells, wherein the risen glutathione levels within the cancer cells led to the disintegration of particles. Anticancer drug doxorubicin was packed within the disulfide-linked nanoparticles (CFO--Dox-NPs), which upon disintegration of the particles resulted in the site-specific release of the loaded anticancer drug inside the cancer cells, thereby demonstrating stimuli-responsive drug delivery. The drug-delivery was attempted in both non-cancer and cancer cells, wherein enhanced absorption of FA-derivatized NPs (FA-CFO—NPs) was observed in cancer cells- C6 glioma and B16F10 melanoma

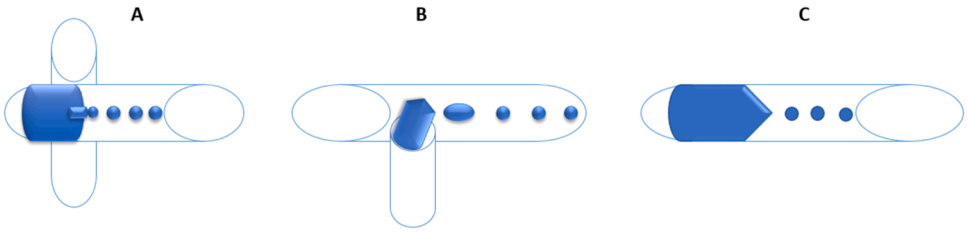

Fig. 2. Flow joints in microfluidic systems (A) Flow focusing junction (B) T-junction (C) Co-flow junction.

cells as against normal HEK293T cells considering the overexpression of FA receptors on the surface of cancer cells [28].

2.2. Droplet- and multi-droplet-based carriers

Droplet-based microfluidics is built on the principle of compartmentalization and is often used to control small volumes [30]. This technique has the advantages of easy production and adaptability and facilitates enhanced exposure to a wide variety of biological and chemical reagents [31]. The basic principle in droplet formation is the dispersion of one phase into another immiscible phase [32]. Three types of devices are commonly seen in use: flow focus joints, T-joints, and co-flow joints. A flow focus joint resembles the one shown in Fig. 2A: the blue dispersed phase moves from left to right and is intersected perpendicularly by the transparent phase flowing at a high flow rate [31]. The droplets then break into smaller pieces, the size of which can be adjusted by varying the flow rates of the two processes (Fig. 2**A**) [33]. The second common structure is the T-junction (Fig. 2**B**). In the example structure shown in Fig. 2B, the blue dispersed phase moves in the vertical direction, while a horizontal, transparent, continuous phase is sheared by the action of surface tension. Those molecules undergo vaporization to form droplets. The co-flow technique is often used to form core-shell structures based on the principle of droplet aggregation with a drain junction (Fig. 2**C**). All three junction types can be used to create complex microfluidics devices, and are all used in the drug-delivery design.

3. Microneedles in microfluidics devices for efficient drug delivery

Advances in materials science and biomedical technology have opened up newer avenues for personalized medicines. One of the most important achievements is miniature-sized microneedles, mostly used for drug delivery purposes [34]. These microneedles have been successfully embedded and tested in microfluidic devices for drug delivery and biomarker detection [35]. Depending upon the property of microneedles, these are divided into solid, coated, dissolving and hollow microneedles.

3.1. Solid microneedles

Solid microneedles (MNs) were first reported in 1971 for use as a method of skin pretreatment by creation of pores [34]. A sharp needle penetrates the skin, creating micron-sized channels and delivering a drug that is then absorbed by the capillaries [36]. The advantage of this method is the safety of transporting and introducing drugs into the body, limiting the potential for infection or introduction of harmful substances because the MNs form extremely small pores that close after the needle is withdrawn [36]. Solid MNs can be made of various materials such as stainless steel [37], silicon [38], metal [39], and polymer [40], and manufactured using laser micro-machinery [18], micro-molding [1] and lithography, and sculpting [41]. Integration of solid MNs in microfluidic channels can deliver easy drug loading and delivery mechanisms [42–44]. With the help of various material chips and microneedles made of polydimethylsiloxane (PDMS), SU-8, silicon, and tungsten, these fabricated advanced devices have demonstrated their uses as a delivery carrier in inflammation treatment [42], for real-time protein detection [43] and for detection of cells in suspension [44].

3.2. Coated microneedles

Coated MNs have sparked immense interest among researchers because they can deliver biomolecules such as proteins and DNA with minimum invasion. But there is a lack of research on coating processes and applications [45]. To explore the utility of coated MNs as transdermal drug delivery vehicles, Liang et al. [46] developed four dip-coating approaches using a dam board, roller, fixture, and limit. They established that the amount of drug that could be loaded onto an MN prepared using those approaches was 15–16 ng, and that the fixture device method produced the lowest deviation of drug loading, around 12.3%. Their *in vitro* drug delivery analysis showed 90% drug loading efficiency of the designed MNs. They affirmed that dip-coating with a fixture could be useful for homogeneous drug loading and was suitable for mass production [46]. Certain issues remain unresolved, such as dose control, homogeneity, and suitability for mass production. To address those issues, Chen et al. [47] developed a novel protocol that used micro-molding to produce coated MNs on a large scale with good uniformity and controlled drug loading. First, they affixed soluble drug carriers to MNs at a specific dose, and then they subjected them to moist conditions to achieve homogeneity. Their mechanical property analysis established that the strength of the coated MNs was suitable for skin penetration, and their *in vivo* trials with diabetic mice showed that insulin-coated MNs had hypoglycemic effects similar to those of subcutaneous injections. Therefore, the micro-molding technique

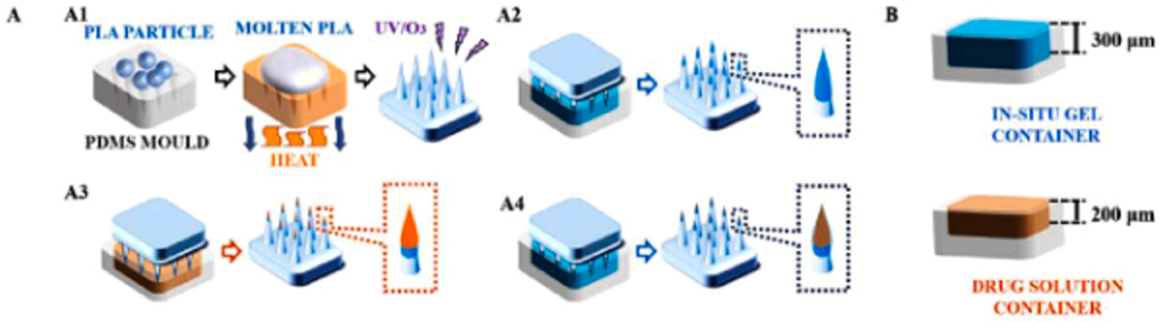

Fig. 3. Schematic diagram of GEC-MN fabrication. (A) The procedure to fabricate the GEC-MNs were as follows: (A1) production of PLA-MN base, (A2) inner gel encapsulation coating, (A3) drug solution coating, and (A4) outer gel encapsulation coating. (B) the depth of the solution containers is 300 µm for the sodium alginate *in situ* gel and 200 µm for the drug solution. The figure was adapted from Zhou et al. [48] copyright © 2021 Elsevier B.V.

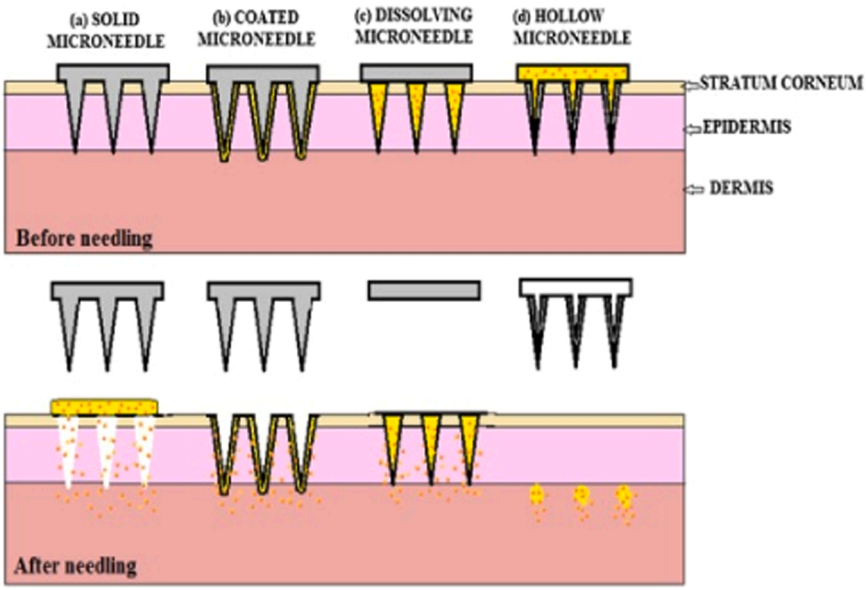

Fig. 4. Types of microneedles (a) Solid microneedles use the poke and patch approach for pretreatment of skin; (b) Coated microneedles use the coat and poke approach by injecting a needle surface-coated with a drug solution; (c) Dissolving microneedles are made of biodegradable polymers; (d) Hollow microneedles are filled with a drug solution and deposit the drug in the dermis. The figure was adapted from Waghule et al. [34] copyright © 2019 Elsevier B.V.

could be used for large-scale production of homogeneous, coated MNs with controlled drug loading [47].

Zhou et al. [48] tested the sustained release of a water-soluble drug from coated MNs with gel encapsulated–coated MNs (GEC-MNs) that enveloped a water-soluble drug in a sodium alginate coating. The complexation enhanced the efficiency and stability of drug delivery [48] **(**Fig. 3**).**

Gao et al. [49] designed a unique poly (ethylene glycol) diacrylate (PEG-DA) MN coated with gelatin/sucrose film to effectively enhance the skin penetration of drugs. Initially, a prepolymer solution was introduced into the mold cavity, and then an MN patch coated with a film was synthesized through photo-induced polymerization. The MNs were tested with rhodamine B, bovine serum albumin, doxorubicin, and indocyanine green to ascertain their delivery and therapeutic effects. This fabrication approach for film-coated MNs was simple and rapid [49].

The fabricated microfluidics including silicone MNs coated with Cr/Au was successfully tested for their chemical delivery capability. The designed microfluidics have also recorded neural signals when injected *in vivo* animal models suggesting its potent role in neuroscience research [50]. Similarly, a PDSM material chip coated with gold and an enzyme layer integrated above the chamber has demonstrated its effectiveness as a glucose and lactose biosensor [51]. In another study, novel SU-8 resin-coated MNs were incorporated above the microfluidic channels for applications including drug administration and body fluid collection [52].

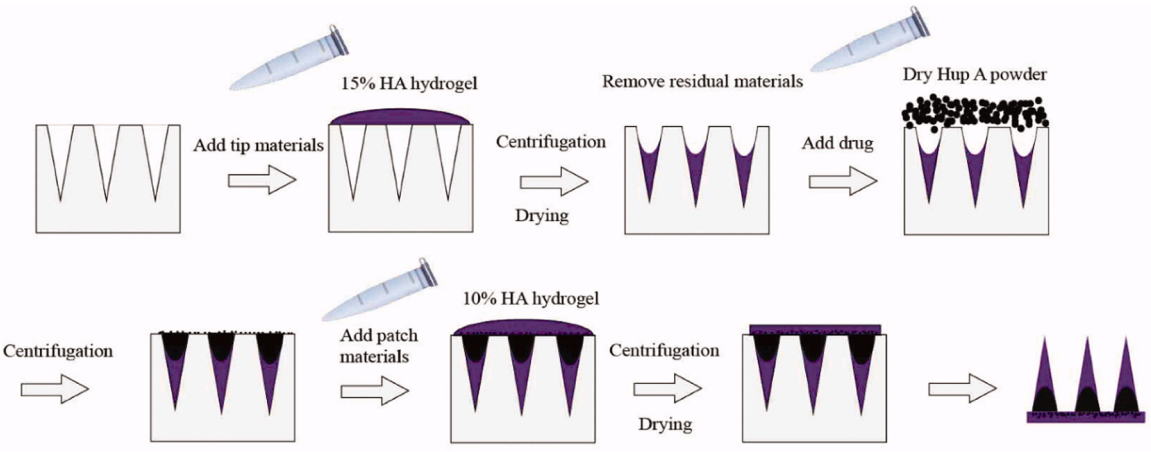

Fig. 5. Schematic illustration of Hup A-HA microneedle preparation process. The figure was adapted from Yan et al. [55] copyright © 2020 Informa UK Limited.

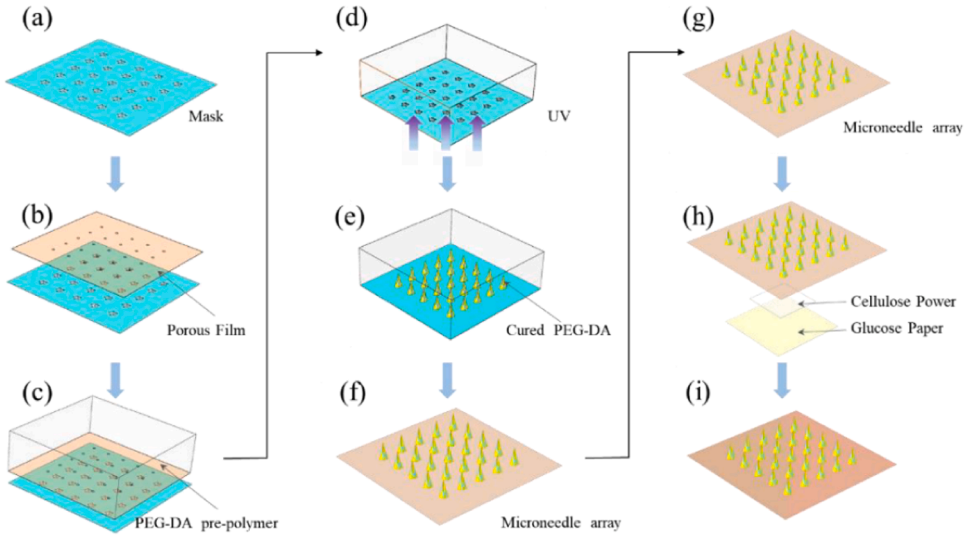

Fig. 6. Fabrication of MN patches. (**a**) A PEG-DA film with micro-holes. (**b**) The PEG-DA film was aligned with a photomask. (**c**) The PEG-DA solution was combined with the film and photomask. (**d**–**f**) UV exposure through the photomask produced MN arrays on the PEG-DA films. (**g**–**i**) An MN array was integrated with glucose test paper, and cellulose powder was added in the middle. The figure was adapted from Wu et al. [57] copyright © 2022 MDPI (Basel, Switzerland).

3.3. Dissolvable microneedles

Dissolvable MNs are made of biodegradable polymers within which drug is encapsulated. After the MN penetrates the skin, the drug is released. Because this process does not require MN removal, it offers the simplicity of a single-step drug release controlled by polymer degradation [34] **(**Fig. 4).

A two-step casting protocol to synthesize tip-loaded MNs/layered MNs was developed by Jian Zhuang et al. to enhance the drug consumption rate and decrease waste. The MN tip was made of rhodamine B to simulate a drug, and the backing was designed using soluble materials such as polyvinyl alcohol, polyvinylpyrrolidone, and hyaluronic acid. The structural preparation and insulin delivery efficiency were analyzed. The authors established that their tip-loaded dissolving MNs could reduce waste and improve drug delivery efficiency by 30% [53]. Allopurinol is a first-line treatment for hyperuricemia and gout, but it poses side effects such as the hepatic first pass effect. Therefore, Chen et al. [54] developed allopurinol-encapsulated dissolving MNs to achieve constant drug release and prevent the hepatic first pass effect, which could enhance the bioavailability of the drug. They fabricated their devices by casting a suspension solution with a regulated dosage. *In vitro* and *ex vivo* analyses established that allopurinol dose had no effect on release of the dissolving MNs and offered higher drug delivery efficiency than existing methods. Their pharmacodynamic analysis ascertained that the dissolving MNs achieved a constant release and anti-hyperuricemia effect [54]. Certain drugs, such as Huperzine A, which is

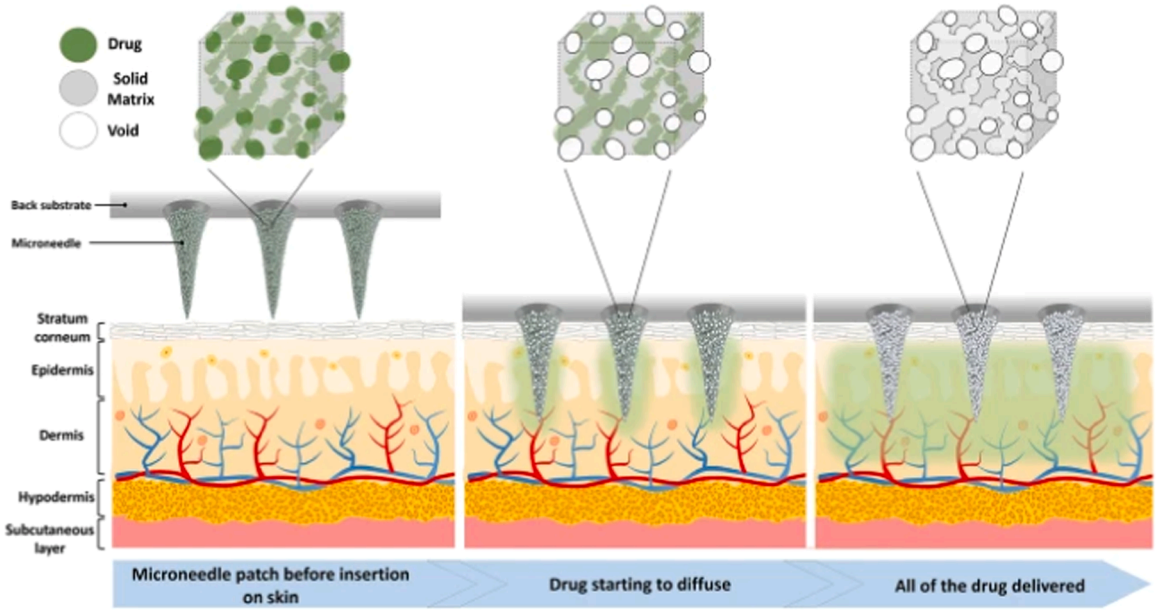

Fig. 7. Schematics of polymeric porous microneedles with solid drugs embedded within the solid matrix before insertion into skin, after insertion into skin, and after sustained release. The figure was adapted from Sadeqi et al. [61] copyright © 2022 Springer Nature Limited.

prescribed to treat Alzheimer's disease, have low bioavailability. Therefore, Yan et al. [55] developed dissolving MNs to deliver Huperzine A. Their *in vitro* analysis showed that more than 80% of the total Huperzine A penetrated the skin over a span of three days, demonstrating constant drug release [55] **(**Fig. 5**)**.

Droplet microfluidics was exercised through photolithography on a PDMS-based chip. In this microfluidic device, HA-PG microdroplets were fabricated and explored for sustained drug release [56].

3.4. Hollow microneedles

Hollow MNs contain a cavity and tilted opening for use in drug delivery or to extract interstitial fluid from skin. They offer advantages over solid MNs by improving drug delivery. They are usually fabricated from glass, metal, silicon, ceramic, or a micro-electro-mechanical system (MEMS). Silicon hollow MNs offer exceptional physical properties but pose drawbacks in the form of raw material costs and a tedious synthesis process. Wu et al. [57] developed an approach to fabricate hollow MNs through photolithography and combined their MNs with glucose paper to sample and analyze skin interstitial fluid, creating a colorimetry glucose sensor that could be useful for diagnosing diabetes **(**Fig. 6**)**.

A unique device known as 3DMNMEMS was developed by Economidu et al. [58]. The device integrated 3D printing and MNs with MEMS. These were the first hollow MNs to be fabricated using 3D printing via stereolithography and incorporated into MEMS. *In vivo* analysis showed that insulin delivered through the 3DMNMEMS enhanced control over glycemia in diabetic animals compared to injections [58]. MNs can deliver numerous drugs through skin penetration, but they lack the force required for drug delivery within tissues. To resolve that issue, Chen et al. [59] used a 3D-printed ultrasonic MN array device comprising hollow MNs and an ultrasonic transducer, which facilitated the force required for the hollow MNs to deliver drugs within tissues. Their drug delivery tests established that the ultrasonic MN array device improved drug delivery efficiency. Moreover, it decreased the force required to insert the MNs, which also decreased tissue damage [59]. Xenikakis et al. [60] designed hollow MNs through vat polymerization to deliver insulin transdermally. Computer-aided design software was used to fabricate hollow MNs with two geometries, a curved pyramid and a syringe-like shape. They found that the shapes of MNs play a role in insulin transportation [60].

Various drawbacks are associated with MNs, such as penetration stability and complications in developing large, flexible MN arrays. Moreover, only certain kinds of drugs can be loaded to specific limits. To address those drawbacks, Sadeqi et al. [61] fabricated polymer-based hollow MNs using biocompatible, photo-curable resins. Those MNs have the unique capability of carrying solid, concentrated anesthetic and non-steroidal anti-inflammatory drugs such as lidocaine and ibuprofen. Various studies have shown promising results for transdermal drug delivery over a large area (Fig. 7).

Hollow MNs pose limitations because they are tedious to synthesize. Therefore, Ghate et al. [62] proposed polymeric hollow MNs through single-step drop-casting on pyramidal stainless-steel needles, which enabled them to achieve hollow MNs of a desirable height on an acrylic base. To deliver multiple doses, the hollow MN base was incorporated in a pack that could deliver 5 μl of drug upon every 30° clockwise rotation. This system was used to deliver vitamin B12 *ex vivo* and insulin *in vivo*. The study showed potential for mass production of hollow MNs and a pack with potential as a wearable device [62].

Hollow MNs embedded microfluidics was used for nonenzymatic glucose measurement for diabetic patients [63]. In another

Table 2
Microneedles-based microfluidics systems reviewed with microneedle type, fabrication method, material of chip/microneedle and its application.

MN type	Fabrication method	Chip Material	MN material	Application	Reference
Solid MNs	Microfabrication	PDMS	SU-8	Inflammation treatment	[42]
	Microfabrication	Oxide layer + metallic layer	Silicon	Label free real-time protein detection	[43]
	–	PDMS	Tungsten + parylene	Detection of cells in suspension	[44]
Coated MNs	Microfabrication	PDMS	Silicon + Cr/Au	Chemical delivery	[50]
	Microfabrication	PDMS	Gold + enzyme layer	Glucose and lactate sensor	[51]
	SU-8	PDMS	SU-8 resin	Drug delivery and body fluid sampling	[52]
Dissolvable MNs	Photolithography	PDMS	HA-PG	Sustained drug releasing	[56]
Hollow MNs	Microfabrication	–	–	Nonenzymatic glucose sensor	[63]
	Direct laser writing	PMMA	Photosensitive material	Fluid injection and extraction	[64]
	3D printing + DRIE	PDMS	Glass	Localized microinjection	[65]

MN, microneedle; PDMS, polydimethylsiloxane; HA-PG, pyrogallol-modified hyaluronic acid; PMMA, polymethyl methacrylate; DRIE, deep reactive-ion etching.

application, photosensitive material hollow MNs integrated above the microfluidic channel was used as a system for fluid injection and extraction. As a proof of concept, a solution of rhodamine B was used for proving the functionality [64]. Hollow MNs have even been explored for localized microinjection and heart monitoring of intact fruit flies. In this study, a neurotransmitter drug, serotonin, was injected close to the heart chamber and the heartbeat was monitored [65]. All mentioned studies incorporating microneedles in microfluidics systems are summarized in Table 2.

4. Microfluidics for direct drug delivery

Effective drug delivery is needed because it can significantly increase the bioavailability and bioaccessibility of drugs and can also reduce side effects. Microfluidics technology has revolutionized biochemical studies. Advances in microfluidics technology are bringing new insights to both academic and industrial scientists. Microfluidics technologies use nano- and microscale fabrication methods to develop highly controllable and reproducible fluidic microenvironments [66]. Microfluidics technology is a promising technique to improve the delivery of nanomedicines and nanoparticulate drugs [67]. One possibility is tiny chip-scale devices that offer precise drug delivery to a target at a specific dosage with controlled release [68]. Microfluidics can significantly enhance the pharmacokinetics and pharmacodynamics of drugs [69]. Depending on the application and functional characteristics, microfluidic devices can take forms of a lab-on-a-chip [70,71], a microreactor [72], or an organ-on-a-chip [73,74]. Again, depending on the application, microfluidics chips can be prepared using a wide range of materials and fabrication methods [75]. Two methods of drug delivery, localized and transdermal approaches, are discussed in detail.

4.1. Localized drug delivery

Localized drug delivery is an effective form of disease treatment in many organs, including the eyes, ears, and nose. Despite numerous physiological and anatomical barriers, unique clearance pathways and insights about those organs can be used to control the effectiveness of drug delivery. For example, to treat hearing loss, Wang et al. [76] used well-designed microfluidics techniques to develop dexamethasone sodium phosphate–encapsulated gelatin methacryloyl (Dexsp@GelMA) microgel particles as otic drug delivery vehicles with finely tunable size. Their findings suggest that adhesive and intricate microfluidics-derived GelMA microgels can be effective in drug delivery.

A study used intact *Drosophila melanogaster* larvae at different developmental stages to demonstrate localized microinjections and heart monitoring through a novel hybrid microfluidics device. Those researchers used a MATLAB-based heartbeat quantification technique to examine the effects of a neurotransmitter (serotonin, 0.01 mM) and it was observed that it significantly increased heart rate. Their results suggest that the heartbeat monitoring technique and microfluidics injection can be used for hemolymph circulation studies, dye angiography, and *in vivo* screening of intravenous drugs in *Drosophila melanogaster* [65].

Hydrogels are extremely porous networks of hydrophilic polymer chains that allow diffusion of small molecules and bioparticles [75,77]. Hydrogels offer biocompatibility, low cytotoxicity, biodegradability, controllable pore size, high permeability, and compatibility with aqueous environments [77,78]. Doxorubicin is used as a powerful chemotherapy drug for cancer treatment. Because the best application of doxorubicin remains debatable, Ma et al. [79] developed innovative, natural doxorubicin-loaded hydrogel microparticles using microfluidics electrospray technology. Their *in vivo* study showed that the doxorubicin-loaded microparticles had adequate biocompatibility. Their results suggest that natural biomass–based hydrogel microparticles are highly promising delivery mechanisms for chemotherapy drugs and could provide a platform for local therapy.

4.2. Transdermal drug delivery

The stratum corneum layer of the skin acts as a barrier to the external environment and limits the bioavailability of therapeutic agents. Several slightly invasive methods are available for transdermal drug delivery, such as iontophoresis, reverse iontophoresis, electroporation, and MNs [80]. Transdermal drug delivery system (TDDSs) have been widely used for several years. It was predicted

that the market for TDDS would reach USD 7.1 billion by 2023, up from USD 5.7 billion in 2018, for a compound annual growth rate of 4.5% [81]. MNs are a novel TDDS with various advantages, such as low infection risk, reduced pain, ease of application, and controlled release and enhanced bioavailability of therapeutic agents [82].

Diabetes mellitus affects millions of people worldwide and is a serious chronic disease that requires patients to receive frequent insulin injections and provide blood samples to monitor blood glucose levels. Various studies have reported on the use of MNs, a relatively painless TDDS, for self-managing diabetes [80,83]. A chitosan/poly (N-isopropylacrylamide-co-acrylic acid)/cellulose technique has been used for transdermal delivery of multiple drugs. Nanoparticles (200 to 300 nm) containing tretinoin and clindamycin phosphate were used, and the results indicate sustained, controlled release of the drugs at the minimum inhibitory and bactericidal concentrations better than those of samples fabricated via the bulk mixing method. The finding suggests that chitosan-based nanoparticles could be used for transdermal multidrug delivery applications [84].

Yeung et al. [85] devised a fabrication system to create hollow MNs interfaced with microfluidics structures in a single step, lowering the cost and increasing the print speed and throughput compared with earlier methods. Their study suggests that their architectures could be implemented in other biomedical devices to simplify the use of transdermal drug delivery applications. Subbiah et al. [86] synthesized, characterized, and tested phospholipid-based deformable nanovesicles (DNVs) carrying the fluorescently labeled hydrophilic bisphosphonate drug AF-647 zoledronate (AF647-Zol). To examine the ability of the DNV-encapsulated drug to be delivered transdermally to a local site, AF647-Zol DNVs were lyophilized, resuspended, and applied topically as a paste to the calvarial skin of mice [86]. Their findings suggest that microfluidics reactor–synthesized DNVs can be produced with excellent yield and high encapsulation efficiency, reproducibility, and stability after storage, and that they are a valuable vehicle for localized transdermal drug delivery.

Jiang et al. [87] reported a low-cost, flexible, passive, polymeric pump for topical drug delivery that uses wound pH as an induction for localized drug release. The pump enables slow release (<0.1 μL min-1) of aqueous antibacterial substances for up to 4 h and withstands up to 8 kPa of backpressure. *In vitro* experiments showed a 58-fold reduction in live *Pseudomonas aeruginosa* after 24 h of pump-aided antibiotic treatment [87]. A different microfluidics diffusion chamber device has been developed for comparison with traditional methods. It also has various advantages, such as low cost, small drug consumption, low sample volume, and rapid and reproducible results [88]. Another study used a three-layer skin equivalent made with human HaCaT keratinocytes, an electrospun polycaprolactone mesh, and a collagen-I gel and compared it with excised human skin samples. A miniaturized dynamic diffusion cell ("skin-on-a-chip" microfluidics device) was used to measure the permeability of the samples to 2% caffeine cream [89]. The outcomes suggest that both the established skin equivalent and the microfluidics diffusion chamber can form an appropriate base for the additional development of more complex tissue alternatives.

5. Smart integrated microfluidics systems for drug delivery

Traditional drug administration and delivery face several challenges, such as decay and loss due to quick release when passing through different biological barriers. Physiological barriers in the human body hinder the efficient delivery of some specific pharmaceuticals to target organs via traditional modes such as intramuscular injection, oral administration, and inhalation [3]. Those barriers allow only minute quantities of a drug to reach the target organs, tissues, cells, or subcellular organelles [79]. Critical challenges with conventional drug delivery modes include reduced bio-allocation and distribution, restricted solvability, low assimilation, and drug accumulation [3]. Traditional drug delivery processes and models include bulk manufacture and development, chemical identification and analysis, pharmacological and toxicological analyses, and clinical trials. These cumbersome steps limit the adaptation and translation of drug delivery models from bench to bedside. Microfluidics technology offers a unique setup for regulating drug delivery using a standardized system that modulates drug release at predictable speeds and monitors the real-time effects of drug delivery to the target site [90]. Delivering a drug via microfluidics in a regulated way influences its effectiveness, absorption capacity, and pharmacokinetics and limits side effects. Microfluidics technology enables the micromanipulation of fluids at the nanoliter scale to optimize flow rates and allow advanced inbuilt sensors for temperature control and exchange [91].

5.1. Fabricating microfluidics devices and carriers

The fabrication of a microfluidics chip can use a single kind of material or a combination of materials. For instance, PDMS, poly (methyl methacrylate) (PMMA), polycarbonate (PC), cyclic olefin copolymer, polyether ether ketone, polyimide plastic resin, glass, quartzose, and silicon can all be used to fabricate microfluidic chips. A blend of two materials, such as PMMA and PC or PDMS and PC, can also be used to fabricate microfluidics devices. Single emulsions are developed by mixing two immiscible liquids. For example, water-in-oil droplets are created when water is applied as a continuous phase, and oil is in the dispersed phase. Double emulsions allow development of customized droplets with specific sizes and geometric features. Such droplets can be used to encapsulate polar and nonpolar molecules. Multiple emulsions have been developed to encapsulate food materials and drugs [92].

Organ-on-a-chip-based microfluidics platforms are fabricated by the soft lithography technique. These devices are lined with channels to load cells or tissues, pump reagent, and inject solutions, as demonstrated in testes-on-a-chip devices. Use of these systems in pharmacotoxicity analyses offers opportunities to reduce animal testing by adhering to the principles of replace, reduce, and refine [91].

Chip-based drug delivery models are best suited for proteins, peptides, and DNA-based drugs, which are at risk of early deactivation when they are subjected to time-consuming, multistep processes [93]. Some example applications are described here.

Table 3
Reviewed nanocarrier systems produced by microfluidics systems with types of nanoparticles, microfluidic type, formulated nanoparticles with its application.

Types of NPs	Microfluidic device type	Type of NPs formulated	Application	Reference
Lipid-based NPs	SHM	Different protein (insulin, OVA or BSA) loaded liposomes (PC, DMPC, DPPC, CHOL, PS and DSPC)	Protein encapsulation	[102]
	N/A	DOX loaded liposomes (HSPC, CHOL and DSPE-PEG2000)	Anticancer	[101]
	SHM	Cisplatin/curcumin loaded liposomes (DMPC, DPPC and DSPC)	Anticancer	[103]
	5-input chip	DOX/UMB loaded liposomes (DSPC, CHOL, and DSPE-PEG2000)	Anticancer	[104]
Polymeric NPs	SHM	Curcumin PLGA NPs	Anti-inflammatory	[107]
	SHM	Curcumin PLGA NPs	Anti-inflammatory	[108]
	2D HFF	Paclitaxel PLGA NPs	Anticancer	[109]
	3D HFF	Ribavirin PLGA NPs	Antiviral	[110]
	2D HFF	Cyclosporine PLGA NPs	Mucolytic	[111]
Inorganic NPs	N/A	DOX-gated MSN	Anticancer	[112]
	N/A	DOX-gated MSN	Anticancer	[113]

NPs, nanoparticles; SHM, staggered herringbone micromixer; OVA, ovalbumin; BSA, bovine serum albumin; HSPC, phosphatidylcholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; CHOL, cholesterol; PS, L-α-phosphatidylserine; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DOX, doxorubicin; HSPC, L-α-phosphatidylcholine; DSPE-PEG2000, 1,2 distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000; UMB, umbelliprenin; HFF, hydrodynamic flow focusing; MSN, mesoporous silica nanomaterial.

- On site analysis: Involves assessing, sorting, and evaluating amino acids from lysed cells. A microfluidics system was developed to lyse, sort, and quantify the protein content in a single cell [94].
- Protein crystallization: To produce a supersaturated fluid that does not change the native characteristics of a molecule, different precipitation factors are added, and conditions such as pH and temperature are optimized. Microfluidics devices are being developed to evaluate crystallization conditions via a partial contact dispensing mechanism; one such device can analyze 96 crystallization conditions [95].
- High-throughput screening: Microfluidics-based high-throughput screening is performed in multi-well plates at the microscale level using multiplexed systems, microwell arrays, plug-based systems, and gradient-developing systems [96].
  - Multiplex systems include a fluidic rotatory mixer that enables multiple steps to be performed together. These systems are being used to detect food pathogens and test for the presence of toxicants or pesticides [97,98].
  - Microwell-based microfluidics systems are being used for drug screening and signaling analyses. For example, a microfluidics chip can be used in drug screening to conduct cytology and toxicity analyses of cancer drugs such as paclitaxel and doxorubicin [99].
  - Plug-based systems can be used for investigations with small sample volumes. For instance, blood group and type sub-classifications can be performed with small blood samples [100].

5.2. *Nanoparticles as drug delivery carriers*

Drug delivery carriers are being developed using microfluidics systems with different nanoparticles, including the lipid-based, polymeric, and inorganic nanoparticles discussed below.

a) Lipid-based nanoparticles share features with plasma membranes in drug delivery systems, *i.e.*, good bio-adaptability, diffusion capacity, and drug transport ability. The size of a nanoparticle can influence drug delivery and its therapeutic effect. Traditional procedures for developing liposomes of similar size involved complex steps. However, microfluidics does not require complex steps for developing liposomes [101–104]. Microfluidics-driven method for manufacturing, purification and monitoring of liposomes encapsulating proteins was developed in which three different proteins, namely insulin, bovine serum albumin and ovalbumin were successfully entrapped in liposomes [102]. In another study, small doxorubicin-loaded liposomes were prepared using microfluidics and tumor penetration capability was determined in the tumor xenograft model [101]. Similarly, curcumin-loaded liposomes were fabricated using the microfluidics approach and anticancer activity was achieved [103]. This approach was also efficient against human breast cancer cells when PEGylated DSPC liposomes were co-loaded and tested with doxorubicin and umbelliprenin [104].
b) Polymeric nanoparticles, both natural and synthetic, are being used extensively for drug delivery. They allow ample time for drug absorption because they interact with mucus via electrostatic, van der Waals, and hydrophobic forces. Polylactic-co-glycolic acid (PLGA) is the most popular polymer-based substance. Polymeric nanoparticles are linear, free from toxins, biocompatible, and already used for clinical purposes. The surfaces of these polymers can be transformed to enable target functions for specific therapeutic treatments. Polymeric nanoparticles offer merits such as good size regulation, size control, and framework. In addition, these nanoparticles have useful applications in microfluidics-based nanoprecipitation mechanisms [105–107]. Microfluidics systems can deliver stabilized polymeric nanoparticles for multiple applications such as the encapsulation of curcumin in PLGA for

Table 4
Several studies of robotic microfluidic in drug development.

Device	Objects	Main findings	Reference
Robotic microfluidic	Droplet	The automated procedure of droplet microfluidic using fluid-handling robotic	[119]
Robotic microfluidic interface	Automated procedure	Introducing the microfluidic-robotic without pipette interface to connect robotic device and liquid storage	[120]
Robotic microfluidic	Colloidal nanomaterial	Introducing the design and performance of microfluidic reactor and autonomous robotic	[121]

anti-inflammatory [107,108], encapsulation of paclitaxel in PLGA for anticancer [109], encapsulation of ribavirin in PLGA for antiviral [110] and encapsulation of cyclosporine in PLGA for mucolytic activity [111].

c) Inorganic nanoparticles, including quantum dots, graphene, iron oxide, black phosphorus, carbon, and silica, offer efficient drug delivery due to their excellent physicochemical characteristics. Two key features of these particles are regulated synthesis and surface engineering, which enable optimal flexibility for functional designs [90]. The use of mesoporous silica nanoparticles is becoming increasingly popular for targeting and treating diseases such as cancer. For example, a doxorubicin-gated mesoporous silica nanoparticle system was developed for drug delivery at cancer-infected sites [112,113].

All reviewed studies related to nanocarrier systems produced by microfluidics systems are summarized in Table 3.

Microfluidics platforms are being developed to screen the effects of drugs on multiple cell lines and tissues derived from multiple organs in a single integrated system (Kimura et al., 2018). Several such body-on-chip systems have been developed by various research groups to explore drug exposure effects [114–116]. As discussed, microfluidics has many applications in the field of drug delivery because it offers high-quality precision and nanoscale fluid dynamics that can deliver minute quantities of fluids to cells. This technology is being used to test the efficacy of drugs and their therapeutic or toxic effects on cell types. Thus, microfluidics technology can be used to create smart, integrated drug delivery systems that could transform the pharmacology and medical landscape.

6. Microfluidics-integrated robotic drug delivery carriers

The technology underlying microfluidics combines micro-electromechanical and fluid mechanics systems [117]. Microfluidics can control milliliter to nanoscale liquid flows via micro-pneumatic systems; thus, microfluidics is a versatile tool for synergizing robots and other dynamic systems, particularly in soft robotics, where fluidic pressure is a main way of actuation [118]. Currently, few publications describe the integration of robotics and microfluidics, especially in drug development, because microfluidics and soft robotics are new technical concepts that have emerged only during the past two decades or so [117] (Table 4).

Apart from their synergic role, several platforms combine robotics and microfluidics systems, such as robotic MNs [122], fluid-handled robots [123], and 3D printers [124]. As explained above, MNs are a promising technology for transdermal drug delivery that offer minimal invasion. Yeung et al. [85] successfully used 3D-printed microfluidics to hollow a wide range of MN features at the micro- to centimeter scale. Fluid-handled robots are involved in automated processes that attach a protein to microbeads. Although functionalized microbeads are important in smart drug delivery systems, using robotic microfluidics to alter the manual steps of microbead treatment, including surface activation, residual chemical removal, protein addition, and mixing, is an effective strategy to prevent human error and reduce production time requirements [123,125]. Moreover, during the nanoparticle synthesis procedure, a fluid-handled robot can perform several steps, such as aspirating and dispensing liquids into wells and tubes, with higher precision than humans can manage. Scientists are working to integrate robotic devices with conventional devices to optimize the function of fluid-handled robots in numerous laboratory techniques [125].

A distributed system is one of two main approaches for integrating microfluidics and robotics. Because recombinant drugs need specific distributions and preservation, which limits access to them, especially during emergencies and in remote areas, the combination of robotics and microfluidics could be a natural way to solve challenges remaining in drug delivery. Automated and decentralized systems could improve global access to drugs at a suitable cost by offering proper mechanical control without human intervention and by helping to prevent toxic exposures [126]. Fluidic automation also contributes to large-scale drug synthesis, including biologics such as cytokines or hormones [127].

One of the main disadvantages of modern drug delivery systems such as droplets and nanoparticles is the complicated procedures required to manufacture them. The robotics-integrated microfluidics device developed by Tran et al. [119] separated multi-step procedures into sub-steps that can be conducted automatically without user intervention. High efficiency and high reproducibility are critical advantages of liquid-handling robotics to the development and accessibility of new drug delivery systems. Although difficulties remain in combining microfluidics and robotics, their integration is a future trend. Moreover, integrating smart digital or artificial intelligence with robotic microfluidics devices is predicted to be a potential strategy for maximizing the advantages of microfluidics in drug development, particularly drug delivery systems.

7. Artificial intelligence/machine learning guided microfluidics for drug delivery

Technological advances in the field of personalized medicine have shifted to focus on the application of artificial intelligence (AI) and machine learning (ML) for drug delivery [125]. In the pandemic era, non-priority laboratory-based experimental and research

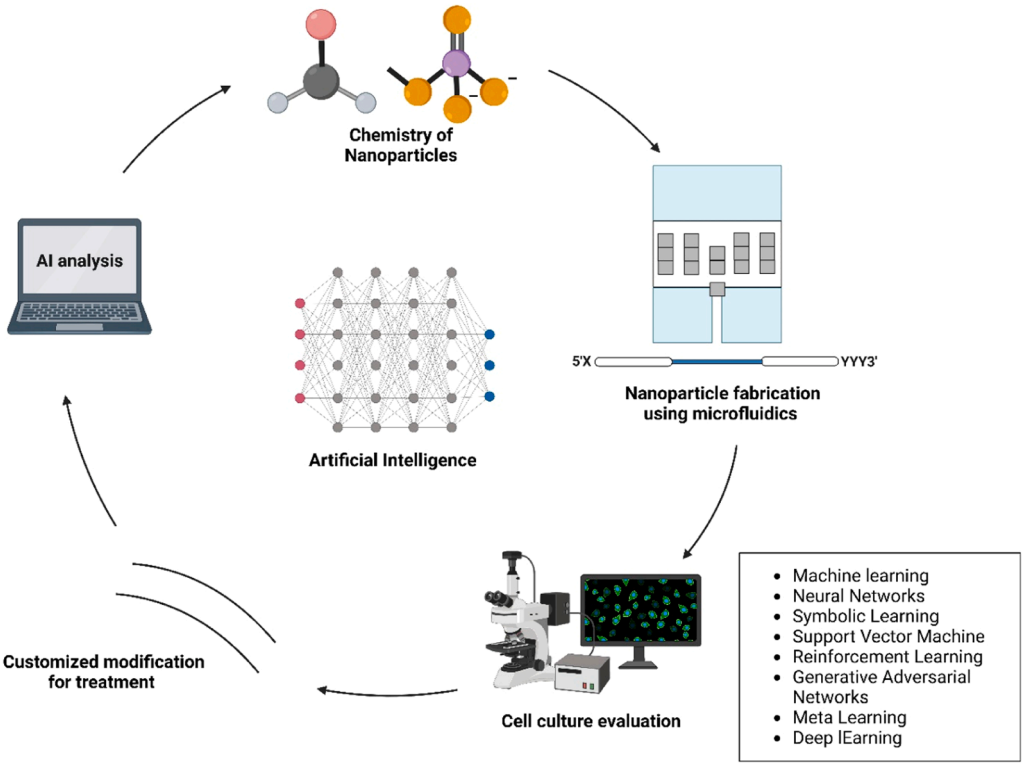

Fig. 8. Multistep flowchart demonstrating application of Machine Learning tools and Artificial intelligence in microfluidic based nanoparticle fabrication and drug delivery process. Figure was created with Biorender.com.

Table 5
Machine Learning in Microfluidics.

Steps	Iterative Learning steps	Automated robotic system
Community repositories	Community repositories required with optimized designs, fabrication protocols and datasets with predictive modeling	Protocol definition
Transfer learning	Regularly update community repositories to expand predictive knowledge	Component selection and sizing
Creation of standardized designs	Understand microfluidic performance across the field	Component linking
Dataset generation	Rapid prototyping	Machine-guided microfluidic operation

studies were halted, and remote work and online research trainings and activities accelerated globally. In that scenario, interest in applying AI and ML to research activities grew tremendously because they could be implemented remotely. To create functional prototypes for pharmacology and medical applications, robotic systems were developed and used. Conventionally, nanoparticle synthesis is conducted using laboratory-based physical procedures that consume many infrastructural resources. A sophisticated AI/ML-guided robotic model could create and install a system for nanoparticle fabrication, evaluation, and optimization (Fig. 8). Integrating AI and ML with software-controlled laboratory equipment enables remote laboratory operations. Operators should be aware of their ethical responsibilities regarding the owner's intellectual property rights and computational propriety and prevent unauthorised access by keeping access information confidential. Necessary technical and manual steps should be taken to prevent network security breaches by strengthening authentication systems, protecting access information and unnecessary software functions, and training all staff involved in the project. Any modification in a nanoparticle fabrication process can alter the characteristic features of the nanoparticle, including its structure, dimension, charge, surface properties, and particle volume [125]. Therefore, the application of microfluidics to nanoparticle fabrication could reduce variation between batches.

AI and ML are synonymous concepts, with ML often considered to be an application of AI [128]. AI collectively represents computer-based reasoning and problem solving, knowledge representation, planning and social intelligence, perception, ML, robotic technology, and natural language processing. AI can reduce the time and costs of complex and expensive drug delivery processes. With data computerization and digitization, AI applications in the field of medical data can eliminate complicated bottlenecks in data analysis and interpretation. AI allows computer programs to act as a human brain; in contrast, ML is based on predictive models derived from previously known datasets and inputs that are provided to the system as described in Table 5 [129]. Instead of being

programmed, a machine applies algorithms such as Markov models, naïve Bayes, and decision trees to learn on its own [128]. Combining AI and ML techniques is a key driver for microfluidics-based drug delivery and analysis, as shown in Fig. 8. Nanotechnology and nanomedicine offer innovative solutions for problems with medical treatment. Automating the various steps involved, such as nanoparticle and drug fabrication, data evaluation, and drug development, can transform medical treatments and provide desired results for patients. AI algorithms need sample results that could be developed using automated developmental steps. Experimental results and data can be used to make the predictive data models needed for ML algorithms, which could eventually guide the development of better and more effective nano-compounds.

One example of ML application occurred during the microfluidics-based synthesis of indomethacin-loaded PLGA nanoparticles, which was optimized by ML [130]. Another example is the development of magnetically actuated intelligent hydrogel-based child--parent microrobots for targeted drug delivery [131]. Different challenges currently hinder the adoption of ML in research. One major challenge is the absence of enough previous results or data for ML output, which can be overcome by opening laboratory doors so that experts from various other research areas can derive maximum output and datasets. Merging ML tools, developing an integrated microfluidics setup, and adapting AI to develop automated systems would be a step forward. AI and ML technologies can be applied to develop high-quality microfluidics systems, perform efficient drug delivery, and investigate the effects of various drugs on different cell types and bodily organs.

8. Conclusion and future perspectives

Microfluidics-based drug delivery systems enable the fabrication of drug-releasing particles that assists the delivery of the drugs to target cells, tissues, or organs. Several research groups around the world have been using microfluidics chips for cell culture, growing organs, drug toxicity testing, and regenerative medicine. Pharmaceutical companies are now investing funds to integrate microfluidics in the production of drug-releasing materials for sustained drug release [132]. An example for this is the use of microfluidics technology to manufacture mRNA lipid nanoparticles used in vaccines [133]. Several pharmaceutical companies, including Pfizer & BioNtech, Moderna, and Sanofi, are developing technologies to aid sustained release of the COVID-19 vaccine [11]. microfluidics drug delivery technology has a promising future, and innovations in areas of sustained drug release, vaccine development, and targeting therapeutics for better treatment are expected to accelerate given that efforts are being put from not just the academic labs but also from industry.

CRediT authorship contribution statement

Gargi Bhattacharjee: Writing – review & editing. **Nisarg Gohil:** Writing – review & editing. **Malvika Shukla:** Writing – review & editing. **Swati Sharma:** Writing – review & editing. **Indra Mani:** Writing – review & editing. **Alok Pandya:** Supervision, Writing – review & editing. **Dinh-Toi Chu:** Supervision, Writing – review & editing. **Nhat Le Bui:** Writing – review & editing. **Yen-Vy Nguyen Thi:** Writing – review & editing. **Khushal Khambhati:** Writing – review & editing. **Rupesh Maurya:** Writing – review & editing. **Suresh Ramakrishna:** Writing – review & editing. **Vijai Singh:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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