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Nuts and Bolts: Microfluidics for the Production of Biomaterials

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Please submit a plain text version of your cover letter here.	Dr. Jovia Jing Editor Advanced Materials Technologies Dear Dr. Jovia Jing, I am submitting the revised manuscript (Invited Review, No. admt.201800611) entitled “Nuts and Bolts: Microfluidics for the Production of Biomaterials”” for consideration and publication as a Review paper in Advanced Materials Technologies. We would like first to thank you and the reviewer for the positive and constructive comments for the improvement of our article. All the comments raised in the reviewer’ reports have been fully addressed. Please find enclosed the reply to the reviewer’ reports, which also describes the changes made in the manuscript. We believe that the changes introduced to the manuscript have further improved it and we hope that the manuscript can now be accepted for publication in your valuable journal. Sincerely yours, Hélder Santos _____ Dr. Hélder A. Santos, D.Sc. (Chem. Eng.), Associate Professor, Head of Division Head of the Division of Pharmaceutical Chemistry and Technology Head of the Nanomedicines and Biomedical Engineering Group Head of Preclinical Drug Formulation and Analysis Group Drug Research Program, Faculty of Pharmacy, University of Helsinki, Finland; & Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Finland @: helder.santos@helsinki.fi, http://www.helsinki.fi/~hsantos/ https://scholar.google.com/citations?hl=en-EN&user=K3Pj_gwAAAAJ

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Nuts and Bolts: Microfluidics for the Production of Biomaterials

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Abstract

Nanotechnology holds the promise of bringing revolutionary therapeutic strategies into the clinic. However, an enormous fraction of the currently proposed nanotechnology-based therapies suffers from lack of reproducibility, complexity, high costs, and scale-up related issues. For these reasons, the research community has been moving towards the miniaturization of biomaterials and fabrication methods. Customizable microfluidic-based products have gained tremendous relevance in the development of biomedical technologies. This review provides an overview of different materials that can be used for the fabrication of microfluidic devices, as well as the other parameters influencing the production of biomaterials and biosensors. Moreover, several advanced microfluidic-based technologies that have been designed to overcome the current challenges of cancer, immunotherapy, and diabetes therapy, among others are described. Then, the pros and cons of microfluidics as alternative to conventional preparation methods, and the challenges of translating this technique to an industrial context are highlighted. Overall, microfluidic technologies and their accessibility to the research community offer a set of exciting opportunities to bridge the development of innovative therapies and their commercialization in the foreseeable future.

1. Introduction

Nanotechnology is an ever-growing field that brings closely together biology and advanced technologies, with the potential to provide ground-breaking solutions in the treatment of several diseases, such as cancer or diabetes. It allows for the manipulation of materials at the nanoscale with an extraordinary degree of precision, and it holds the promise to revolutionize the field of medicine through manifold applications, from pharmaceutical products to medical devices.[1] Over the years, nanotechnology has been enabling the delivery of poorly water-soluble drugs, including some previously considered as undevelopable, and to allow for targeted drug delivery, thereby improving drug bioavailability and specificity, while minimizing unwanted side effects.[1-2] Additionally, nanosized biomedical devices such as prostheses and implants have been showing a tremendous potential in a wide range of applications.[3] Such potential has been translated into an enormous amount of publications, patents, and start-up companies with one common goal, bring nanotechnology into the clinic. However, all the enthusiasm surrounding the new biomedical technologies is often deflated before any clinical/commercial achievement.[4] Despite the fairly predictable improvement of nanotechnology-based therapies over conventional treatments, some constraints related to the technologies themselves have now started to be heard, including complexity and costs of the manufacturing processes, which create a significant hurdle for drug companies.[1, 4] For these reasons, the scientific progress that is translated into clinical applications is reduced.[5]

Indeed, conventional methods of preparation of nanotechnology-based therapies suffer from complex experimental set-ups, poor controllability and reproducibility, and are further associated with scale-up feasibility issues.[6] The long development and evaluation processes, together with their associated high costs are undeniably hindering the launching of novel nanotechnology-based systems into the market.[6] It is therefore crucial to find alternative fabrication techniques that can help nanotechnology to have the greatest clinical impact in the foreseeable future. Here, microfluidics seems to be the new player.

A tremendous technological progress has taken place in the biomedical field over the past decades, particularly towards the miniaturization of biomaterials and their respective fabrication methods, in order to replace conventional macrosized benchtop equipment.[7] Microfluidics has emerged in the early 1950s,[8] but its application was only largely widened after George M. Whitesides research group reported, in 1998, the preparation of microscopic channels based on rapid prototyping of poly(dimethylsiloxane) (PDMS) masters using high-resolution printing and contact lithography.[9]

The miniaturization of fluidic operations and their high applicability is indeed particularly compelling, and therefore, represents a new shift in the fabrication of advanced technologies that can replace conventional therapies. The current developments of microfluidic-assisted fabrication technologies are based in a variety of theoretical considerations and advanced knowledge, involving the geometries of the microfluidic devices, the materials used for their preparation, basic fluidic principles, flow patterns and regimes inside the microfluidic channels, and have been described in a great number of related publications in this area.[6, 10]

In this review, we first discuss the materials used for the fabrication of microfluidic devices, and other parameters influencing the production of biomaterials. Additionally, we acknowledge the most recent applications of microfluidics in cancer, immunology, and diabetes research areas, among others. Finally, we address the pros and cons of microfluidics, and the challenges of translating this technique to an industrial context.

2. Material Development

During the last decades, multiple microfluidics platforms different for the fabrication material, for the geometrical configuration and for the operative conditions have been reported in literature. In this section we will review the nuts and bolts of this technique applied to the development of drug delivery systems.

Microfluidics chips have been fabricated over a variety of substrates, including glass, polydimethylsiloxane (PDMS), polycarbonate, poly (methyl methacrylate) (PMMA), cyclic olefin copolymer, polyetheretherketone, polyimide plastic resin, stainless steel, single-crystal silicon.[11] Each of these materials presents advantages and disadvantages, based on the final application of the platform. As an example, polymeric flexible substrate (PDMS, PMMA, *etc.*) have been extensively employed in point-of-care diagnostics thanks to their favorable properties (mainly flexibility, optical transparency, heat conductivity, electrochemical resistance).[12] However, PDMS chips are less suitable for application in the synthesis of drug delivery systems due to their reactivity towards organic solvents, the low withstandingness to high pressures, the absorption of small inorganic molecules, and the problematic, and only temporary, hydrophilization of the channels.[13] Glass platforms, on the other hand, resist to chemical solvents, do not swell, and present low electrical conductivity, smooth surface, surface easily modifiable.[13a, 14]

The fabrication methods differ between the different platforms: in general, they rely on *e.g.* lithographic methods, etching, molding.[13a] A summary of these methods is presented in **Table 1**.

Table 1. Fabrication methods of microfluidics platforms based on the fabrication material. Modified and reprinted with permission.[13a] © 2013, Elsevier B.V.

Platform Material	Fabrication Methods	Final Surface	Ref.
Single-crystal Silicon	Photolithograpy, Anisotropic Wet Etching, Deep Reactive Ion Etching	Hydrophilic, Microgrooves, Straight-through Microchannels, Micronozzles	[15]
Poly (dimethylsiloxane) (PDMS)	Soft Lithography, Combination of Micromilling, Molding and Soft lithography to Create Circular Channels	Hydrophobic, can be Hydrophilic by Plasma Oxidation, Sol-Gel Coating with Silica or Titania, Layer-by-layer Deposition of Polyelectrolytes, UV Polymerization of Acrylic acid, UV Irradiation, Chemical Vapour Deposition, Antifouling Coatings	[11b, 16]
Glass (different types)	Mechanical cutting, Chemical Dry Etching, Isotropic Wet Etching	Hydrophilic	[17]
Cyclic Olefin Copolymer (COP)	Hot Embossing, Injection Molding, Nanoimprint Lithography, Laser Ablation	Hydrophobic	[18]
Polyurethane	Soft Lithography	Hydrophobic	[19]
Poly (methyl methacrylate) (PMMA)	Hot Embossing Lithography, Injection Molding, Mechanical Cutting, Laser Ablation,	Hydrophobic	[20]

	Stereolithography, Mechanical Micromilling		
Stainless Steel	Mechanical Cutting	Hydrophilic	[21]
Glass Capillaries	Micropipette Pulling, Microforge, Manual Sanding of the Orifice, Gluing to the Glass Slide	Hydrophilic, with Modifications Hydrophobic	[14, 22]
Stainless Steel Platform for Glass Capillaries	Micropipette Pulling, Microforge, Manual Sanding of the Orifice	Hydrophilic, with Modifications Hydrophobic	[23]
Paper (Paper based-microfluidics)	Cutting, Wax printing, Inkjet printing, Photolithography, DLP Printing	Hydrophilic, with Modifications Hydrophobic	[24]

Moving forward, the next element characterizing a microfluidics platform is its geometry, as presented in **Figure 1**. Microfluidics platforms are usually based on the exploitation of the geometries to achieve a laminal flow within the channels. A laminar flow corresponds to flow regimens presenting low Reynolds number (Re), dimensionless parameter describing the interaction between inertial to viscous forces in the channel.[25] In these conditions, the viscous forces are predominant over the inertial ones, allowing a high degree of control over the fluidic, and thereby, on the nanosystems engineered by microfluidics.[6, 10a, 26] Depending on the final drug delivery system, a suitable geometry can be engineered. As an example, microparticles are produced by droplet, emulsion-based microfluidics, with different platform configurations depending on the need for single or multiple emulsions, which correlates with the need for a core shell structure in the microparticle (Figure 1a).[11a, 27] Microfluidics devices have also been used in the layer-by-layer production of microcapsules (Figure 1d). For example, core particles were coated either by liquid crystals and/or by polymers, with intermediate washing steps.[28] Alternatively, microfluidics can serve in the soft lithography of particles, both in continuous and discrete flowing.[29]

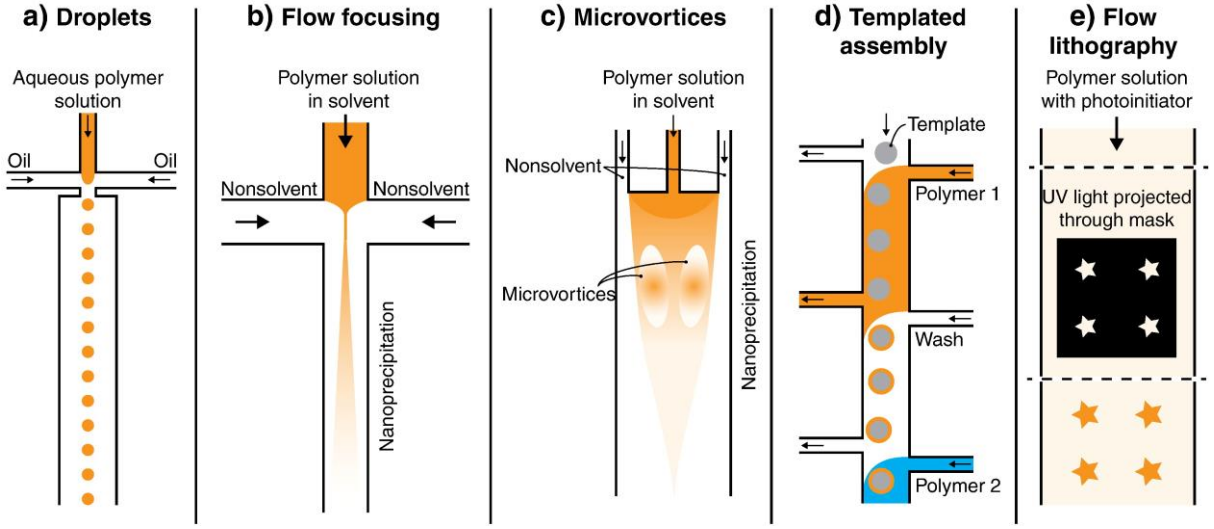

Figure 1. General types of geometries employed in the production of micro- and nanoparticles. **a)** Production of microparticles by droplets emulsion; **b)** and **c)** preparation of nanoparticles by flow focusing and microvortices nanoprecipitation, respectively; **d)** engineering of multistage particles by subsequent coating with different polymers in subsequent T-junctions; **e)** production of particles by flow lithography. Reprinted with permission.[30] © 2014, Elsevier B.V.

The term "nanoprecipitation" identifies the formation of nanoparticles caused by a change in the type of solvent, from good solvent to non-solvent.[31] Research in the process highlighted the presence of three different stages, namely nucleation, nucleus growth, and nanoparticle formation, which are highly dependent on the mixing time between solvent and non-solvent.[32] The laminar and jetting flow regimens in microfluidics devices enable a very rapid micromixing, which in turn enables a high degree of control on the size and size distribution of the particles produced.[6] Nanoprecipitation is usually achieved in platforms displaying flow focusing geometry.[32-33] In PDMS microfluidics platforms, the continuous phase coming from the lateral channels squeezes the dispersed phase into a thin stream, resulting into enhanced mixing in lower time, as depicted in **Figure 2a**.[34] The dispersed phase contains the polymer dissolved in a suitable solvent, while the continuous phase is an antisolvent for the

polymer, while still being miscible with the dispersed solvent.[10a] This platform can produce polymeric NPs with homogenous size distribution.[32] However, in this configuration, the flow is focused only in 2D, which may cause precipitation of the particles along the walls of the channel, blocking it.[35] Thereby, a further development involved the engineering of a 3D flow focusing device (**Figure 2b**).[35] In this device, the dispersed phase containing the polymer is first focused vertically by two layers of the same solvent, before being horizontally focused by the antisolvent.[35]

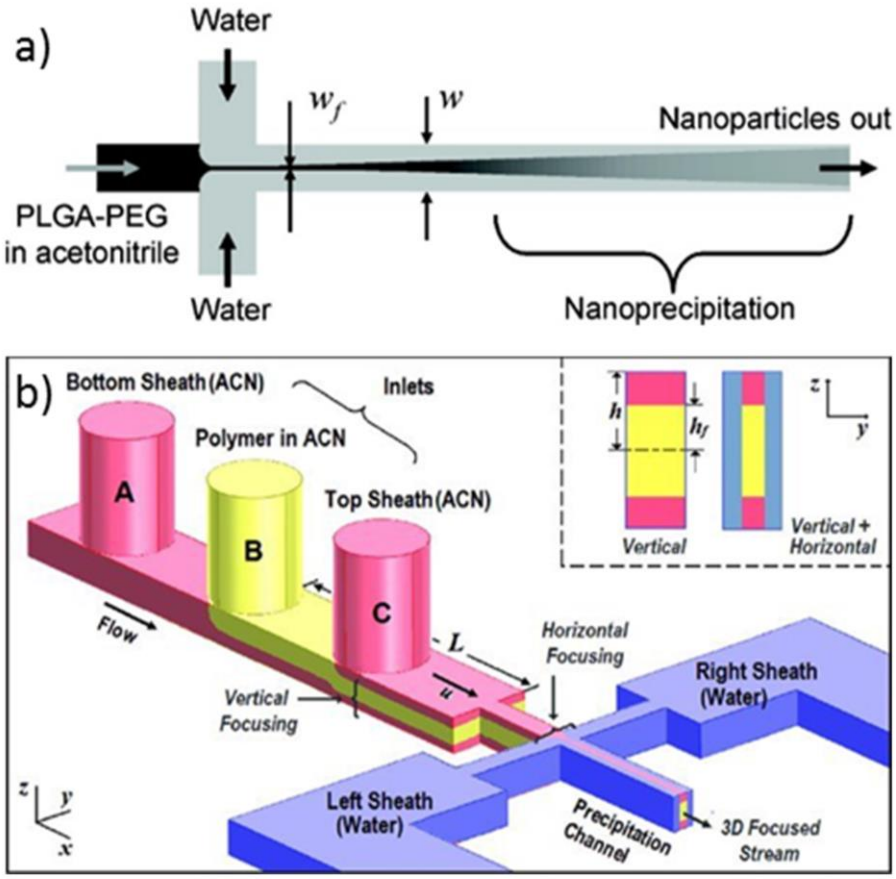

Figure 2. Hydrodynamic flow focusing (HFF) devices in PDMS. **a)** 2D-HFF: the flow is focused only horizontally by the antisolvent. Reprinted with permission.[32] © 2008, American Chemical Society; **b)** 3D-HFF, the flow is first focused vertically by two layers of solvent, before the horizontal focusing achieved by the continuous phase. Reprinted with permission.[35] © 2011, Wiley–VCH Verlag GmbH&Co. KGaA, Wienheim.

An alternative approach can achieve the 3D hydrodynamic flow focusing into glass capillaries platforms. For example, concentric capillaries are assembled and fused into a bigger polymeric tube (**Figure 3a**);[36] in an alternative, easier configuration, where a glass capillary is first pulled and sanded to present an orifice of predetermined dimensions, before being coaxially aligned within a bigger capillary (**Figure 3b**).[23, 37]

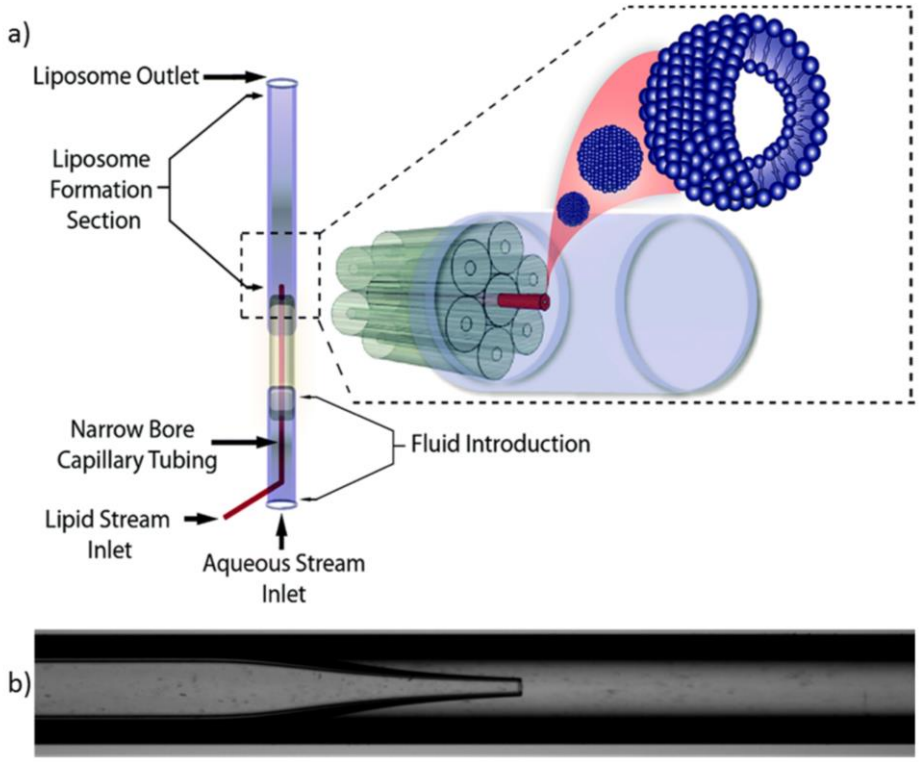

Figure 3. Glass capillary 3DHFF configurations. **a)** Platform constituted by 7 capillaries assembled and fused together into a polymeric tube for the production of liposomes. Reprinted with permission from.[36] © 2014, The Royal Society of Chemistry; **b)** Microfluidics chip constituted by glass capillaries into a flow focusing configuration: a smaller, pointed, capillary is coaxially aligned within a bigger capillary. Reprinted with permission.[23] © 2016, Elsevier B.V.

In particular, the second type of glass capillary platform presented is highly versatile, allowing the possibility to achieve nanoprecipitation in conditions of microvortexes, at lower Reynold numbers, and in jetting regimen, for higher Reynold numbers, increasing the overall

yield up to 250 g day^{-1}.[37] Further engineering of this platform produced a microfluidics chip composed by two sequential precipitation areas, to produced core-shell particles with extremely high yield (up to 700 g day^{-1}).[38]

In the production of NPs by nanoprecipitation in microfluidics, the viscosity of the dispersed phase plays a role in the final size of the systems. As a rule of thumb, an increase in the viscosity determines an increase in the size of the NPs, due to the prolonging of the mixing time determined by a decrease in the diffusiveness of the more viscous solvent (**Figure 4a**).[37, 39] A similar effect is observed also when the viscosity of the continuous phase is increased by addition of surfactants and other stabilizers: more viscous solutions (*e.g.*, poly vinyl alcohol) lead to an increase in the particle size; when positively charged phospholipids are employed, they tend to adsorb on the surface of the particle, changing its surface charge to positive (**Figure 4b and c**).[37]

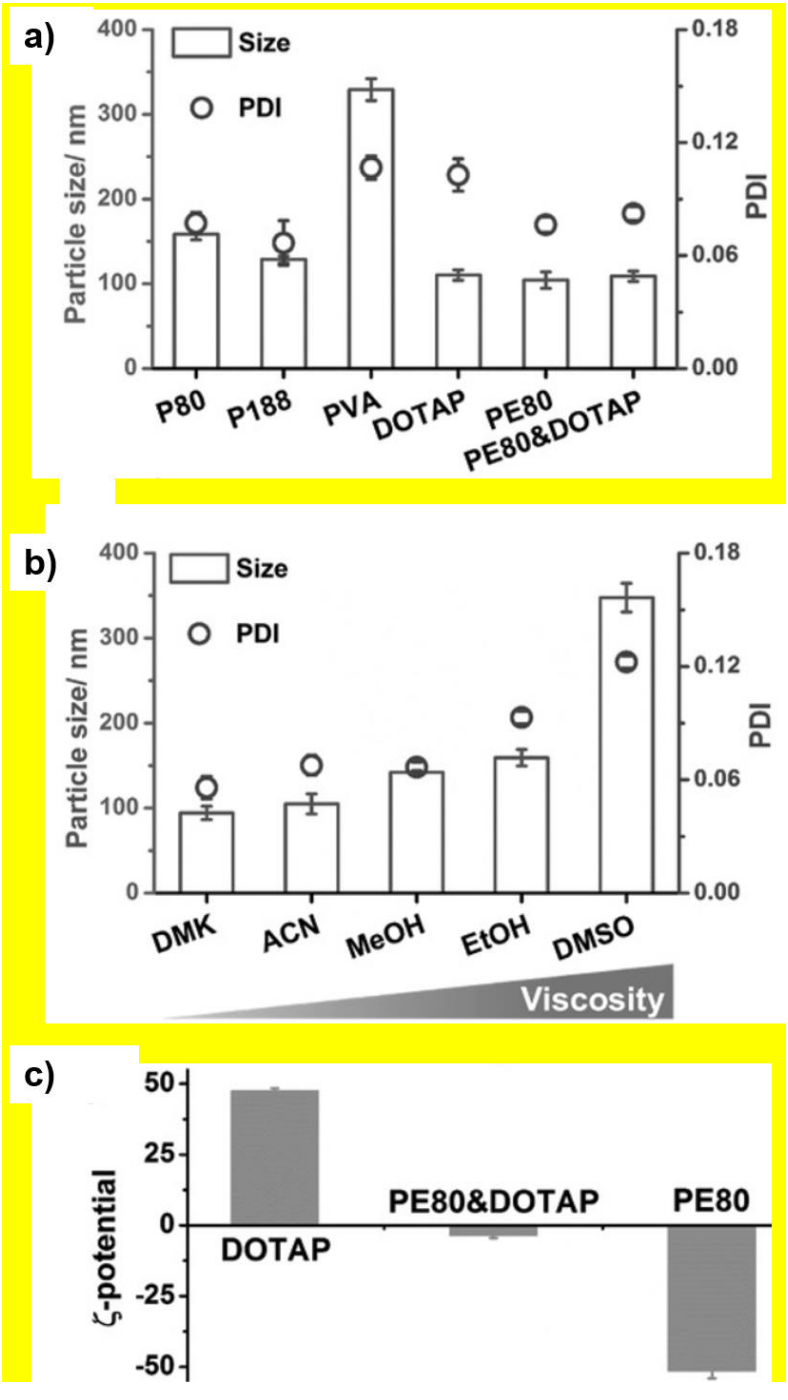

Figure 4. Parameters affecting the size of NPs produced by nanoprecipitation. **a)** Effect of the viscosity of the dispersed phase: solvents with different viscosities were tested in the preparation of acetalated dextan NPs. **b)** Effect of the viscosity of the continuous phase: the addition of surfactants with higher viscosity produces an increase in the particle size; **c)** effect of the adsorption of differently charged phospholipids. Phospholipids are successfully

adsorbed on the particles, as shown by the change in the particle's surface charge. Reprinted with permission.[37]

In summary, the size and yield of NPs, amongst other parameters, are influenced by the type of platform chosen (type of material and geometry), together with parameters belonging both to the dispersed phase (*e.g.*, viscosity of the organic solvent) and the continuous phase (viscosity of the continuous phase, type and charge of surfactants/lipids added to the solution). In the next section we will review applications of such platforms in the formulation of NPs for biomedical applications.

3. Applications

Microfluidics, both as platform for the preparation of biomaterial and as sensor, has been employed in a variety of applications in different human diseases, like cancer, immunological pathologies, diabetes, *etc*.[40] In this section, we will review recent applications in these different pathologies.

3.1 Cancer

Cancer still represents one of the leading causes of death worldwide, although the death rate has been constantly declining over years with the therapeutic advancements.[41] Nanomedicine has been hailed as great promise to enhance the efficacy of the treatments while reducing the side effects.[42] Moreover, NPs have the potentiality to actively target tumors cells overexpressing certain targets.[43] Another advantage brought by the use of nanosized carriers is the possibility to co-deliver different chemotherapeutics, with a precise dosing ratio, and, in some cases, in sequential order.[43] However, only 1% of the administered dose accumulates in the tumor, opening concerns about the safety and degradability of the 99% ending up off-target.[44] Other obstacles for a successful cancer nanomedicine include the heterogeneity of

the disease, the behavior and interaction of a NP in a biological environment, production methods and translation from lab scale to industrial production, and the difficult pathway for the approval by the regulatory authorities.[45]

Microfluidics represents a solution to some of the above-mentioned obstacles. More specifically, enables the production of NPs with excellent reproducibility and low batch to batch variations, while providing also excellent *in vitro* platforms to study whole libraries of NPs (**Figure 5a**) and the interactions between these particles and the biological environment.[43] However, despite the reports of platforms able to produce up to 700 g day^{-1},[38] the scale up of some of these microfluidics systems remains problematic.[43] Different strategies to enable such scale up in the engineering of emulsion droplets and microparticles have been already proposed,[13a] while solutions for NPs are currently being investigated.[38, 46] Glass capillary microfluidics platforms were employed to develop stimuli-responsive NPs delivering or co-delivering chemotherapeutics, achieving also ultrahigh drug loading (**Figure 5b and c**).[47] Specifically, these platforms enabled the successful delivery of 3 different chemotherapeutics (sorafenib, methotrexate, paclitaxel) in a nano-in-nano system constituted by porous silicon NPs encapsulated within a pH-responsive polymeric layer and modified with targeting moieties (Figure 5b).[47a] Moreover, the same platform was adapted for the precipitation of pH-responsive nanocomposites for the delivery of sorafenib.[47b] Finally, two sequential platforms produced nano-in-nano vectors constituted by a drug nanocrystal core coated by a polymeric shell (Figure 5c).[47c]

Prickly NPs represent a recent player in the field of NPs.[48] They present irregular shape and destroy intracellular membranes, controlling cancer cell proliferation. To improve the safety of these particles, they were encapsulated into a pH-responsive polymer modified with a targeting moiety to carbonic anhydrase IX, membrane protein overexpressed in tumor cells.[49]

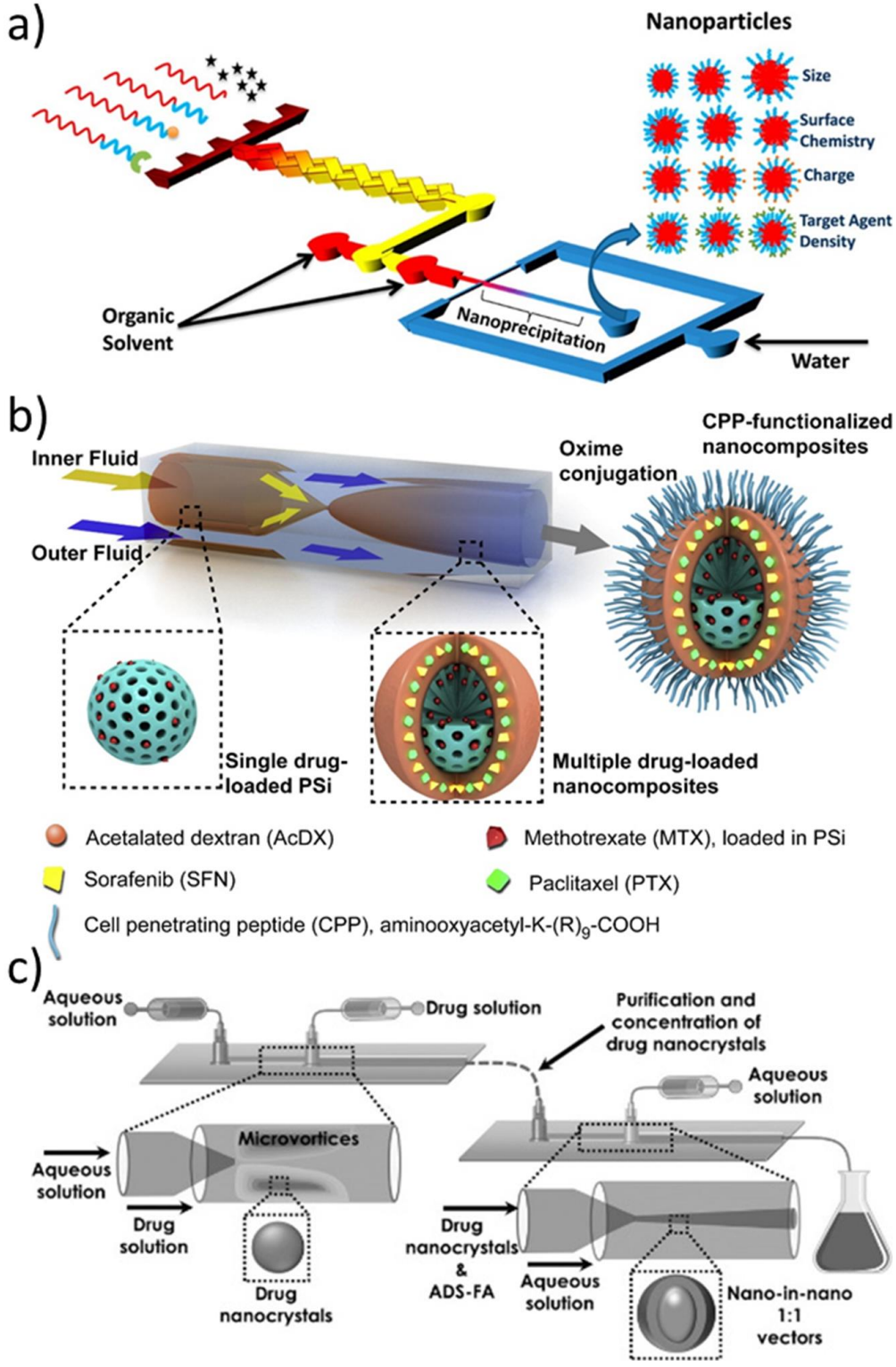

Figure 5. Microfluidics platforms for the synthesis of cancer nanomedicines. **a)** PDMS platform enabling the synthesis of combinatorial libraries of polymeric NPs. Reprinted with permission.[50] © American Chemical Society; **b)** glass capillary system engineering a nanoplatform for the targeted combinatorial delivery of three different chemotherapeutics

(sorafenib, methotrexate, and paclitaxel). Reprinted with permission.[47a] © 2014, Elsevier Ltd.; **c)** sequential platforms for the production of core-shell NPs constituted by sorafenib drug nanocrystals in the core coated by a polymeric layer. Reprinted with permission.[47c] © 2017, Wiley–Verlag GmbH & Co. KGaA, Weinheim.

Rigid nanovescicles for the delivery of hydrophilic cargoes were developed by Zhang *et al.*.[51] The authors developed a polymeric microfluidics platform with three separate nanoprecipitation areas for the sequential formation of reverse micelles, a polymeric shell around the micelles, and, finally, a lipidic layer coating the polymeric shell. This platform enabled the simultaneous loading and delivery of doxorubicin and a siRNA, achieving control over the tumor growth in an *in vivo* setting.[51]

Another interesting platform engineered by microfluidics is formed by hybrid NPs: PLGA was modified with biphosphonate to achieve the targeting to the bone tissue, and in particular to bone metastases.[52] The particles were further loaded with iron oxide NPs and with paclitaxel.

Sequential nanoprecipitation in microfluidics was adapted for the preparation of chitosan NPs,[53] followed by a coating with enteric polymers for drug delivery to colon cancer. The external layer allows the particles to pass through the harsh pH of the stomach, before freeing the chitosan particles at colonic pH, where the NPs swell and release paclitaxel.[54]

Finally, microfluidic platforms are currently under investigation as 3D models of the tumor and tumor microenvironment.[55] These systems allow the study of the mechanisms responsible for the metastases formation and the behavior of cancer cells into blood vessels.[56] Alternatively, patient-derived myeloid cells were successfully grown on microfluidics platforms, establishing systems for the personalized evaluation of the therapeutic efficacy of drugs.[57]

In conclusion, microfluidics represents an excellent alternative to bulk methods, both for the synthesis of NPs and for the creation of tumor models, addressing and removing some of the obstacles preventing a larger diffusion of nanomedicines in cancer therapy.

3.2 Immunotherapy

Immunotherapy refers to treatments aimed to interfere with patient's immune systems, with the aims of reactivating it (e.g., in case of viral infection and cancer) or to induce tolerogenicity (in case of autoimmune diseases).[58] Recently, NPs have gained attention as delivery systems for immunomodulatory molecules and as nanovaccines, due to their favorable biodistribution and properties that activate the immune system.[58]

So far, the use of microfluidics for the preparation of nanovaccines has been limited. Glass capillary platforms were employed for the synthesis of a core-adjuvant particle, followed by membrane extrusion to coat it with membranes derived from cancer cells (**Figure 6**), obtaining a personalized cancer vaccine.[59] This platform was able to activate human immune cells *in vitro*.

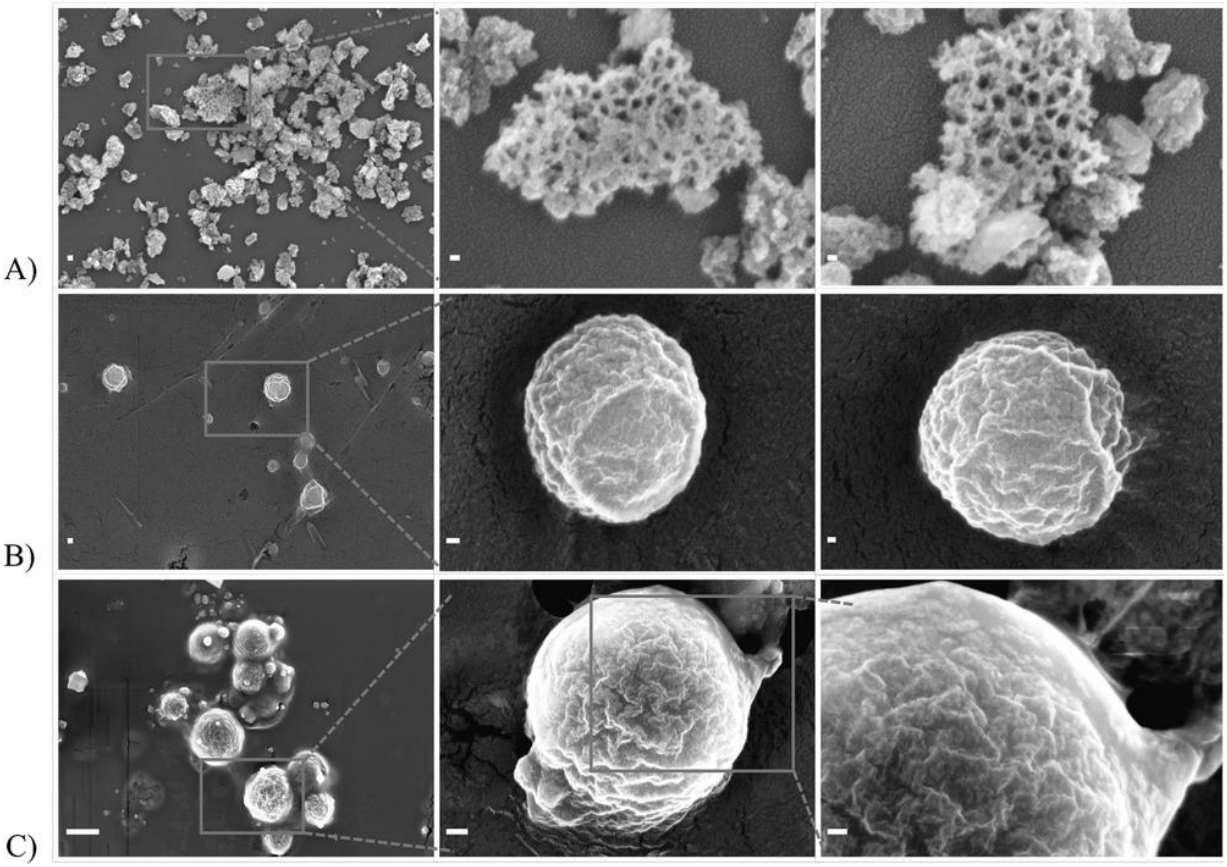

Figure 6. High resolution scanning electron microscope images of **a)** thermally oxidized porous silicon nanoparticles (TOPSi); **b)** TOPSi nanoparticlesencapsulated within a layer of acetalated dextran by nanoprecipitation in glass capillary microfluidics (TOPSi@AcDEX); **c)** final system after the coating with cell membrane derived from cancer cells. Reprinted with permission.[59] © 2016 WILEY–VCH Verlag GmbH & Co. KGaA, Weinheim.

Another application of microfluidics platforms in immunotherapy is the modelling of the interactions between cancer and immune cells in the tumor microenvironment.[60] In particular, in one of the first examples, the migration of cells between two compartments, one containing tumor cells and the other splenocytes, was assessed in a microfluidic platform, to investigate the direction of the migration in wild type and knock out splenocytes (**Figure 7a**).[61] A similar platforms was employed to examine the effect of immunogenic cancer cell death onto immune cells activation.[62] Schematics for other platforms have been proposed by Boussommier-Calleja *et al*.[60] One system would present one channel, lined with endothelial

cells flanked by two gel sections containing on one side immune cells and, on the other cancer and organ-specific cells. A cytokine gradient would be established throughout the system. The purpose of this system would be to evaluate cancer cell metastases to the blood vessels and the immune cells migration towards the site of the tumor. A more simple design would evaluated the interactions into a 3D tumor microenviroment model without the cytokine gradient.[60]

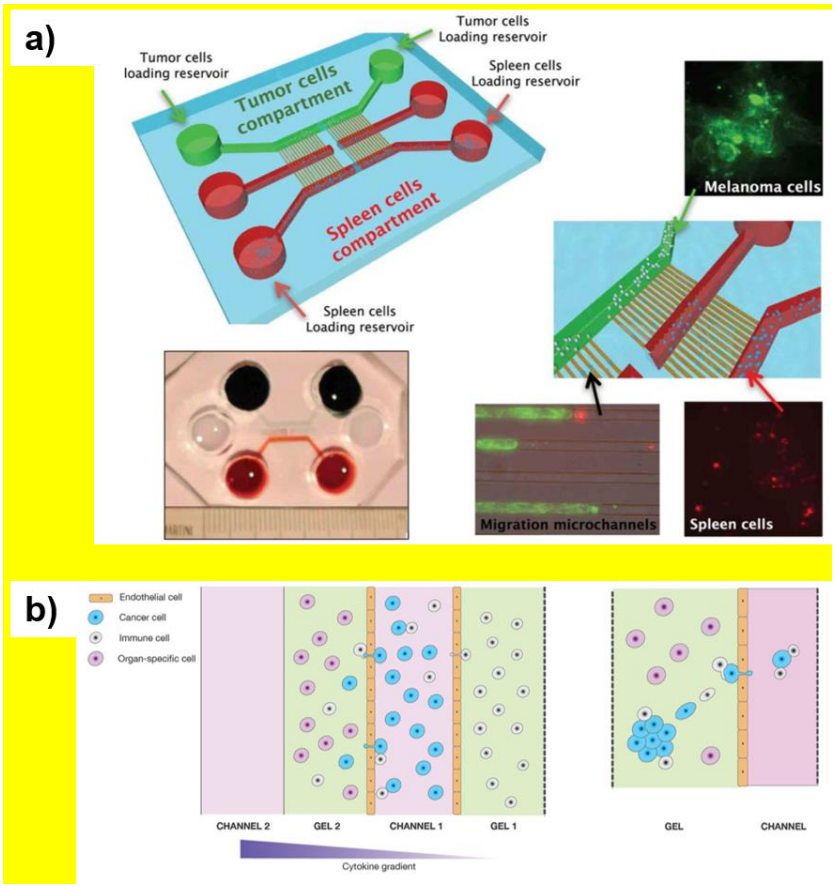

Figure 7. Examples of microchips to study the interaction between cancer and immune cells. **a)** microfluidics platform investigating the migration of splenocytes towards cancer cells. Reprinted with permission.[61] © 2014, Springer Nature, published under the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License; **b)** ideas to evaluate the interaction between the formation of metastases and immune cells under simulated blood flow conditions. Reprinted with permission.[60] © 2015, Elsevier Inc.

In summary, microfluidics has excellent potential to provide solutions for application in immunology, from the preparation of precisely controlled nanovaccines, to their evaluation into complex systems mimicking the tumor microenvironment and the immune organs. Thereby, we envision a growth of microfluidics applications in the immunology field in the next years.

3.3. Diabetes

Diabetes mellitus (DM) is a diagnostic term used for a group of heterogeneous disorders caused by abnormal glucose homeostasis.[63] Low insulin secretion by the cells or low insulin binding efficiency to its cell membrane receptors leads to high glucose levels, also known as hyperglicemia.[64] Chronic hyperglycaemia, in turn, often causes long-term complications such as dysfunction or even failure of several organs, including the eyes, kidneys, nerves, heart, and blood vessels.[65] The majority of DM cases can be categorized into two etiopathogenetic groups, type 1 and type 2.[65] Type 1 DM treatment relies on insulin replacement by self-injection, with doses adjusted on the basis of self-monitoring of blood glucose levels, in order to mimic the natural insulin fluctuation levels through the day.[66] Type 2 DM treatment focuses on delaying the progression of the disease via regulation of ingested food, physical exercise, weight management, and smoking cessation.[66-67] Oral and injectable medication such as the glucagon-like peptide-1 (GLP-1) are also recommendable to improve insulin production and function.[66, 68] However, the progression of the disease frequently ends up requiring insulin administration as well.[65] The therapeutic strategies currently used in DM treatment are, therefore, mostly based on the parenteral administration of drugs. Hence, pain and anxiety caused by daily injections, together with numerous associated adverse effects, including infections, insulin edema, local hypertrophy, hypoglycaemic episodes, and atherosclerosis, among others, dramatically reduce patient

compliance.[69] Here, we review the developing role of microfluidics in diabetes therapies, from diagnosis to management.

3.3.1 Microfluidics in the Diagnosis of Diabetes

Diabetes detection is most commonly made by blood glucose tests, however, irregular glucose levels are not a good indicator when it comes to early diagnosis of the disease.[70] It is urgent to find efficient methods for screening asymptomatic diabetes patients, and thereby reduce the subsequent physical, psychological, and economical burden. Several immune indicators are considered reliable for early diabetes detection, however, they require the use of complex test methods performed in relatively large facilities.[70] In 2012, Yao *et al.*[70] reported a cyber-based telemedicine system for insulin quantification in an integrated PDMS micro-fluidic system for early detection of diabetes. With the use of two pneumatic micropumps and immune microbeads in a micromixer, the authors could target insulin for further quantification by chemiluminescent assays (**Figure 8**). The controlling of the platform via wireless allowed the remote monitoring of the performance of the device, data collection and analysis by specialized medical doctors located off-site.[70]

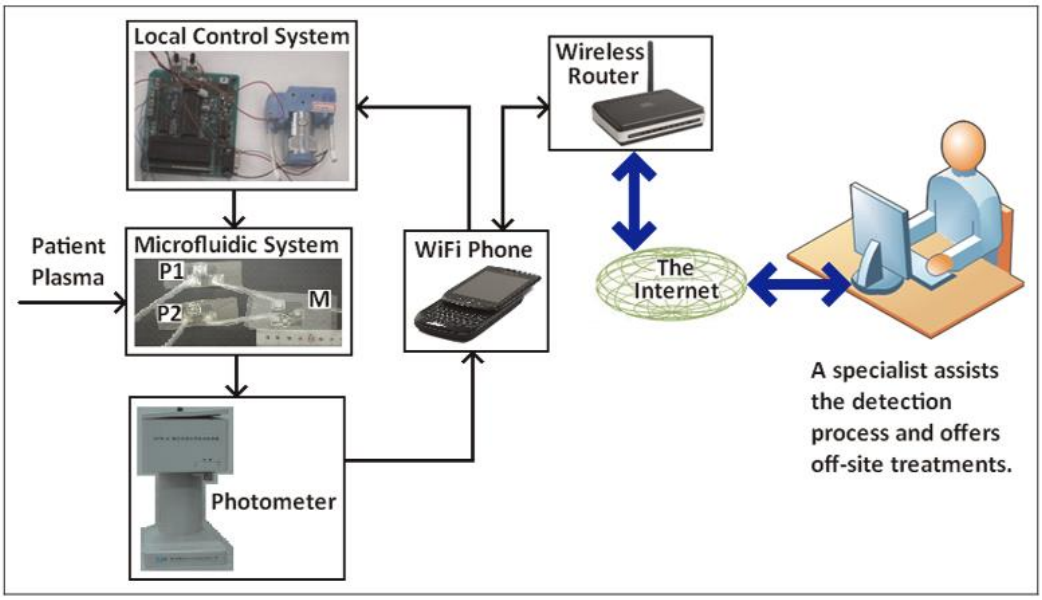

Figure 8. Architecture of the microfluidic-based telemedicine system. The insulin detection was performed in a PDMS-based microfluidic system equipped with beads for insulin targeting, and further supported by electronics and communication hardware for exchanging the control parameters and the results with off-site specialists. Reprinted with permission.[70] © 2013, SAGE Publications.

Yin *et al.*,[71] in 2016, reported an optical fiber sensor integrated microfluidic chip for ultrasensitive glucose detection. The device was based on a long-period grating (LPG) sensor fabricated in a small-diameter single-mode fiber (**Figure 9**). Poly (ethylenimine) (PEI) and poly (acrylic acid) (PAA) were added to the surface of the LPG sensor layer-by-layer, by self-assembly, for support and signal enhancement. A negatively charged glucose oxidase layer was further immobilized on the outer layer for glucose sensing. The biosensor was then embedded into the microchannel of the chip. Results showed the efficient detection of ultralow concentrations of glucose, in a remarkably fast and low sample consumption manner.[71]

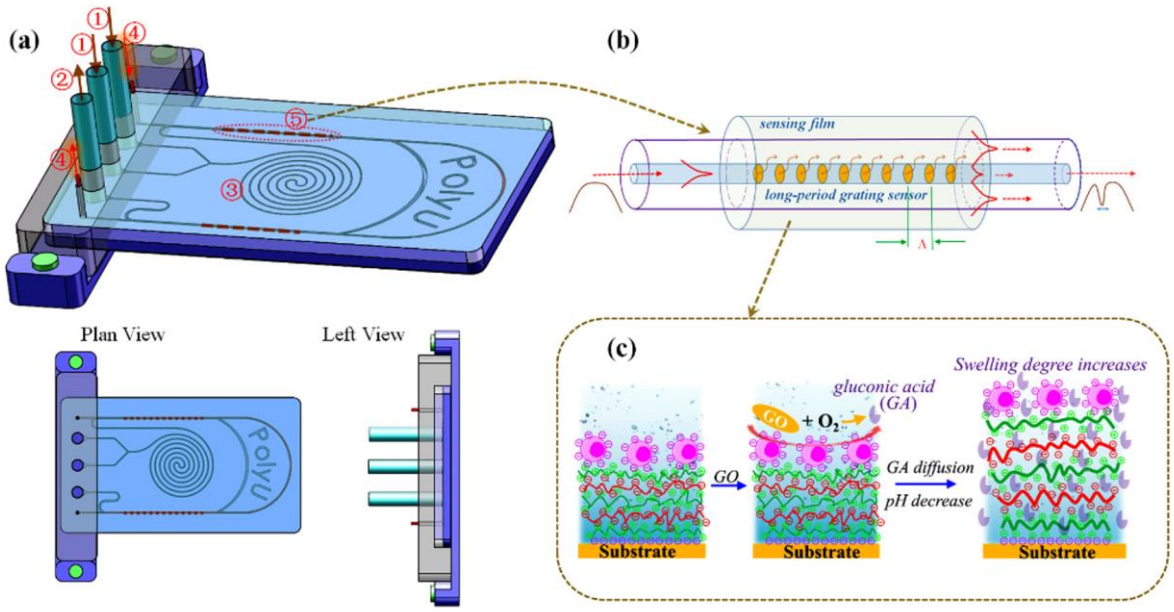

Figure 9. a) Schematic design of the optical fiber biosensor integrated microfluidic chip: (1) two inlets, (2) outlet, (3) spiral mixture, (4) optical fibers, (5) embedded LPG sensor; **b)** The

mode coupling and optical resonance in the LPG biosensor; **c)** Working mechanism of the multilayer film for glucose sensing and signal enhancement. Reprinted with permission.[71] © 2016, Optical Society of America.

Type 1 DM is characterized by increased levels of autoantibodies against insulin, glutamic acid decarboxylase, and insulinoma associated protein- 2, among others, and their detection enables clinical diagnosis of the disease.[72] In 2017, aiming at early diagnosis and predictive screening of diabetes in order to allow for efficient disease management before the appearance of life-threatening symptoms, Duan *et al.*[73] created an enhanced suspension assay multiplexed in microfluidic channels. The authors designed a microfluidic array that generated individual porous droplets made of a polyethylene glycol (PEG) smart hydrogel, and with detection chemistries optimized for the target biomolecules (**Figure 10a**). For this purpose, droplet hydrogels were spatially arrayed in a microfluidic serpentine array for multiplexing. Flow switching was used to direct unwanted droplets to a waste channel, while redirecting the droplets to the traps when the correct detection chemistry was generated. Then, the microfluidic flow was used to push assay reagents to the substrate surface. After UV curing, porogen molecules were washed away by buffer perfusion, generating the porous hydrogel droplets (**Figure 10b**). The validation of the device was completed by the addition of target antibodies into the microchannels, which provided varying fluorescence intensities according to the amount of antibody detected (**Figure 10c**).[73]

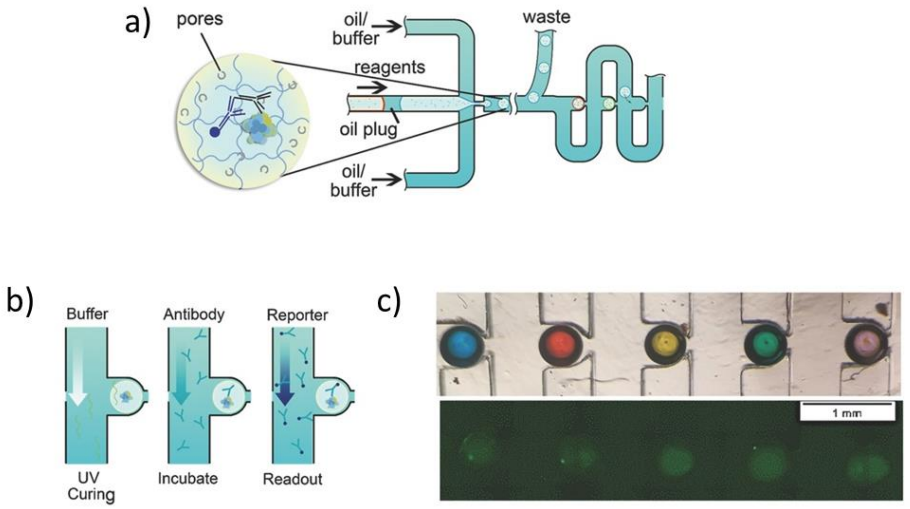

Figure 10. Quantitative microfluidic droplet array for diabetes detection. **a)** Individual droplets were generated with detection chemistries optimized for target molecules. The droplets were then spatially arrayed in designated traps within the serpentine channels. **b)** The serpentine microchannels allowed trapped droplets to be perfused by assay reagents. After UV curing, buffer perfusion was used to wash away the porogen molecules (yellow stripes), creating a porous hydrogel droplet. Target antibodies (blue) and reporter antibodies (green) were then introduced into the microchannels. **c)** The result of spatial multiplexing was an array of droplets targeting individual diabetes antibodies, illustrated on the top panel by the generation of multicolored droplets. In the actual assay (lower panel), only a single reporter wavelength was required, providing fluorescence intensities that varied at each spatial position per its respective antibody detection. Adapted with permission.[73] © 2017, WILEY–VCH Verlag GmbH & Co. KGaA, Weinheim.

Other examples of the most recent advances in the use of lab-on-chip technologies for the detection of diabetes can be found in **Figure 11**. Evans *et al.*[74] reported the development of a filter paper-based analytical device (µPAD) modified with silica nanoparticles. The µPAD improve the signal intensity and uniformity for clinically relevant enzymatic reactions, including glucose (**Figure 11A**).[74] Sechi *et al.*,[75] for instance, created a 3D µPAD by

combining wax printing fabrication techniques with the basic principles of origami. The assay is based in multiple and simultaneous colorimetric assays, can accommodate one or more analytes, and has been successfully used to test blood glucose levels (**Figure 11B**).[75] Kugimiya *et al.*,[76] in turn, proposed a fluidic microchip biosensor for amino acid detection using enzymatic reactions coupled to spectrophotometric measurements of hydrogen peroxide.[76] Two microchips with two chemical inlets each, and a serpentine for reaction were connected in series under optimized temperature settings (**Figure 11C**). The analysis could be completed within 30 minutes. Interestingly, beyond the molecular biomarkers, DM detection has also been achieved by the analysis of the stability and deformability of erythrocytes.[77] For this reason, Zhan *et al.*[78] developed simple microfluidic tool for examining erythrocyte fragility based on the characterization of osmotic lysis kinetics under hydrodynamic focusing (**Figure 11D**).[78] The latter generates rapid dilution of the buffer and produces lysis of erythrocytes during their flow. The release of intracellular contents is monitored and quantified by their light intensity throughout the channel.

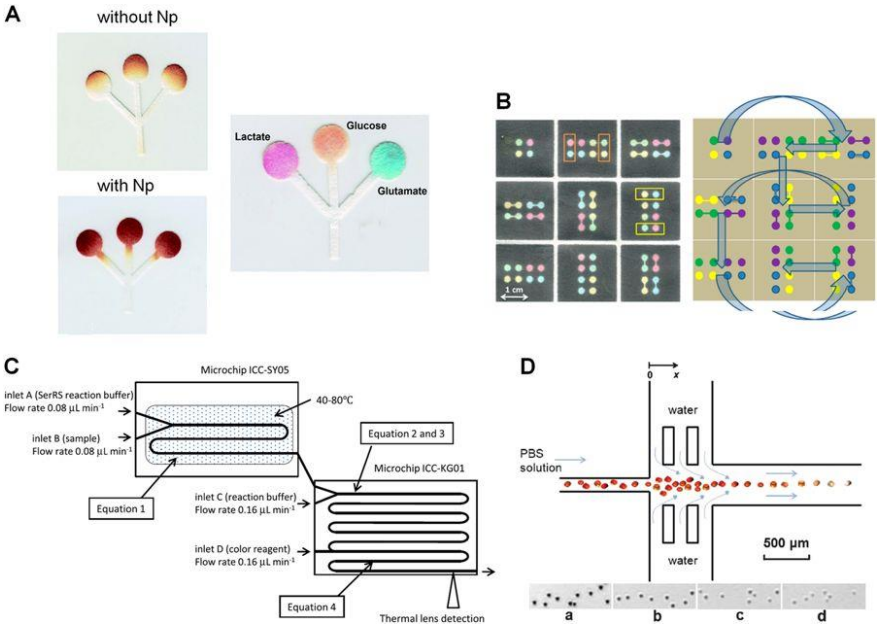

Figure 11. Examples of microfluidic devices for diabetes detection applications. **A)** Illustration of a µPAD to test glucose with and without silica nanoparticles; **B)** Easy and low-cost three-dimensional microfluidic paper device for glucose assay (flow pattern shown in the

right image); **C)** A microfluidic biosensor for the detection of amino acids using enzymatic reactions coupled with spectrophotometric detection; **D)** A simple microfluidic tool for studying erythrocyte fragility by analyzing osmotic lysis kinetics under hydrodynamic focusing. Reprinted with permission (https://creativecommons.org/licenses/by/4.0/legalcode) .[74-78]

Earlier this year, Furutani *et al.*[79] proposed a compact disc (CD)-shaped microfluidic device for multiple and rapid enzyme-linked immunosorbent assays (ELISA), which are widely used for the quantification of specific proteins. The device can perform the analysis of up to 15 samples simultaneously within 16 minutes, and consists of six different layers containing the microchannels, the reagent reservoirs, and the parts for reaction.[79] Also very recently, Professor Vipul Bansal, Director of the Ian Potter NanoBioSensing Facility at the Royal Melbourne Institute of Technology, announced that researchers from the same institute have developed a cost-effective and simple-to-use detection kit based on a microchip and a sensor coated with nanoparticles, capable of detecting blood markers that indicate the early loss of beta cells.[80] Such pioneering study was done in collaboration with Dr. Ravi Shukla, and currently also with engineers at the Micro Nano Research Facility (MNRF), aiming at the miniaturization of the sensor into a microfluidic chip. The kit is expected to be implemented as a standard test for newborns, in order to diagnose the disease in its earliest stages.[80]

3.3.2 Microfluidics in Diabetes Management

While devoting a significant amount of resources and money towards the development of efficient diabetes detection strategies, researchers from all over the world have also been investing tremendous efforts towards the creation of advanced drug delivery systems and technologies that can help several millions of people sentenced to lifelong medication. As mentioned above, DM can cause severe complications, including blindness, kidney failure,

heart attack, stroke, and lower-limb amputations, among others.[81] A proper management of the disease is therefore extremely important, not only to ensure an adequate glycemic control, but also to prevent future complications.[82]

The unprecedented advances of microfluidics have been expanding the amount of alternatives that can revolutionize diabetes management. Huang *et al.*[83] reported the development of a microfluidic system capable not only of measuring glucose concentrations in real-time, but also of automatically injecting insulin. This glucose sensing and insulin injection (GSII) chip was fabricated using micro-electro-mechanical-system (MEMS)-based technologies, comprising two modules: a PDMS-based microfluidic control module, including micropumps, microvalves and microchannels, and a sensing module, consisting of glucose sensing electrodes, a flow sensor (**Figure 12**).[83] With the GSII, 30-100 µL of sample were enough for an accurate determination of the glucose concentrations as low as 1.61 mM by the incorporated glucose sensor. Afterwards, precise amounts of insulin could be injected through a stick needle attached to a micropump while monitored by the flow sensor.

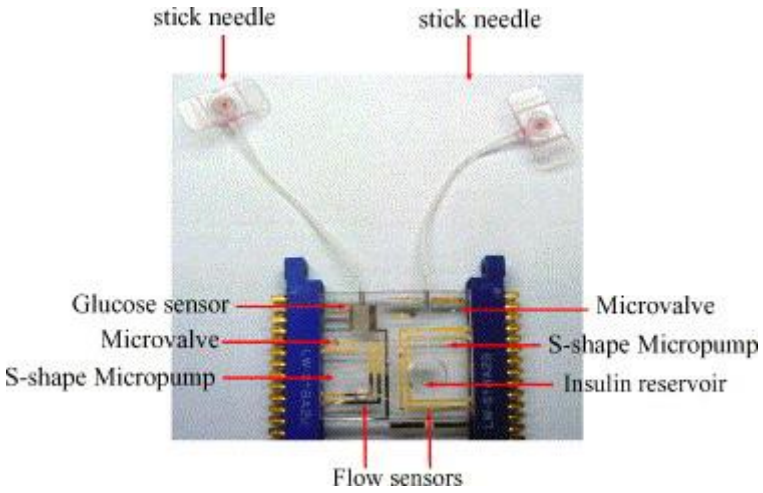

Figure 12. Photograph of the GSII microfluidic system, composed of a PDMS-based microfludic control module (microchannels, micropumps and microvalves), and a sensing module (electrochemical glucose sensor and flow sensors). Reprinted with permission.[83] © 2006, Elsevier B.V.

The adequate monitoring of blood glucose levels is an essential part of managing diabetes.[82] Following the discovery that the whole-blood glycated hemoglobin, particularly the glycated hemoglobin A1c (HbA1c) is the best marker for long-term glycaemic control,[84] Lee *et al.* [85] proposed a microfluidic device for self-monitoring of both blood glucose and HbA1c. In this device, the loaded blood sample was absorbed in the PDMS microchannel due to its hydrophilic nature. The application of AC voltage on the dielectrophoretic (DEP) electrodes incorporated in the device attracted red blood cells, while plasma remained flowing, therefore separating the blood into two main components.[85] The plasma was driven to the detection zone by the use of micropumps and microvalves for glucose detection. Afterwards, distilled water was injected into the microchannel for red blood cell lysis, the DEP force was turned off, the microvalves of HbA1c were opened, and the cell lysates were transported into the HbA1c detection zone. This system encompasses both sample pre-treatment and sensing, and could be used by diabetic patients for self-monitoring of blood glucose and HbA1c levels in a quick and automatized manner.[85]

As mentioned above, type 1 and type 2 DM therapies rely mostly on the use of injectable treatments that, for numerous reasons, suffer from low patient compliance.[86] Particularly, insulin subcutaneous injections are used as an attempt to replace the physiological insulin secretion, and dominate the popular perception of the life of a diabetic patient. Therefore, alternative routes of administration represent an important area of study. The use of advanced drug delivery systems such as micro- and nanoparticles has been demonstrating ground-breaking solutions for this problem, particularly aiming at the oral and pulmonary routes of anti-diabetic drug administration.[64, 66, 87] In fact, the emergence of quantum effects of nanosized materials renders them with useful physical, chemical and biological properties.[10d, 66] A wide variety of materials and production strategies have been applied in the development of particulate-based drug delivery systems for diabetes treatment, including the use of

mucoadhesive and mucopenetrating polymers,[88] adsorption enhancers and enzymatic inhibitors,[89] pH-responsive platforms,[90] and targeted drug carriers,[91] among others.

However, the high batch-to-batch variations, and the scale-up-related limitations of bulk production lead to unsatisfactory production rates, while microfluidic techniques are envisaged to overcome the restrained production of such promising drug delivery platforms.[10c] In 2015, Araújo *et al.*[89d] proposed the microfluidic preparation of GLP-1 and dipeptidyl peptidase-4 (DPP4) inhibitor loaded composites for diabetes therapy based on a flow-focusing geometry (**Figure 13**). The authors started by loading GLP-1 into poly(lactic-co-glycolic acid) (PLGA) and porous silicon (PSi) nanoparticles, which were further coated with chitosan, for mucoadhesive purposes. Afterwards, cell penetrating peptides (CPPs) were conjugated to the chitosan, due to their ability to harmlessly enter the cells, with the aim to increase transcellular transport.[92] Then, glass-capillary microfluidic techniques were used to encapsulate the nanoparticles together with dipeptidyl peptidase 4 (DPP4) into an enteric polymer (hydroxypropyl methylcellulose acetyl succinate; HPMC-AS; Figure 13).[89d] The produced nanoparticles showed high encapsulation efficiency and, importantly, an exquisite degree of reproducibility due to the microfluidics technique used. These studies were supported by the succeeding *in vivo* tests performed by the same authors, in which the PLGA-based particles were administered to a diabetic rat model.[89c] Results showed an increase in the hypoglycemic effects in a sustained and prolonged manner after the administration GLP-1 loaded nanoparticles, supporting the successful development of an oral protein/peptide delivery system for diabetes therapy.[89c]

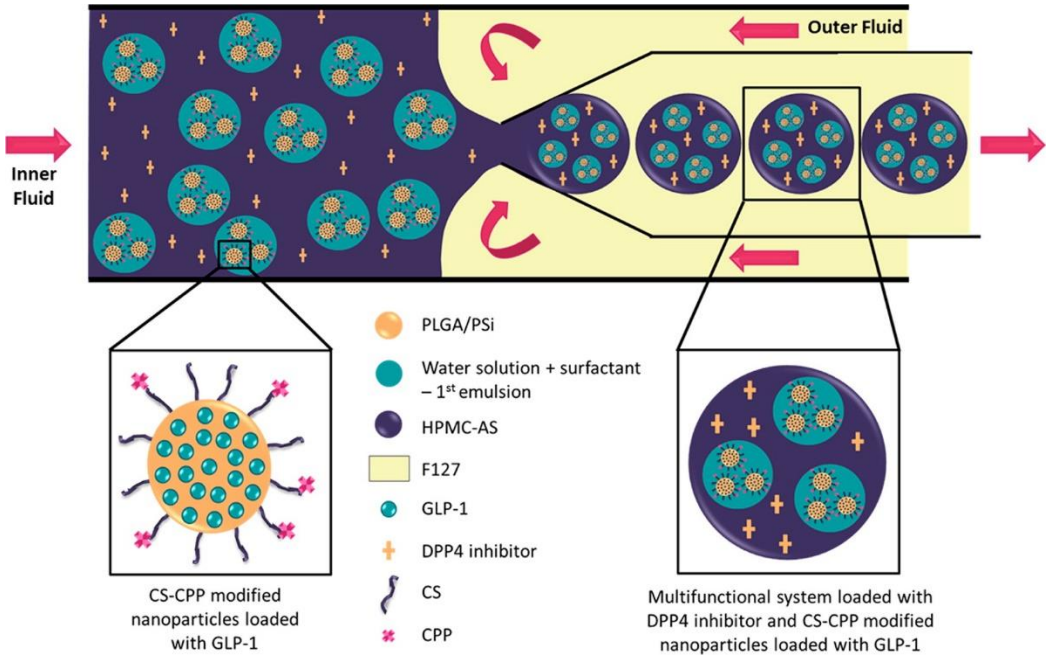

Figure 13. Schematic representation of a microfluidics glass capillary technique with a flow focusing geometry for the production a multifunctional drug delivery system for diabetes. The inner fluid consisted of GLP-1 loaded chitosan-coated PLGA or PSi nanoparticles in HPMC-AS solution, where the enzymatic DPP4 inhibitor was also dissolved. The outer continuous fluid consisted of an aqueous solution (F127) for stabilization of the final particles. Reprinted with permission.[89d]

3.3.3 Other Applications in Diabetes Therapy

Since impaired glucose metabolism can lead to low-grade inflammation and activation of the innate immune system, diabetic patients have increased risk of cardiovascular complications.[93] For this reason, leukocyte enumeration if frequently assessed in diabetic patients, but it is urgent to identify cell-based biomarkers to quantify specific immune functions of these cells, beyond their counting.[94] In this sense, in 2016, Hou *et al.*[95] described the creation of a microfluidic system for a single-step, rapid, and label-free isolation of neutrophils, which are the key effector cells of the innate immune system. The authors used Dean Flow Fractionation (DFF), a microfluidic cell sorting technology developed by the same

authors for size-based separation of diseased cells from the blood,[96] for neutrophil purification. The DFF device is a 10 cm long PDMS layer, and consists of a two-inlet and four-outlet spiral microdevice (**Figure 14**). With the use of Dean Forces, the neutrophils migrated to the outlets according to their size. After purification, the authors could evaluate their rolling behavior in E-selectin, which is a critical step in leukocyte recruitment during inflammation.[95]

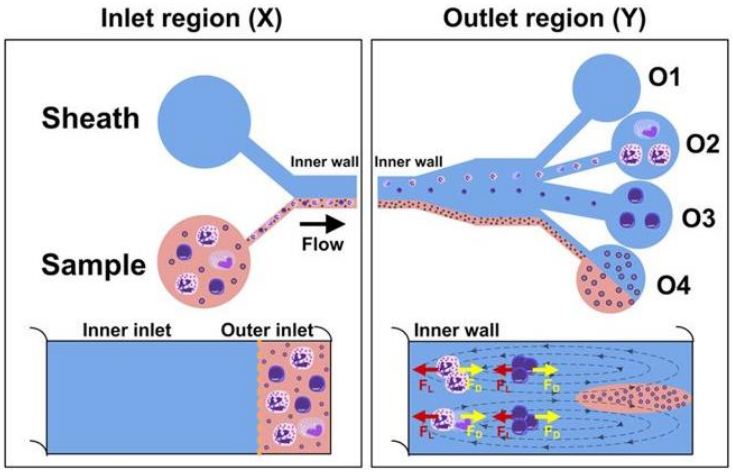

Figure 14. Schematic representation of the microfluidic chip for size-based separation of neutrophils, consisting of a two-inlet and four-outlet spiral PDMS microdevice. Adapted with permission (https://creativecommons.org/licenses/by/4.0/legalcode).[95]

Blood glucose level is regulated by a set of endocrine hormones released by different cells, with the intestinal and pancreatic cells playing a crucial role in this process.[97] Whereas insulin secreting β-cells are located in the pancreas, small intestine L-cells are the responsible for stimulating its secretion. Taking advantage of the outstanding versatility of microfluidics, Nguyen *et al.*[97] proposed an endocrine system aiming at the screening of drugs for diabetes therapy by measuring insulin release over time. The authors used a microfluidic perfused 3D cell-culture chip for co-culturing intestinal and pancreatic cells, in order to manage the effect of glucose on the secretion of GLP-1 by the intestinal cells, and insulin from the pancreatic ones. For this purpose, an epoxy-based photoresist was used to create a master, which in turn

was used to produce a PDMS layer through soft lithography techniques. After plasma treatment, the PDMS layer was attached to a glass slide. Small metal tubes were used to connect the inlets and outlets to flexible polymeric tubes. The microfluidic channel was divided into a wide central cell culture compartment and two wide side channels by elliptical micropillars. Results showed that the GLP-1 producing cells stimulated insulin secretion by the pancreatic cells, as well as that higher glucose concentration levels in the media lead to faster and higher insulin secretion.[97] Importantly, the authors suggest that the ability to study dynamic profiles of insulin secretion can be used to effectively screen new drugs by evaluating their effects on insulin production.

In the same line, Bauer *et al.*[98] developed a microfluidic two-organ-chip model, incorporating both pancreatic and liver cells. The fabrication of the device was based on PDMS molding, in which two cell culture compartments were connected by a microchannel. An on-chip micropump was used to create a pulsatile flow. The microfluidic chip allowed for the *in vitro* evaluation of the physiological crosstalk between pancreatic islet microtissues and liver spheroids, and the authors could show that insulin secreted in the circulation by the pancreas stimulates the glucose uptake by the liver cells.[98] Also, absence of insulin in circulation led to an impaired glucose consumption by the liver. Likewise, a decrease in glucose concentration also compromised insulin secretion, suggesting a functional feedback loop between the two tissues.

3.4. Other applications

Recently, RNA interference through the delivery of small interfering RNAs (siRNAs) has revealed great potency in silencing gene expression in different pathologies; however, in spite of the great efficacy of the method itself, the translation into treatment is challenged by the lack of approaches to safely and efficiently deliver the nucleic acids to target cells.[99] Microfluidics enabled the encapsulation of nucleic acids in both lipid and polymeric particles,

but the method was still far from optimal for the synthesis of such nanosystems, since the resulting formulation was too diluted, with the formation of precipitate.[35] Moreover, further steps (*e.g.*, sonication or dialysis) were still insufficient to produce particles homogeneous in size.[100] Thus, new engineering was urgently needed to achieve high-throughput screening synthesis of siRNA-loaded NPs. Chen *et al.*[101] developed a microfluidic system producing a library of 70 different lipid nanoparticles with a loading degree up to 80% of siRNAs through stepwise ethanol dilution. Following *in vitro* and *in vivo* gene silencing assays, the authors demonstrated the presence of a high rate of false negatives, up to 90%, highlighting the importance of the preliminary formulation screening in the development of nanosystems for siRNA encapsulation.

By adapting a similar microfluidic device, Rungta *et al.*[102] synthetized lipid nanoparticles encapsulating siRNAs against GRIN1, the gene encoding the GluN1 subunit of the NMDA receptor (NMDAR). The authors then assessed whether the delivery of siRNAs by the lipid nanoparticles successfully manipulated the expression of proteins involved in neuronal processes *in vitro* and *in vivo*. The herringbone structure of the microfluidic micromixer resulted in the production of lipid nanoparticles presenting a diameter between 50 and 60 nm and encapsulating the desired siRNA. The transfection was successful and led to robust knockdown of GluN1 expression both *in vitro* and *in vivo*, as demonstrated by western blots and functional disruption of NMDAR synaptic currents.

Other recent applications of microfluidics have been developed for the treatment of human immunodeficiency virus (HIV), acute liver failure (ALF) and inflammatory bowel disease (IBD). Indeed, Liu *et al.*[103] engineered a theranostic platform for ALF, a clinical syndrome resulting from the rapid loss in hepatocyte function, by using a glass capillary device. The microfluidic platform allowed the single-step co-encapsulation of porous silicon and gold nanoparticles into a polymeric matrix made of acetalated dextran (AcDEX) (**Figure 15**).

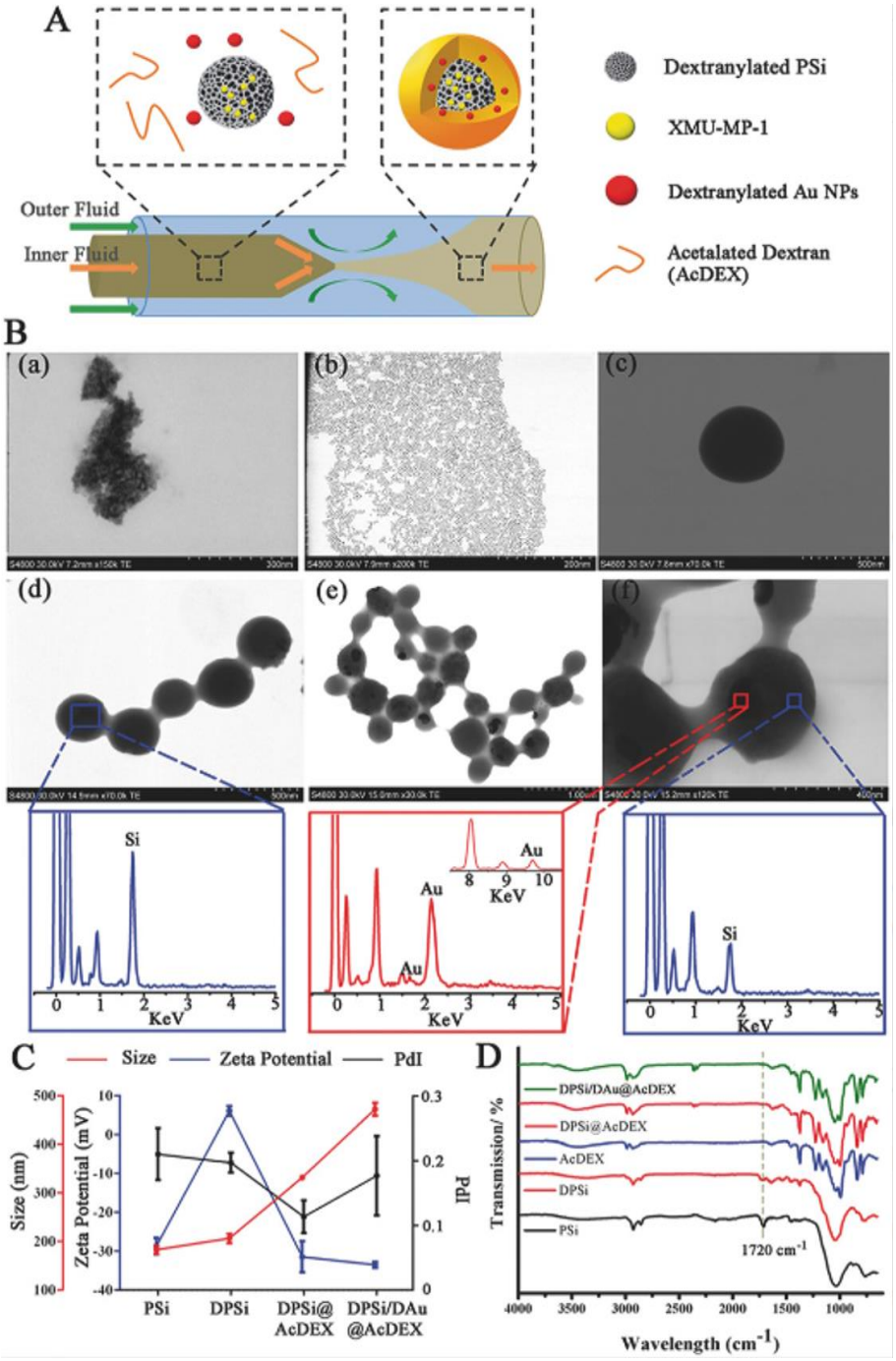

Figure 15. Preparation and characterization of DPSi/DAu@AcDEX. **A)** Schematic illustration of the microfluidic platform used to prepare DPSi/DAu@AcDEX. **B)** TEM images of **a)** DPSi, **b)** DAu, **c)** AcDEX, **d)** DPSi@AcDEX and **e,f)** DPSi/DAu@AcDEX with respective EDX spectra witnessing successful encapsulation **C)** Size (nm), PdI, and surface zeta-potential (mV) values at each synthesis step. **D)** FTIR spectra of PSi, DPSi, AcDEX, DPSi@AcDEX, and DPSi/DAu@AcDEX, demonstrating the change in surface chemistry.

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A novel small drug molecule, XMU-MP-1, was loaded in the pores of the porous silicon nanoparticles, improving both the efficacy of the compound and its biological profile, reducing the side effects. The role of gold nanoparticles was to image the affected tissue by CT-imaging. However, both drug encapsulation and use of gold nanoparticles present challenges: in fact, the confinement of drug molecules into the pores of porous silicon, while preventing their release proves challenging. As for gold nanoparticles, they should be small to penetrate into the cells and enable imaging, but this size range is also resulting into rapid excretion from the body. Considering this, Liu *et al.* decided to encapsulate both the nanosystems into a polymeric matrix of AcDEX, to prevent their own intrinsic limitations. Since this polymer features pH responsive properties, both drug molecules and gold nanoparticles are released inside the cells upon degradation of the polymeric matrix at endocellular pH. Altogether, the results obtained showed that the produced nanocomposite is actually a potential theranostic platform for ALF.

Another interesting platform produced single-step reassembling of high density lipoproteins (HDL) with antithrombotic effect, allowing also the simultaneous loading of simvastatin, gold, iron oxide NPs, quantum dots, or fluorophores, to produce a biocompatible theranostic platform.[104]

Regarding the application in HIV, Martins *et al.*[105] prepared a brain targeted poly(lactic-co-glycolic acid) (PLGA) nanosystem encapsulating Efavirenz (EFV) with both a conventional and a microfluidic method to compare the two different production methods (**Figure 16**).

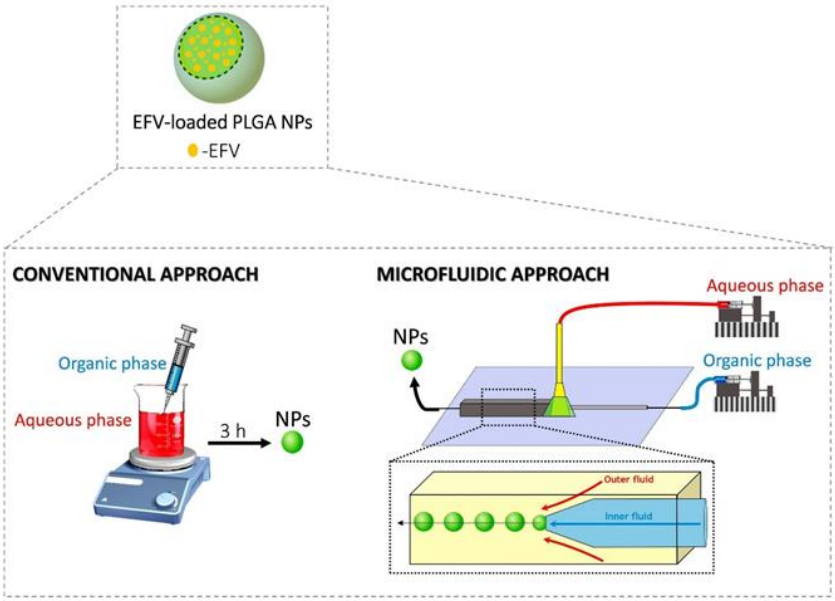

Figure 16. Schematic representation of both bulk and microfluidic methods for the preparation of EFV-loaded PLGA NPs. In both it occurs nanoprecipitation of a mixture of PLGA in DMSO (organic phase) into an aqueous phase, consisting of a solution of Tween®80. Reprinted with permission from.[105] © 2018, Elsevier B.V.

The encapsulation of EFV in the system it is crucial for its biodistribution to the brain, since the drug is not able to pass the blood brain barrier (BBB). Compared to the conventional method, the nanoparticles prepared by nanoprecipitation in a glass capillary device with co-flow geometry, resulted more efficient in encapsulating the drug (80.7% versus 32.7%) while being smaller in size (73 nm versus 133 nm). Since the particles produced by microfluidics exhibited better properties, Martins *et al.* continued their investigations only with those systems showing that they present a sustained release profile with only 50% of EFV released in 24h. Moreover, the final system, modified with a transferrin-receptor binding peptide, showed increased interaction with BBB cells and improved 1.3.-fold the permeability of the drug.

Microfluidics platforms have been developed to engineer nanoparticles for the treatment of IBD, a chronic and to date still incurable inflammatory disease of the intestine. Unfortunately,

current therapies are directed exclusively to address the symptoms of the disease while being characterized by heavy systemic side effects; thus, nanomedicine can represent a valid alternative, aiming to address the delivery of those drugs exclusively in the site of inflammation. In a recent study, Li *et al.*[106] prepared a composite nanosystem to overcome the intrinsic limitations of the different materials, engineering the particles to complete their journey across the gastro-intestinal tract and finally reach their target site. The system was based on porous silicon nanoparticles functionalized with hyaluronic acid (HA) to achieve retention in the intestinal mucosa. The nanoparticles were then coated by hydroxypropyl methylcellulose acetate succinate (HPMCAS) LF type, to achieve gastro-protection and dissolution of the protective capsule in the intestine at pH 5.5. Budesonide (BUD) was encapsulated in the pores of the PSi nanoparticles, together with ascorbyl palmitate (AP): AP is a small molecule, which forms a gel and induces the enzyme-responsive release of the drug once the system reaches the target site and meets enzymes that are upregulated and released in inflamed tissue. Microfluidic technique came into play during the encapsulation step, allowing the easy coating with the gastro-protective polymer HPMCAS-LF (**Figure 17**). As presented in **Figure 17d**, the final system (BUDAP@PSi-HA@LF) looks highly homogeneous in size and the PSi nanoparticles are successfully coated with HPMCAS-LF.

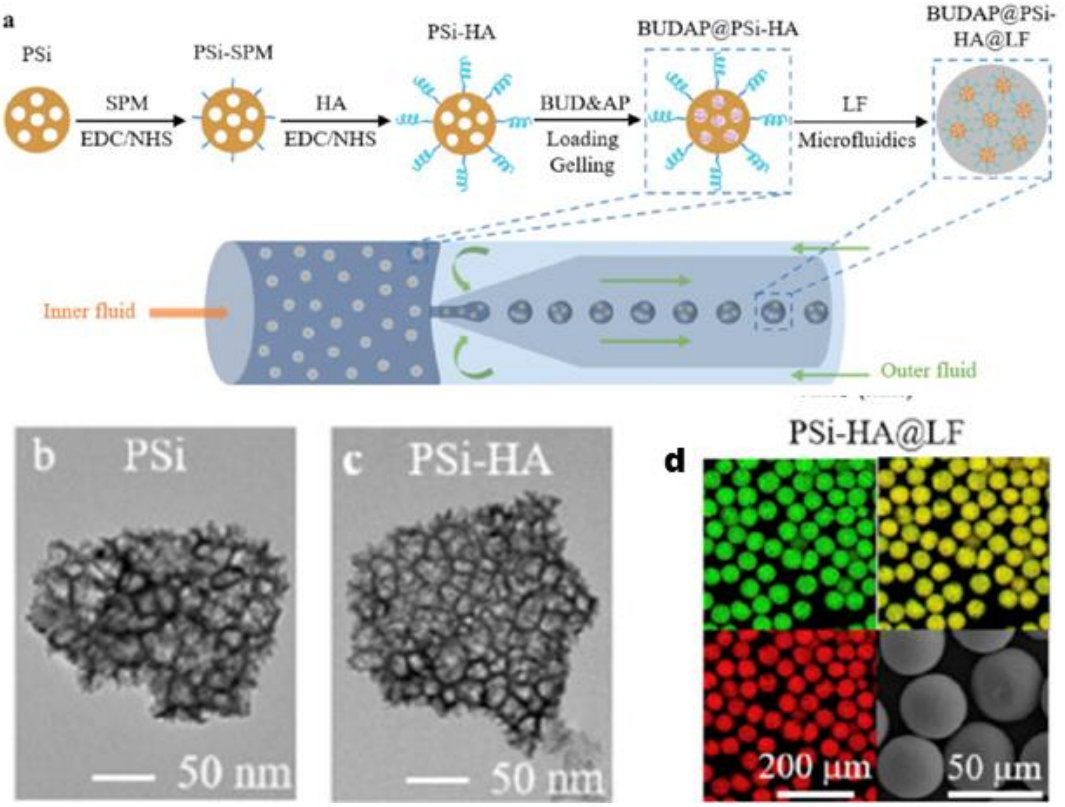

Figure 17. a) Schematics of the synthesis of BUDAP@PSi-HA@LF. **b,c)** Transmission electron microscopy (TEM) image of PSi and PSi-HA, respectively. **d)** Confocal and SEM images showing the monodispersity of PSi-HA@LF and the successful encapsulation of PSi-HA (green because conjugated with FITC) in LF (labelled with TRITC and thus red) through the resulting yellow color after overlaying red and green channels. Adapted with permission.[106] © 2018, Elsevier B.V.

Moreover, the final system showed total recover of the transepithelial electrical resistance (TEER) in the *in vitro* IBD model, demonstrating together with the decrease of release of IL-8 from cells that the composite NPs are able to induce a prolonged therapeutic effect. *In vitro* observations have been then confirmed by *in vivo* studies, which displayed mitigation of weight loss in colitis induced-animals treated with BUDAP@PSi-HA@LF, significant decrease of the disease activity index (DAI) and reduction of IL-1β and IL-6 levels. Furthermore, hematoxylin and eosin (H&E) stained histological sections of distal end of colon

tissues were examined and scored, showing high reduction in disease severity of the sections belonging to tissue treated with BUDAP@PSi-HA@LF (**Figure 18a**).

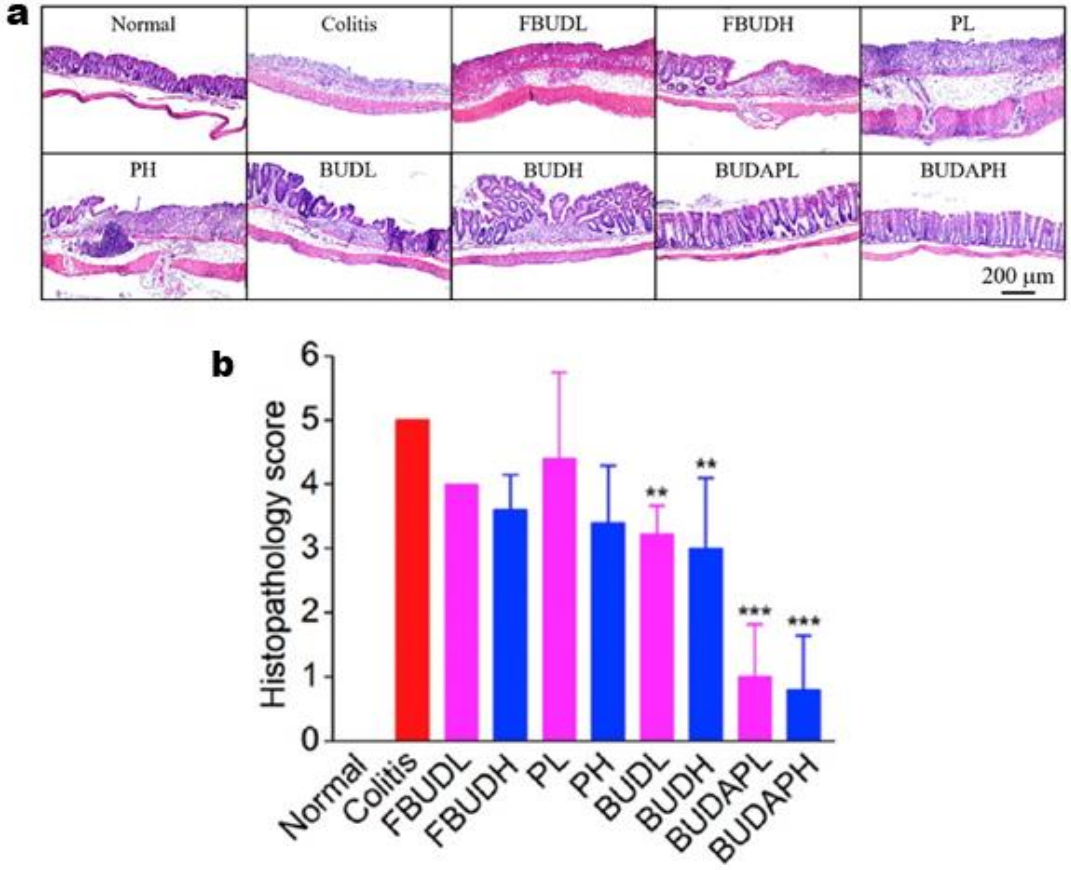

Figure 18. In vivo therapeutic efficacy of different BUD formulations on an IBD model of colitis induced in mice. **a)** H&E histology images of the distal end of colon after 7 d of treatment. **b)** Histopathology score of the distal end of colon after 7 d of treatment. Data are means ± SD (n = 5). *p < 0.05, **p < 0.01 and ***p < 0.001 versus the colitis group. Adapted with permission.[106] © 2018, Elsevier B.V.

Table 2. Summary of the applications of microfluidics in different diseases.

Application	Platform	Summary of the Findings	Ref.
Cancer	Glass capillary, various geometries; PDMS, various geometries	Production of homogenous micro-and nanoparticles, co-loading of various drugs with different physicochemical properties, enhancement of the drug dissolution rate, combinatorial production of different formulations	[6, 10a, 11a, 15b, 32, 35, 38, 39b, 46b, 47a, 47b, 49-50, 107]
Cancer Immunotherapy	Glass capillary, Polymeric, Hydrogel	Production of homogenous cancer vaccines, evaluation of immune cell migration in tumor, 3D models of the tumor microenvironment	[59-61]
Diabetes	Glass capillary, PDMS, Polymers, Paper	Production of multicomposite formulations for the oral delivery of insulin, determination of glucose and insulin concentration, early diagnosis of diabetes, analysis of the neutrophils	[70-71, 73-78, 83, 88a, 89d]
RNA Delivery	Different platforms	Successful encapsulation and enhanced delivery of RNA	[101-102]
Liver Failure	Glass capillary	Production of homogenous nanoparticles, co-encapsulation of imaging moieties, reduction in the drug side effects	[103]
Thrombosis	PDMS	Production of synthetic high density lipoproteins, with the simultaneous encapsulation of drug and theranostic moieities	[104]
HIV	Glass capillary	Production of homogenous particles, enhanced crossing of the blood-brain barrier	[105]
Intestinal Bowel Syndrome	Glass capillary	Production of multicomposite particles for the local delivery of drug, improved efficacy compared to the drug only	[106, 108]

4. Advantages, Disadvantages, and Industrial Application

There is no doubt that nanomedicine has gained increasing interest over years. The possibility to easily tune particle size, shape and functionalization represent the key factor making drug delivery systems attractive tools for the treatment of different pathologies.[109] However, despite the general enthusiasm spreading all over the world and the high innovation reached during the years, the translation of nanoparticles into clinic still represent a great challenge. At the base of this failure is the current lack of fabrication methodologies to control nanoparticles' properties (especially size and homogeneity in size), batch-to-batch reproducibility, and large-scale production. All these factors tremendously affect the translation into clinic, together with the inadequacy and/or absence of tools for rapid nanoparticles screening and platforms for *in vitro* testing, able to mimic a complex and dynamic system as the human body and guarantee good *in vitro-in vivo* correlations.[6, 40]

In this scenario, microfluidics offers a solution to the limitations imposed by bulk fabrication methods. The control over flow permit to obtain nanoparticles with high homogeneity in size

distribution and high encapsulation efficiency. Moreover, the technique offer advantageous reduction of reagents consumption due to the miniaturization of the process, which turns out to be very efficient.[10a]

Another benefit offered by microfluidics is the possibility to build up libraries of nanoparticles in a very easy way. Valencia *et al.*[50] indeed, synthetized a library of 45 different nanoparticles, by combining 15 different precursors in different ratios in a microfluidic integrated platform, meanwhile Wang *et al.*[110] were able to obtain 648 nanocarriers within just 2.5 hours by using a system able to sample, dilute, meter and mix different precursors in an automated way. The possibility to build up nanoparticles libraries in such a fast and easy way represents a great advantage in the nanoparticles' optimization process. In fact, when fabricating nanoparticles different features should be taken into account, like composition, size, charge, shape, softness and drug loading and thus, the optimization process is very time consuming and represents a challenge for conventional fabrication methods. The possibility to integrate the microfluidic device with a multi-inlet micromixer enables to mix different precursors before nanoprecipitation. Moreover, different parameters, like geometry of the device, polarity of the solvents, flow rates, concentration and composition of the precursors, can be tuned in order to obtain particles with specific characteristics.[6]

Another advantage provided by microfluidics is the ability to produce nanoparticles in a highly reproducible manner. The fact that the entire process in automated and regulated by pumps operating in a continue flow regimen, drops dramatically the possibility to have batch-to batch variations.[111] Moreover, the recent employment of a coaxial turbulent jet mixer by Valencia *et al.*[111b] allowed to produce nanoparticles up to 3 kg per day, showing that microfluidics it is also easy to scale-up and thus, appropriate for industrial scale production.

Despite that, there are still some drawbacks to face in order to have a total translation of microfluidics into industrial application. For example, choosing the material for chip

preparation it is not easy. The most common used, PDMS, is easy to manipulate and engineer in more complex channels structures, cheap and optically transparent.[11b] However, this material is sensitive to organic solvents, acids and bases, giving phenomena of swelling and degradation and has the tendency to absorb small molecules on its surface.[112] Moreover, it needs to be manipulated in clean room. Other options could be silicon or glass. Silicon has a very good chemical compatibility range and it is thermostable,[113] but it is very expensive, fragile and opaque to visible and ultraviolet light.[11b] Glass has several advantages; it is cheap, rigid, optically transparent, chemically inert, resistant to high temperatures and easy to modify.[113] However, it is difficult to handle and does not allow the construction of complex structures. Moreover, it requires production in clean room in some cases.[114] Altogether, it is difficult to choose the right material to build up a chip for nanoparticle fabrication, since all of them present different intrinsic challenges. In addition, the fabrication should be performed in dedicated microfabrication facilities and it requires specific equipment and personnel with particular skills. Finally, it should be also considered that complex designs are often required for parallelization, which is important to achieve large-scale production.[6]

In overall, microfluidics holds great promises in the translation of nanomedicine into clinic and industrial production. The possibility to produce different types of nanocarriers at industrial scale, with very low batch-to-batch variations makes microfluidics a versatile and attractive alternative to conventional fabrication methods. The fast and constant advance of both microfluidics and material science will bring solutions to overcome the production challenges in a not too distant future.

5. Conclusions

This review focuses on the applications of a wide variety of microfluidic devices reported in the literature both as platforms for the preparation of advanced biomaterials and as sensors. Microfluidic technologies gained tremendous relevance in the past decade, and their ongoing

sophistication holds the promise of revolutionizing the current challenges of cancer, immunotherapy, and diabetes therapy. The exquisite control over the miniaturized materials and platforms, the increased batch-to-batch reproducibility, and the possibility for large-scale production have already shed a light on the microfluidics exciting opportunities in potentiating the clinical translation of nanotechnology-based therapies. The acceptance and effective translation of the technique awaits now for the joint efforts between researchers of different fields, from biologists to engineers, the pharmaceutical industries, and clinicians to become a reality.

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Author Photograph(s) ((40 mm broad, 50 mm high, gray scale))

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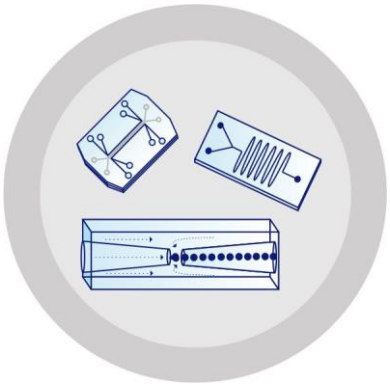
Microfluidics displays multiple applications in the treatment of complex diseases. The nuts and bolts to engineer a platform for the production of drug delivery system are analyzed. Moreover, the application of microfluidics platforms as sensor and for the production of nanotherapeutics in cancer, immunotherapy, diabetes and other diseases are reviewed. Finally, the challenges still remaining for industrial and clinical use are reported.

Microfluidics

F. Fontana[†], J. P. Martins[†], G. Torrieri, H. A. Santos*

Nuts and Bolts: Microfluidics for the Production of Biomaterials



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