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Nanomaterial Preparation by Extrusion through Nanoporous Membranes

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Template synthesis represents an important class of nanofabrication methods. Herein, recent advances in nanomaterial preparation by extrusion through nanoporous membranes that preserve the template membrane without sacrificing it, which is termed as "non-sacrificing template synthesis," are reviewed. First, the types of nanoporous membranes used in nanoporous membrane extrusion applications are introduced. Next, four common nanoporous membrane extrusion strategies: vesicle extrusion, membrane emulsification, precipitation extrusion, and biological membrane extrusion, are examined. These methods have been utilized to prepare a wide range of nanomaterials, including liposomes, emulsions, nanoparticles, nanofibers, and nanotubes. The principle and historical context of each specific technology are discussed, presenting prominent examples and evaluating their positive and negative features. Finally, the current challenges and future opportunities of nanoporous membrane extrusion methods are discussed.

1. Introduction

Nanoporous membranes are excellent templates for nanomaterial fabrication. Over the past three decades, nanoporous membrane-assisted synthesis, also called "template synthesis," has become one of the most important nanofabrication methods, and has significantly contributed to the development of functional nanomaterial research ranging from electrochemical energy storage to drug delivery.[1–20] This template synthesis method generally entails synthesizing the desired materials within the nanoscale pores of a membrane, and depending on the physical parameters of nanopores, the size, shape, and structure of synthesized nanomaterials can be readily controlled. To date, template synthesis on the nanoscopic scale has been successfully utilized to process polymers,[2–6] metals,[7–9,14] semiconductors,[17,18] and other materials.[13,15,16,19,20] However, conventional template synthesis methods commonly require sacrificing the template membrane toward the end of the nanofabrication process to free the synthesized nanomaterials. This template removal step increases the fabrication cost and technical difficulty, and limits the yield of nanomaterials. Moreover, template removal approaches frequently utilize harsh chemical or physical environments (e.g., acids or organic solvents) that could potentially damage the synthesized nanomaterials.[15,19,21] For these reasons, extensive efforts have been devoted to develop new template synthesis methods that can preserve the nanoporous membrane instead of sacrificing it, which significantly improves this nanofabrication process in a greener and more economical manner.

Given that conventional template synthesis methods with a template removal step have been extensively reviewed in the literature,[22–26] the objective of this article is to provide an overview of the recent advances in nanoporous membrane extrusion strategies, including vesicle extrusion, membrane emulsification, precipitation extrusion, and biological membrane extrusion, which have not been systematically reviewed to date. The types of nanoporous membranes used in these applications are discussed, along with the mechanisms, critical parameters, historical context, and prominent examples of each specific nanoporous membrane extrusion strategy. We also discuss the potential that the continuation of innovation in nanoporous membrane extrusion techniques and integration with interdisciplinary approaches can bring with respect to the promotion of industrial and biomedical applications of nanomaterials.

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2. Membranes Used

Three types of nanoporous membranes are most commonly used in nanoporous membrane extrusion methods: track-etch membranes, anodic aluminum oxide (AAO) membranes, and Shirasu porous glass (SPG) membranes. All three membranes are commercially available with associated extrusion devices to meet the need for laboratory and industrial applications.

2.1. Track-Etch Membranes

Track-etch membranes are defined as micro- or nanoporous membranes prepared by the track-etch method.[27] This method entails bombarding a nonporous sheet of the desired material with nuclear fission fragments to create damage tracks in the material, and then chemically etching these tracks into micro- or nanoscale pores. Polymeric and inorganic materials have been demonstrated to prepare track-etch membranes, such as polycarbonate (PC), polyethylene terephthalate (PET), mica, and silicon nitride.[27–31] Track-etch membranes feature cylindrical shape nanopores with pore sizes ranging from 10 nm to 12 μm (**Figure 1**A,B).[32] In additional to a cylindrical shape, other shapes of nanopores (e.g., conical or diamond shape) have also been prepared by varying the chemical etching conditions.[15,33–35] Track-etch membranes have been commercialized for filtration applications, and the most widely used ones are prepared from polycarbonate and polyester.[36–38] The membrane thickness of commercial track-etch polycarbonate (PCTE) membrane is between 6 and 25 μm with nanopores randomly distributed in the polymeric substrate. The pore density of track-etch membranes can be readily tuned from 1 to 10^8 pores per cm^2 by adjusting nuclear fission bombarding tracks.[1,4,22] The advantages of track-etch membranes are their low-cost, chemically inertness, and durability with a maximum tolerated pressure of over 3000 psi.[39] Track-etch membranes, especially PCTE membrane, are widely used in extrusion methods under high pressure (e.g., vesicle extrusion). The major limitation of track-etch membranes is their low porosity (1–20%) that is relatively lower than AAO and SPG membranes (up to 60%).[40] Moreover, the random distribution of nanopores makes it difficult to control the spacing distance between nanopores. This frequently causes two nanopores to merge together (Figure 1A), resulting in a pore diameter coefficient of variation (CV) as high as 76%.[41] This may also cause nanomaterial aggregation during the nanofabrication process.

2.2. AAO Membranes

AAO membranes are prepared from aluminum thin film using an anodization process.[42] During anodization, an aluminum metal sheet is electrochemically etched into a highly uniform and self-organized nanoporous structure arranged in a hexagonal array (Figure 1C,D).[43] Similar to track-etch membranes, the pore shape of the AAO membrane is also cylindrical. The pore size of AAO membranes ranges from 5 to 500 nm with a high pore uniformity (CV ≈ 10–20%).[44–46] The porosity of AAO membranes has a wide range from 3% to 60% and the pore density of AAO membranes can reach as high as 10^{12} pores per cm^2.[44,47] This highly porous characteristic makes the AAO membrane the most commonly used template in conventional template synthesis methods since its higher pore density leads to a higher yield of nanomaterials in comparison with other low pore density membranes. The thickness of commercial AAO membranes ranges from 5 to 200 μm. The major advantage of an AAO membrane is its highly uniform nanopore pattern and extremely high pore density. AAO membranes with a high maximum rupture pressure of 100 000 psi has also been reported.[48] However, AAO membranes are not as chemically inert as track-etch membranes.[49] The commercial AAO membranes only offer a narrow pore size range between 10 and 200 nm and are more expansive than track-etch membranes due to the anodization fabrication process.

2.3. SPG Membranes

SPG membranes are prepared from calcium aluminoborosilicate glass that is made from "Shirasu," a Japanese volcanic ash.[50–53] To prepare SPG membrane, refined Shirasu is mixed with calcium carbonate and boric acid and heated to achieve glass fusion, then phase separation of calcium-borate-rich glass and aluminosilicate-rich glass is formed via annealing. Micro-/nanopores are eventually formed by leaching out calcium borate with acid. Unlike track-etch and AAO membranes' straight-through nanopores, the nanopores in SPG membranes are interconnected with each other in a tortuous path (Figure 1E,F)[54] which provides a higher transmembrane flux. SPG membranes have a wide spectrum of pore sizes ranging from 50 nm to 30 μm (CV ≈ 20%) and a high porosity from 50% to 60%.[50,53,55,56]

The shape of a SPG membrane is usually that of a tube instead of a sheet, and the tube outer diameter is 10 mm with a membrane thickness of ≈0.45–0.75 mm.[50,53,55] The surface wettability of SPG membranes can be modified by reaction with organosilanes, such as octadecyltrichlorosilane.[57,58] The major advantages of SPG membranes are that they are chemically and thermally stable with extremely high pore density. SPG membranes can be easily cleaned and recycled by incineration due to their high thermal stability.[59] However, the nanopore channels in SPG membranes are not so uniform and well-defined as track-etch and AAO membranes, which could limit their size control capability in preparing nanoscale materials.

3. Nanoporous Membrane Extrusion Strategies

3.1. Vesicle Extrusion

Vesicle extrusion is one of the most widely used liposome preparation techniques.[60–62] Its mechanism is based on a nanoporous membrane extrusion procedure whereby pre-formed multilamellar vesicles (MLVs) are forced through nanopore channels in a membrane to obtain monodisperse unilamellar liposome vesicles (**Figure 2**A,B).[63] In a typical vesicle extrusion procedure, natural or synthetic lipids (e.g., 1,2-dioleoyl-sn-glycero-3-phosphocholine, cholesterol, and others) are first

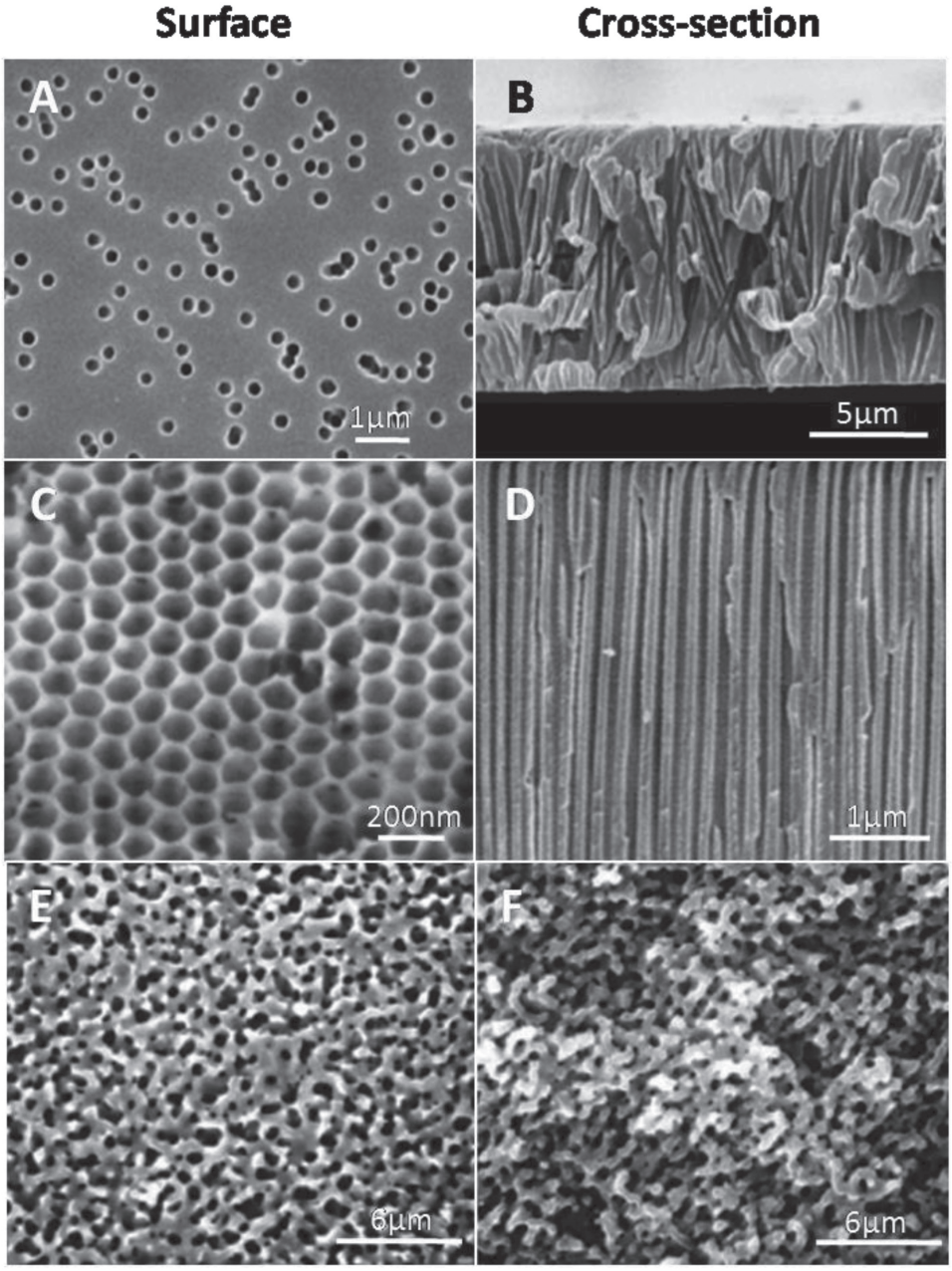

Figure 1. Scanning electron microscopy (SEM) images of A,B) a track-etch membrane (PCTE), C,D) an AAO membrane, and E,F) a SPG membrane. For each type of nanoporous membrane, both A,C,E) surface and B,D,F) cross-section images are presented. Reproduced with permission.[32,43,54] Copyright 2005, Elsevier; Copyright 2010, IEEE; Copyright 2010, Elsevier.

dried into a thin lipid film using an evaporator or inert gas blowing. This lipid film is then hydrated in aqueous solution and phospholipid molecules in the lipid film spontaneously self-assemble into MLVs with sizes ranging from 0.5 to 10 μm during their dispersion into the aqueous solutions. In order to improve their lamellarity, obtained MLVs usually go through 10 freeze/thaw cycles that facilitate lipid molecule rearrangement in the lipid bilayers. These pre-formed MLVs are extruded through double-stacked nanoporous membranes with defined pore sizes of 100 or 200 nm. During this extrusion procedure, MLVs are forced to enter narrow nanopore channels that are significantly smaller than their diameters, leading to the rupture of the MLV's lipid membrane and continuous formation of unilamellar liposomes with a single lipid bilayer inside the nanopore channel. These formed unilamellar liposomes are carried away by the continuous pressure flow and released at the exit of nanopore channels in the reverse side of the membrane. This extrusion process is usually repeated five to ten times to achieve a desired size distribution with the mean diameter of obtained liposomes usually reflecting the diameter of the nanopore. A critical parameter in vesicle extrusion is to make certain that MLVs are extruded at a temperature higher

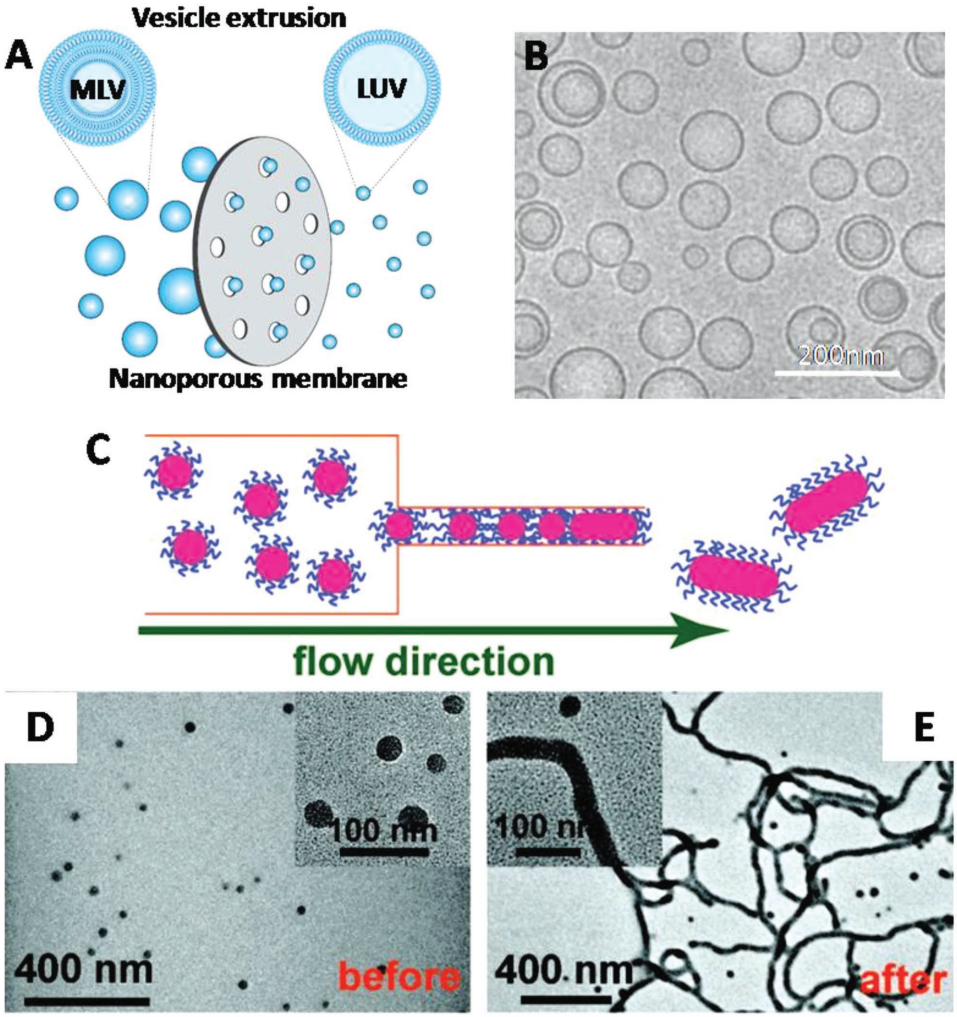

Figure 2. A) Schematic illustration of the vesicle extrusion process for liposome preparation. B) Transmission electron microscopy (TEM) image of liposomes prepared by vesicle extrusion. Reproduced with permission.[63] Copyright 2005, Elsevier. C) Schematic illustration and D,E) TEM images of vesicle extrusion method for preparing 1D cylindrical micelles. Reproduced with permission.[104] Copyright 2009, American Chemical Society.

than their lipids' gel–liquid crystalline phase transition temperature (Tc), which allows the lipids of MLVs to enter the liquid phase and provides enough flexibility in the lipid membrane to form unilamellar liposomes. Track-etched polycarbonate (PCTE) membranes are the most commonly used nanoporous membranes for vesicle extrusion applications.[64–67]

Vesicle extrusion method was originally developed by Olson et al. when they demonstrated the preparation of nanoscale unilaminar liposomes (≈270 nm in diameter) through a sequential extrusion under low pressures (<80 lb in.$^{-1}$) using a series of nanoporous membranes with decreasing pore size.[64] Later, Olson et al. reported the preparation of the chemodrug, doxorubicin, encapsulating liposomes using the vesicle extrusion method followed by evaluating their in vitro and in vivo toxicity and therapeutic efficacy against leukemia cells.[68] Since then, extensive studies have been carried out to investigate the influential parameters (e.g., lipid composition, nanoporous membrane properties, extrusion pressure, etc.) on the vesicle extrusion procedure as well as its industrial and biomedical applications.[69–72] Uniform nanoscale liposomes are the major product of vesicle extrusion and they currently play a pivotal role in drug delivery applications.[73–76]

A liposome is a spherical synthetic lipid vesicle composed of a single lipid bilayer with a nanoscale diameter ranging from 60 nm to a few micrometers. Due to their unique hollow structure, liposomes have been widely used as a drug delivery system to protect and deliver therapeutic agents including chemodrugs,[77,78] small molecule inhibitors,[79,80] siRNAs,[81,82] DNAs,[83,84] proteins/peptides,[85,86] and recently developed CRISPR-Cas gene editing systems.[87–89] The surface of liposomes is frequently modified with polyethylene glycol (PEG) to reduce cell uptake by immune cells and extend the liposome blood circulation time.[90–92] The surface of liposomes can be further conjugated with targeting ligands (e.g., antibodies, peptides, aptamers, etc.)[93–96] to facilitate targeted drug delivery. To date, liposomes are the most widely used nanomedicines in the clinic with over 10 liposome-based drugs approved for treating a variety of diseases from cancers to fungal infections to pain management. Over 20 liposome formulations are currently being tested in clinical trials.[76,90,97] Among all clinically approved

liposomes, PEGylated and non-PEGylated doxorubicin-encapsulating liposomes (trade name: Doxil and Myocet) are the most successful examples. Doxil was approved by the United States Food and Drug Administration (US FDA) in 1995 as the first clinically approved nanomedicine for treating ovarian cancer and multiple myeloma.[98] The clinical studies have shown that these liposome formulations successfully reduced the cardiotoxicity of their chemotherapy payload, doxorubicin. For example, Myocet has shown a substantially improved maximum tolerated dose of doxorubicin compared to free doxorubicin from 480 to 2200 mg m^{-2}, resulting in an 80% lower risk of cardiotoxicity.[99] The most recently approved liposome-based drug is irinotecan liposome (trade name: Onivyde), which increases the survival of patients with metastatic pancreatic cancer, one of the most lethal cancers, for up to four months.[100–102]

Vesicle extrusion has also been extended to produce 1D nanomaterials (Figure 2C). For example, Guo et al. reported the creation of a two-step vesicle extrusion method to prepare lipid nanotubes (LNTs).[103] They first extruded glycolipid *N*-(11-*cis*-octadecenoyl)-a-D-glucopyranosylamine at 90 °C through a 100 nm PCTE nanoporous membrane to form liposomes, and the obtained liposomes were immediately extruded again using a 200 nm AAO membrane to form LNTs. The obtained LNTs featured controlled diameters from 148 to 392 nm with a wall thickness between 48 and 145 nm, and the length of LNTs ranges from few to tens of micrometers. This method has also been used to prepare cylindrical micelle nanotubes. For example, Chen et al. reported that they extruded polystyrene-*b*-polyisoprene diblock copolymer micelles through a 20 nm AAO nanoporous membrane.[104] Due to the confinement of nanopore channel, several spherical micelles fused into a cylindrical tube and pushed out at the outlet of nanopore (Figure 2D,E).[104] The obtained cylindrical micelle features a high aspect ratio of few micrometers in length and 33 nm in diameter. Later, Chen et al. continued to apply this vesicle extrusion method to prepare gold nanoparticle-loaded cylindrical micelles.[105]

Unlike other established liposome preparation methods (e.g., sonicating, stirring, and freeze drying), using vesicle extrusion to prepare liposomes is not limited by lipid solubility and composition, and its reproducibility is extremely high. However, the vesicle extrusion method also has some drawbacks. Vesicle extrusion can only effectively prepare liposomes with a narrow size range of 40–150 nm but liposomes within this size range are considered as the most useful ones for biomedical applications due to their ability to avoid immune cell uptake.[106] The entire procedure for vesicle extrusion method is relatively longer and more complicated than other established liposome preparation methods. In addition, the vesicle extrusion method suffers from a higher product loss caused by the nanopore filtration effect. The filtered lipids and drugs continuously accumulate on the feeding side of the nanoporous membrane during the nanofabrication process, which may clog the nanopore channels in scale-up productions.

3.2. Membrane Emulsification

Membrane emulsification is a nanoporous membrane extrusion method for preparing monodisperse emulsions.[50,55,107] There are two common types of membrane emulsification: direct membrane emulsification and premix membrane emulsification (**Figure 3**A).[53,107–109] In a typical direct membrane emulsification process, a dispersed liquid phase (e.g., oil) is forced to permeate through a nanoporous membrane into another immiscible liquid phase (e.g., water) under a continuous flow. The function of nanopore channels in membrane emulsification is to break up large dispersed phase droplets into small, uniform micro-/nanoscale droplets. Small droplets of dispersed phase are formed at the exits of the nanopore channels and are carried away by the shear stress across the membrane surface from continuous phase. The resulting mixture solution contains insoluble droplets of dispersed phase is called emulsion (Figure 3B),[110,111] and the most common emulsion is an oil (o)/water (w) mixture. This membrane emulsification method has been successfully demonstrated in the preparation of various types of emulsion droplets including o/w, w/o, w/o/w, and o/w/o, etc.[112] The obtained emulsion solution can be further processed into solid nanoparticles via sequential treatments such as polymerization, solvent evaporation, or crystallization.[113]

There are several differences between membrane emulsification and the previously discussed vesicle extrusion method. First, direct membrane emulsification utilizes a nanoporous membrane to emulsify the bulky dispersed phase into uniform nanoscale droplets instead of downsizing pre-formed lipid vesicles. Nevertheless, a premix membrane emulsification method was later developed by adopting a similar procedure to the vesicle extrusion method.[108,114] In the premix membrane emulsification procedure, coarse emulsion droplets are first prepared by mixing two immiscible phases (oil and water phases) using a conventional stirring method after which the coarse emulsion droplets are forced through a nanoporous membrane to be downsized into uniform micro-/nanoscale emulsion droplets (Figure 3A). The advantage of this premix membrane emulsification method is that its emulsion droplets exhibit a better uniformity and dispersity in comparison to those prepared by direct membrane emulsification. Second, in membrane emulsification, nanopore size more closely controls the size of emulsion droplets in comparison with vesicle extrusion. Schröder et al. found a linear relationship between the average membrane pore diameter and the average emulsion droplet diameter.[115] It indicated that the average emulsion droplet diameter formed by membrane emulsification is usually 2 to 10 times larger than its average membrane pore diameter. In addition, the porosity of the membrane surface also has a critical role in preventing coalescence because if two nanopores come too close to each other, the newly formed emulsion droplets at the exits of nanopore will contact and fuse with other droplets, leading to coalescence. The maximum membrane porosity for preventing emulsion droplet coalescence has been calculated by Abrahamse et al.[116] Third, surfactant molecules (e.g., sodium dodecyl sulfate (SDS)) are frequently added into continuous phases to protect the newly formed emulsion droplets from coalescence in a membrane emulsification.[117–119]

SPG membranes are the most commonly used nanoporous membranes in membrane emulsification applications.[50,53,55,57–59] In addition to the SPG membrane, other nanoporous membranes have also been used in membrane

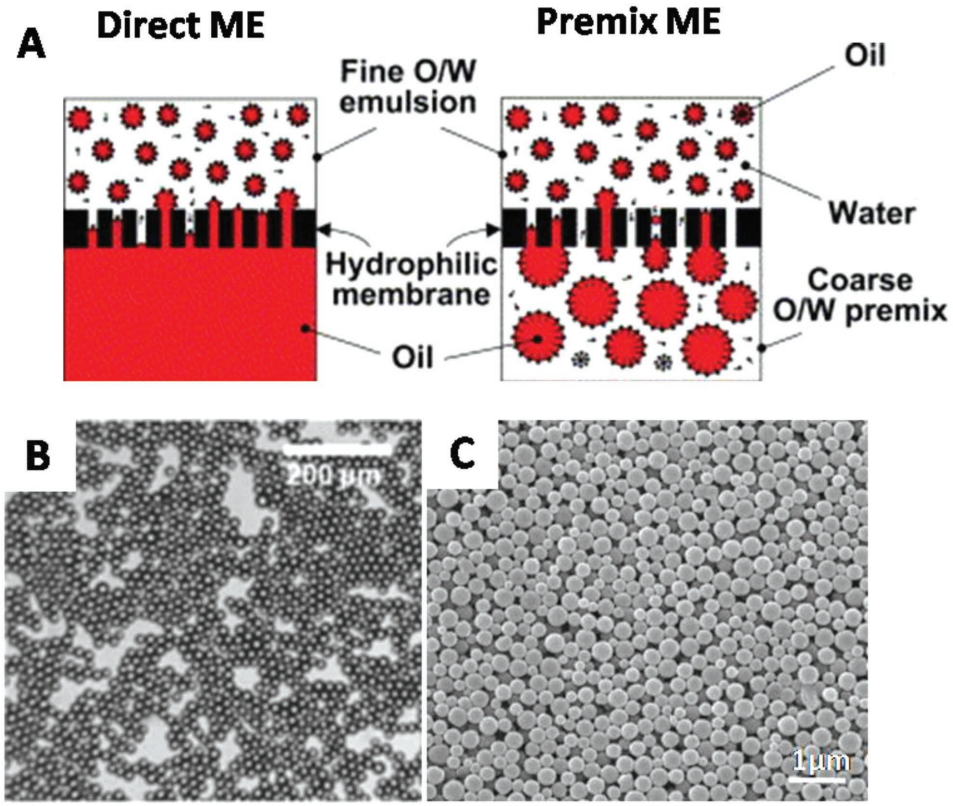

Figure 3. A) Schematic illustrations of direct membrane emulsification (Direct ME) and premix membrane emulsification (Premix ME). Reproduced with permission.[107] Copyright 2005, Elsevier. B) Optical micrograph of w/o emulsions prepared by membrane emulsification. Reproduced with permission.[110] Copyright 2013, Wiley-VCH. C) SEM image of PLA nanoparticles prepared by premix membrane emulsification. Reproduced with permission.[139] Copyright 2008, Elsevier.

emulsification applications. For example, Yanagishita et al. described the membrane emulsification preparation of SiO_2 nanoparticles using an AAO membrane.[120] Park et al. prepared o/w emulsions of kerosene and SDS solution using PCTE membranes with different pore sizes.[121] Kobayashi et al. showed that straight-through silicon microchannels can also be used for membrane emulsification applications.[122]

The direct membrane emulsification method was originally developed by Nakashimai et al., when they reported the preparation of highly uniform microscale kerosene-in-water and water-in-kerosene emulsions using a SPG membrane.[53] Later, Suzuki et al. improved this method by developing the premix membrane emulsification method that significantly increased the uniformity and reduced the droplet size of corn oil/water emulsions.[109] Since then, and over the past two decades, the membrane emulsification method has been rapidly developed to prepare a variety of micro-/nanoscale materials.[109,113] To date, the major products of membrane emulsification are emulsions and micro-/nanoparticles. Several emulsion drugs have been approved by US FDA and European Medicines Agency for treating parenteral nutrition-associated diseases and cancers.[123–125] For example, Soy bean oil nanoemulsion (trade name: Intralipid, droplet size: 300–400 nm in diameter) was approved by US FDA in 1972 for treating parenteral nutrition-associated diseases.[126–128] Chemotherapeutics (e.g., epirubicin and cisplatin) have also been successfully encapsulated in poppy seed oil emulsions (trade name: Lipiodol) using membrane emulsification methods, and have been used for treating unresectable hepatocellular carcinoma via transcatheter arterial chemoembolization.[129–132] In addition to drug delivery, emulsions have also been widely used in many other industrial applications including foods[133–135] and cosmetics.[136–138]

Polymer micro-/nanoparticles can also be prepared by membrane emulsification. Monomers are preliminarily dissolved in the dispersed phase and extruded through a nanoporous membrane into micro-/nanoscale emulsion droplets, which are further solidified via polymerization into uniform particles with a narrow size distribution. An important example is developed by Wei et al. when they successfully prepared polylactide acid (PLA) nanoparticles using the premix membrane emulsification method.[139] The synthesized PLA nanoparticles demonstrated a highly uniform spherical morphology with a narrow size distribution (Figure 3C).[139] The size of these PLA nanoparticles was readily controlled by their transmembrane pressure with a range from 250 to 450 nm. PLA nanoparticle is one of the most studied nanocarriers with substantial potential for clinical applications.[140–142] Prostate-specific membrane antigen (PSMA)-targeted, paclitaxel-encapsulating PLA nanoparticle (trade name: BIND-014), was tested in several clinical trials directed against a variety of cancers including metastatic prostate cancer and non-small cell lung cancer.[143,144] Membrane emulsification has also been shown to prepare nanoparticles

from many other materials. For example, Charcosset et al. reported the preparation of solid lipid nanoparticles with a size range between 70 and 215 nm using direct membrane emulsification with an AAO membrane.[145] Inorganic nanoparticles, such as SiO_2 and silver nanoparticles, have also been successfully prepared using membrane emulsification methods.[120,146] In addition to emulsions and nanoparticles, membrane emulsification has also been demonstrated to prepare more complicated nanostructures. For example, Blouza et al. reported the preparation of spironolactone-loaded polycaprolactone (PCL) nanocaspsules with mean diameters of 320 and 400 nm using a Kerasep ceramic membrane with 100 nm nanopores.[147] Kukizaki and Goto also reported the preparation of SDS-stabilized nanobubbles (360–720 nm in diameter) using membrane emulsification with a SPG membrane.[148]

Conventional emulsion preparation methods include high-pressure homogenization, ultrasonic homogenization, and colloid milling,[149–151] which share a common drawback that requires a high-energy input to disrupt large dispersed phase droplets in zones of high energy density. Additionally, the derivatives of high-energy inputs may negatively impact on the emulsion payloads. For example, ultrasound and heat generated from homogenization could denature bioactive payloads (e.g., proteins or nucleic acids) in the emulsion. In comparison, membrane emulsification provides better control of emulsion droplet size and distribution, mildness of procedure, low energy consumption, and is easy to scale up. However, membrane emulsification also has its own technical limitations. Membrane emulsification has a relatively low dispersed phase flux compared to other emulsification methods, which leads to a lower emulsion production. It is also difficult to prepare emulsion droplets with high viscosity and a uniform emulsion can only be prepared using highly uniform nanoporous membranes.

3.3. Precipitation Extrusion

Precipitation extrusion is a recently developed nanoporous membrane extrusion method for one-step synthesis of nanoparticles and nanofibers.[152–157] In a typical precipitation extrusion procedure (**Figure 4**A),[154] a feed solution containing dissolved solutes is forced through a nanoporous membrane to meet a receiver solution at the exits of the nanopores, in which the solutes are insoluble. At the interface between nanopore exit and receiver solution, droplets of feed solution confined in the nanopore rapidly precipitate forming solid nanoparticles when contacting the receiver solution, and the resulting precipitated nanoparticles are carried away from the nanopore exits by the continuous flow and dispersed in the receiver solution to achieve complete solidification. This nanopore-controlled precipitation of feed solution can be achieved using a variety of mechanisms, including antisolvent, pH-induced protonation/deprotonation and self-assembly.

A signature difference between precipitation extrusion and membrane emulsification is that in the precipitation extrusion, both feed and receiver solution are miscible and the obtained nanomaterials are spontaneously solidified through precipitation in the receiver solution. In contrast, membrane emulsification is performed in two immiscible liquid phases and requires an additional step to convert the emulsion droplets into solid nanoparticles. Precipitation extrusion is a very dynamic process and the precipitation of nanomaterials is governed by both transmembrane flux speed of the feed solution and the nature of precipitation mechanisms. If the precipitation extrusion process is too fast, too many nanoparticles are solidified at the exits of the nanoporous membrane and could clog the nanopore channels. In contrast, if the precipitation extrusion process is too slow, the droplets of feed solution are not fully solidified when they leave the nanopore exit. These unsolidified droplets will coalesce with other droplets when they contact each other to form bulky aggregates. These two critical factors must be finely tuned to achieve optimal outcomes. Track-etch and AAO membranes are both widely used in precipitation extrusion due to their uniform and straight-through nanopore channels. Other membranes such as SPG membranes are difficult to apply to precipitation extrusion because their interconnected nanopore structure easily causes large precipitation to form inside the nanopore channels.

The precipitation extrusion method was first developed by the authors to prepare ultrasmall chitosan nanoparticles using PCTE and AAO membranes.[152] Low molecular weight chitosan oligosaccharides (MW 20 000) were dissolved in an acidic PBS solution as feed solution and extruded into a basic PBS solution to induce deprotonation-mediated precipitation. The obtained chitosan nanoparticles show a close correlation with nanopore size. Later, these investigators continued to use this method to prepare amorphous hydrophobic drug nanoparticles based on antisolvent-mediated precipitation.[154] Three water insoluble drug compounds, silymarin, beta-carotene, and butylated hydroxytoluene, in bulky crystalline powders (Figure 4B), were successfully converted into highly uniform nanoparticles (Figure 4C) with a mean hydrodynamic diameter of ≈100 nm, which can be readily dispersed in the aqueous solution without aggregation.[154] It is worth nothing that precipitation extrusion process not only reduced the drug particle size, but also changed its crystalline structure. The X-ray diffraction analysis revealed that this rapid nanopore-controlled precipitation process converts the crystalline powders of hydrophobic drugs into an amorphous phase that is more favorable for aqueous dissolution and body absorbance (Figure 4D).[154]

Precipitation extrusion has also been explored to produce 1D nanomaterials. We utilized the precipitation extrusion method to cooperatively precipitate calcium phosphate nanoparticles and collagen nanofibrils into biomineralized nanofibers followed by exploring their applications in stem cell differentiation and bone tissue engineering.[153] Important and distinct from other established biomineralization methods, we found that this precipitation extrusion method can produce a unique biomineralized pattern featuring a 67 nm band as calcium phosphate nucleation sites (Figure 4E),[153] which more closely resembles the naturally occurring biomineralized collagen nanofibers found in bone tissues. This unique nanocomposite structure is extremely difficult to prepare using other established biomineralization methods (e.g., polymer-induced liquid-precursor (PILP)) that take up to several days, with an additional need of polyanionic polymers to achieve this.[158–162] By using the precipitation extrusion method, the nanopore-controlled

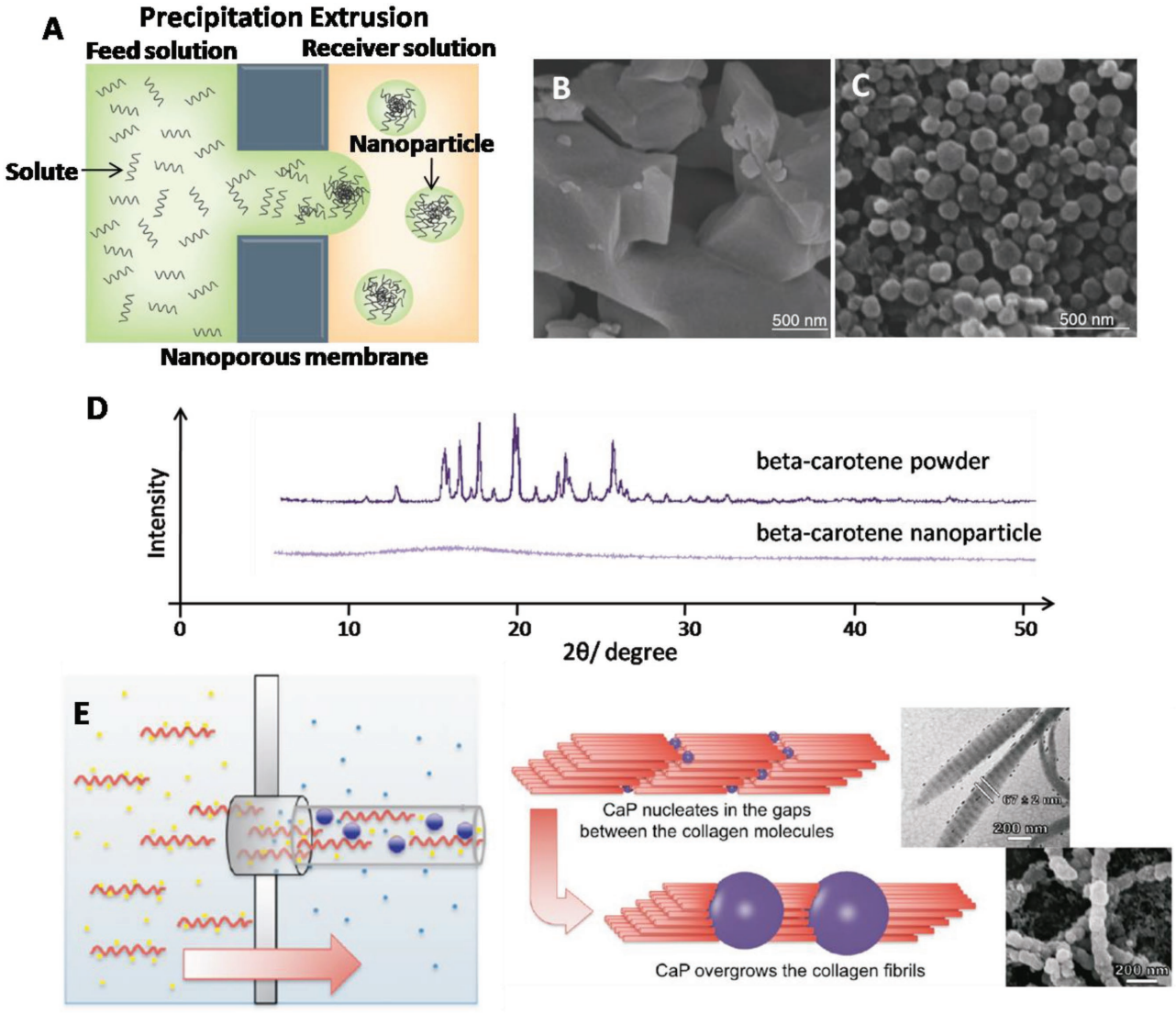

Figure 4. A) Schematic illustration of precipitation extrusion for nanoparticle preparation. SEM micrographs of B) bulk beta-carotene powders and C) beta-carotene nanoparticles prepared by precipitation extrusion. D) Powder X-ray diffraction patterns of beta-carotene power and nanoparticles. Reproduced with permission.[154] Copyright 2013, Future Medicine. E) Schematic illustration and TEM images of calcium phosphate (CaP) mineralized collagen nanofibers prepared by precipitation extrusion. Reproduced with permission.[153] Copyright 2011, American Chemical Society.

precipitation process does not require any polyanionic polymers to suppress bulk crystallization and only required us less than 1 h to complete the experiment, which significantly simplifies and shortens the nanofabrication process. This approach offers promising potential for scale-up production of biomineralized nanomaterials. This precipitation extrusion method has been further extended for preparing composite nanomaterials from other biomaterials including fibronectin, elastin, hyaluronan, and PLA.[155–157] For example, Raoufi et al. reported the use of precipitation extrusion to prepare a variety of biopolymer blended nanofibers with an AAO membrane.[155] Uehara et al. reported the preparation of stereocomplex PLA nanoparticles using the precipitation extrusion method based on antisolvent-mediated precipitation. They blended poly(L-lactic acid) and poly(D-lactic acid) in chloroform as feed solution and extruded it through 100 nm PCTE or AAO membrane into a methanol receiver solution. Owing to the insolubility of PLA in methanol, stereocomplex nanoparticles were precipitated.[157] Interestingly, Powell et al. observed the phenomenon of transient precipitation and dissolution process of calcium hydrogen phosphate nanoparticles inside a conical-shaped nanopore of a PET membrane.[163] This process can be monitored in real time by observing ion current oscillations using patch clamp, which connects nanopore-controlled precipitation with another important application of nanoporous membranes: resistive-pulse sensing. This nanoprecipitation-associated ion current oscillation has been further investigated in the study of nonlinear electrochemical processes and stochastic sensors.[164–168]

Compared with the conventional nanoprecipitation method, the precipitation extrusion method exhibits the following advantages: First, precipitation extrusion provides better control of the nanoparticle uniformity and mondispersity. Precipitation

extrusion is especially efficient in preparing nanoparticles with a size equivalent or less than 100 nm, which represents the most useful size range for biomedical applications. Second, the nanopore confinement enables the precipitation extrusion method to prepare more complicated nanocomposite structures (e.g., biomineralized nanofibers or stereocomplex nanoparticles) than the conventional nanoprecipitation method. Third, precipitation extrusion exhibits an excellent reproducibility compared with the conventional nanoprecipitation method, which could significantly reduce the batch-to-batch difference for scale-up of nanomaterial production. Notably, a recently developed flash nanoprecipitation method has significantly improved the nanoparticle size control and uniformity by using a multi-inlet vortex mixer or a confined impingement jet mixer,[169–171] however, this method frequently requires the addition of amphiphilic block copolymers to stabilize nanoparticles from aggregating. In comparison, the precipitation extrusion method can directly formulate hydrophobic small molecules into stabilized nanoparticles without the help of amphiphilic block copolymers. In addition, precipitation extrusion can be used to prepare both nanoparticles and nanofibers whereas flash nanoprecipitation can only be used to prepare nanoparticles. However, precipitation extrusion has its own limitations: precipitation extrusion is based on finely tuned experimental conditions that require extensive time and labor to optimize (e.g., flow rate, feed solution concentrations, etc.) and may not be applicable to all precipitation mechanisms. Similar to other nanoporous membrane extrusion methods (e.g., vesicle extrusion and membrane emulsification), its production yields are generally lower than the conventional nanoprecipitation method due to the nanopore filtration effect.

3.4. Biological Membrane Extrusion

Cell-derived nanomaterials (e.g., cell membrane-cloaked nanoparticles and extracellular vesicles) have recently emerged as a novel type of nanomaterials and exhibit promising potentials in many biomedical applications from drug delivery to disease diagnosis.[172–175] Cell membrane-cloaked nanoparticles are a prominent example of cell-derived nanomaterials and are defined as a "core–shell" nanocomposite that comprises a solid nanoparticle "core" and a layer of cell membrane "shell".[176–179] These cell membrane-cloaked nanoparticles harness the biological functions of naturally occurring cell membranes and their membrane-bound proteins, which enable them to more efficiently avoid the reticuloendothelial system uptake and to circulate longer in comparison to synthetic PEGylated nanoparticles. This feature represents a novel biomimetic "stealth" strategy. Cell membrane-cloaked nanoparticles are prepared via a nanoporous membrane extrusion method that is highly similar to vesicle extrusion but with modifications. We call it the "biological membrane extrusion" method, which generally involves two steps (**Figure 5**A).[176] The first step is to harvest cell membranes from target cells. This step is usually achieved through whole cell rupture using hypotonic treatment, homogenization, or cell lysis. The obtained cell membrane is separated from cell debris and organelles using centrifugation. In the second step, the obtained cell membrane fragments are extruded through a nanoporous membrane to form uniform cell membrane-derived vesicles ("shell") and these cell membrane-derived vesicles are extruded again with solid nanoparticle ("core") several times. When a cell membrane-derived vesicle encounters a solid nanoparticle within the nanopore channel, both "shell" vesicle and "core" particle fuse together to form a "core–shell" nanocomposite which is released at the exit of the nanopore.

There are several factors that regulate this biological membrane extrusion process. First, the membrane-to-polymer ratio has a direct impact on the membrane coverage of nanoparticles. Luk et al. reported that the membrane-to-polymer ratio must reach at least 100 μL mouse blood per mg polymer for the red blood cell (RBC) membrane to completely coat the 100 nm in diameter poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles.[180] Second, the surface charge of "core" particles also affects the cell membrane coating. Luk et al. investigated the preparation of cell membrane-cloaked nanoparticles using both positively and negatively charged polymers as "core" nanoparticles via the biological membrane extrusion method, and found that only negatively charged nanoparticles readily formed "core–shell" nanostructures.[180] The positively charged nanoparticles electrostatically interact with the negatively charged cell membranes to form microscale aggregates, leading to blockade of nanopore channels during the extrusion process. Importantly, they also found that 84% of obtained RBC membrane-cloaked nanoparticles had the extracellular side of the RBC membrane coated outward, which is called "right-side-out".[180] This directional membrane coating could also be attributed to the electrostatic repulsions between negatively charged cell membranes and negatively charged polymeric nanoparticles. This same group have also demonstrated that this biological membrane extrusion method can be flexibly applied to coat polymeric nanoparticles of different sizes ranging from 65 to 340 nm.[180] To date, track-etch membranes (e.g., PCTE membranes) are the most commonly used nanoporous membrane for biological membrane extrusion applications.

This biological membrane extrusion method was firstly developed by Shingles and McCarty when they extruded plant plasma membranes through a 100 nm PCTE nanoporous membrane to form uniform and monodisperse plasma membrane vesicles with a mean diameter of 103 nm.[181] They also measured membrane sidedness of obtained plasma membrane vesicles using an ATPase activity assay and found that ≈80% of obtained plasma vesicles are in the "right-side-out" orientation, which is significantly higher than the 30% of plasma membrane vesicles prepared by the conventional freeze/thaw method. Hu et al. adopted this biological membrane extrusion method to prepare mouse RBC membrane-coated PLGA nanoparticles for chemotherapeutic delivery.[176] In this study, mouse RBC membrane-cloaked PLGA nanoparticles were prepared using the two-step biological membrane extrusion method described previously: the obtained mouse RBC membrane-cloaked PLGA nanoparticles exhibited a mean diameter of ≈80 nm with a 70 nm "core" particle and a 7–8 nm "shell" lipid layer (Figure 5B).[176] They found that the glycans (e.g., CD47) presented on the RBC extracellular surface could help RBC membrane-cloaked PLGA nanoparticles avoid being taken up by macrophages in the blood circulation and the half-life of RBC membrane-cloaked PLGA nanoparticles in the circulation

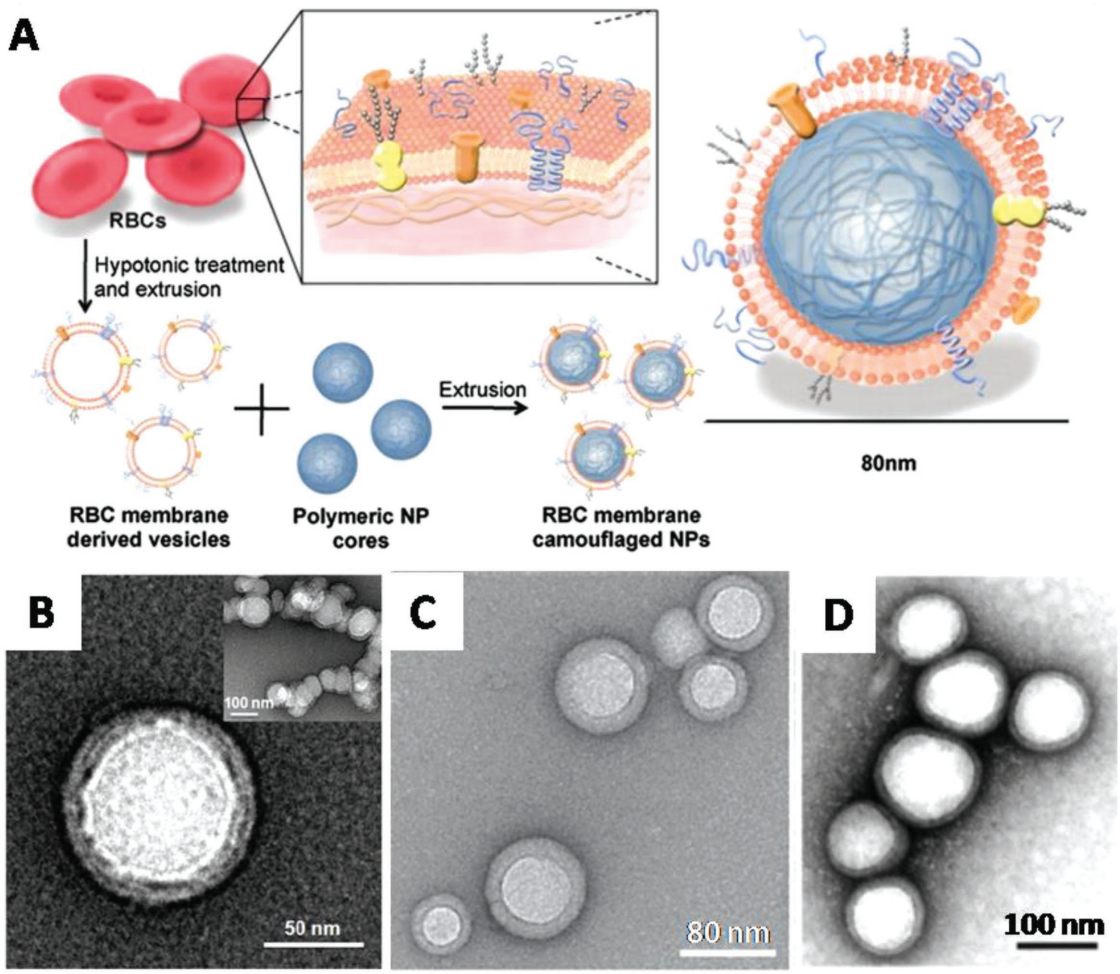

Figure 5. A) Schematic illustration of biological membrane extrusion for preparing red blood cell (RBC) membrane-cloaked nanoparticles. TEM images of B) mouse RBC membrane cloaked PLGA nanoparticles, C) human RBC membrane-cloaked PLGA nanosponges, and D) human platelet membrane cloaked PLGA nanoparticles. Reproduced with permission.[176,177,179] Copyright 2011, National Academy of Science; Copyright 2013, Nature Publishing Group; Copyright 2015, Nature Publishing Group.

is 39.7 h, more than twice as long as that of PEGylated PLGA nanoparticles (≈15.8 h), which can be attributed to the "stealth" function of RBC membrane coating. Later, they continued to develop a human RBC membrane-cloaked nanosponge to absorb membrane-damaging toxins from blood circulation (Figure 5C) and significantly improved the survival rate of toxin-challenged mice.[177,178] Since then, the biological membrane extrusion method has been extensively studied for use in the preparation of cell membrane-cloaked nanomaterials using all types of cell membranes. For example, Hu et al. reported the preparation of human platelet membrane-cloaked nanoparticles (Figure 5D) and found that these nanoparticles exhibited platelet-like behaviors that selectively recognize and bind to damaged human and rodent vasculatures, an interesting feature which can be translated into promising disease-targeting therapeutics.[179] Other cell membranes, such as cancer cell membranes,[182] neutrophil membranes[183] and hybrid cell membranes,[184] have also been used to prepare cell membrane-cloaked nanoparticles to facilitate tumor-targeted treatments. Interestingly, a recent study reported the use of the biological membrane extrusion method to directly extrude chemodrug-loaded live macrophages through a series of microporous membranes.[185] Unlike conventional two-step biological membrane extrusion, this macrophage extrusion process was performed at the whole cell level without membrane isolation. The resulting macrophage membrane-derived vesicles exhibited a nanoscale diameter of ≈130 nm with high chemodrug encapsulation.

Biological membrane extrusion is a simple and straightforward method to prepare cell-derived nanomaterials. Compared with conventional methods (e.g., free/thaw or sonication),[186–188] cell-derived nanomaterials prepared by biological membrane extrusion demonstrate a better uniformity and dispersity with smaller sizes. Importantly, biological membrane extrusion significantly improves the membrane sidedness compared to conventional free/thaw method with a "right-side-out" orientation ratio of over 80%. Furthermore, biological membrane extrusion is usually performed in a relatively mild environment that helps to preserve the bioactivities of native proteins and nucleic acids in cell-derived nanomaterials. In comparison, conventional free/thaw and sonication methods frequently denature

these biomolecules and cause bioactivity loss in the obtained cell-derived nanomaterials. However, compared to conventional methods, biological membrane extrusion suffers from a higher protein loss caused by the nanopore filtration effect, leading to a lower yield of cell-derived nanomaterials.

4. Summary and Future Perspective

In summary, the nanoporous membrane extrusion method has proven to be a facile and powerful approach for preparing nanomaterials with extraordinary size control and reproducibility. These nanomaterials can be prepared from broadly available materials including lipids, polymers, inorganic materials and even live cells. This review has presented four major types of nanoporous membrane extrusion methods that are now available for use in preparing a wide variety of nanomaterials including liposomes, emulsions, nanoparticles, nanofibers/tubes and cell-derived nanomaterials. The applications of these nanomaterials widely range from drug delivery to tissue engineering to disease diagnosis.

To date, many fundamental scientific questions and technical challenges still exist with respect to nanoporous membrane extrusion methods. The potential merits of employing nanoporous membrane extrusion are yet to be realized in industrial nanomaterial production, and many newly developed nanoporous membrane extrusion methods (e.g., biological membrane extrusion) are still in their infancy. Extensive efforts should be devoted to improve nanoporous membrane extrusion by simplifying usage, reducing costs, and expanding its applications. Furthermore, advanced biomechanistic studies are required to improve our capability to use nanoporous membrane extrusion to construct sophisticated cell-derived nanomaterials with excellent biological functions. On the basis of this review, research in the near future should been focused on, but not limited to, meeting following key challenges.

First, scale-up production of nanomaterials using nanoporous membrane extrusion remains technically difficult. The major issue is that the yield of nanoporous membrane extrusion is generally lower than other established nanofabrication methods while having a better size control. This is mainly due to the fact that almost every nanoporous membrane extrusion strategy relies on utilizing nanopore channels to control the size of products, leading to filtration residue of raw materials to continuously accumulate on the feeding side of the nanoporous membrane, eventually block these nanochannels. When this occurs, the nanofabrication process must be stopped in order to replace the membrane ultimately reducing the yield and increasing the cost of nanomaterial production. One possible way to solve this problem is to perform nanoporous membrane extrusion in supercritical fluids (SCFs) rather than liquid phases. SCF is defined as a substance in its supercritical phase that both its temperature and pressure are beyond respective critical points.[189–191] Unlike other liquid phases (e.g., water, oil, or organic solvents) currently used in the nanoporous membrane extrusion methods, SCF has a unique physicochemical property that it can dissolve solutes like a liquid, and the extremely low viscosity and high diffusivity of SCF enable it to effuse more easily through a nanoporous membrane like a gas. Meanwhile, at supercritical state, the density and solvating power of SCF can be easily and continuously tuned by adjusting its temperature and pressure, allowing it to prepare nanomaterials using different mechanisms (e.g., antisolvent, emulsification, self-assembly, etc.).[192–195] To date, the SCF technique itself has already been used to prepare many nanomaterials including nanoparticles, nanoemulsions, and liposomes.[192–205] In these studies, SCF can be used either as a solvent for rapid expansion of supercritical solution[206] process or as an antisolvent for supercritical antisolvent[207] process based on the solubility of nanomaterials in SCFs. However, the drawback of SCF-based nanofabrication is its poor control over the uniformity and dispersity of obtained nanomaterials, and it is also technically challenging for SCF to prepare nanomaterials with sizes smaller than 200 nm, which is considered to be the most useful size for biomedical applications. This is mainly due to the fact that most SCF-based nanofabrication approaches rely on microscale nozzles to break up the droplets of solute solution (SCF or other solvents), and microscale nozzles usually have a poor control over droplet sizes. Therefore, we can envision that if we could combine nanoporous membrane extrusion with SCF techniques, we could significantly reduce the viscosity of feed solutions by using SCFs to improve its hydrodynamic permeability through nanoporous membrane. In addition, uniform and monodisperse nanopore channels could help to more efficiently break up feed solution droplets and downsize them into nanoscale, which, in turn, substantially improves the production yield with better size control.

On the other hand, preparing nanomaterials from an extremely small concentration (e.g., nanomolar) and/or volume (e.g., nanoliter) of raw materials, also called "ultra scale-down production", is also challenging. In the rapidly developing field of disease diagnosis research, many native disease biomarkers (e.g., urine and blood proteins, circulating tumor DNAs and others) exist at very low concentrations (e.g., nanomolar or lower) with short half-life times (e.g., minutes or shorter).[208–215] One possible way to detect these biomolecules at ultralow concentration is to capture these biomolecules using micro-/nanoparticles at single molecular levels and then amplify the detection signals based on the unit of micro-/nanoparticles. One prominent example was reported by Rissin et al. who utilized magnetic microparticles to capture and detect serum proteins at a concentration as low as 14 fg mL^{-1}.[216] Given the important role of nanomaterials in biological detection, it will be very beneficial if it is possible to formulate biomolecules at ultralow concentration/volume into nanoparticles without further dilution and then, use the versatile armory of nanoparticle-based detection techniques to detect and analyze these biomolecules in a more efficient and precise manner. In order to achieve this goal, an ultrasmall scale nanofabrication method is required to prepare nanoparticles with high uniformity and dispersity which remains a challenge to conventional nanofabrication methods. To the authors' knowledge, one possible way to solve this issue is to integrate nanoporous membrane extrusion with the Lab-on-a-Chip technique. The rapid advances in Lab-on-a-Chip technique have allowed scientists to handle chemical reactions at mciro-/nanoliter volumes,[217–219] and have also been widely used for single molecule detection and analysis.[220–223] For example, Landry et al. recently developed

a single-wall carbon nanotube array on a microfluidic chip for single-molecule detection of protein efflux from microorganisms.[223] Though several techniques have been developed to prepare nanomaterials on microfluidic chips, including micromixing,[224–226] flow focusing,[227–231] and pulse jetting,[230,232,233] none of these techniques can achieve the scale-down production of nanomaterials at the nanoliter level. For example, the most commonly used flow focusing technique usually requires a minimum volume of tens of microliters of raw material solution to initiate nanofabrication.[227–231] A recently developed Lab-on-a-Chip nanofabrication method, called transient membrane ejection, is of particular interest to us.[234] In this method, Ota et al. forced a lipid-containing oil phase to infuse into a microchannel junction and deposit as a lipid film inside the microchannel. They then ejected this lipid bilayer from the microchannel using a water phase to form monodisperse uniform lipid vesicles. Kurakazu and Takeuchi further improved this technique in a manner that enables it to tune vesicle size by controlling the volume of the microchannel.[235] However, the size of the lipid vesicle prepared by this transient membrane ejection method is still at the microscale instead of the nanoscale. Considering the fact that the size, shape, and pore density of nanoporous membranes can be easily and precisely controlled, we expect that the transient membrane ejection method can be adopted to prepare nanoparticles from biomolecules at ultralow concentrations and volumes by replacing microchannels with a nanoporous membrane. We envision that integrating nanoporous membrane extrusion with the Lab-on-a-Chip technology will enable us to achieve the goal of ultra scale-down nanomaterial production.

We are optimistic about the recent advances in nanoporous membrane fabrication technologies.[236–243] For example, the utilization of self-assembly block polymers has enabled us to prepare highly uniform and ordered nanoporous membrane with a pore size as small as 1 nm. These newly developed block polymer-based nanoporous membranes provide a narrower pore size distribution, higher porosity, tunable chemical and physical properties, with many other advanced functions, providing the field with new experimental tools and research opportunities in nanoporous membrane extrusion methods. We envision that with advances in nanoporous membrane fabrication techniques and integration of interdisciplinary approaches, the nanoporous membrane extrusion method could ultimately reach the industrial level for the scale-up of nanomaterial production, which has the potential to be utilized in many important biomedical applications, among other possible uses.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cells, emulsion, extrusion, liposomes, nanomaterials, nanoporous membranes

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[1] C. R. Martin, *Chem. Mater.* **1996**, *8*, 1739.
[2] C. R. Martin, R. V. Parthasarathy, *Adv. Mater.* **1995**, *7*, 487.
[3] R. V. Parthasarathy, C. R. Martin, *Nature* **1994**, *369*, 298.
[4] C. R. Martin, *Adv. Mater.* **1991**, *3*, 457.
[5] W. Liang, C. R. Martin, *J. Am. Chem. Soc.* **1990**, *112*, 9666.
[6] Z. Cai, C. R. Martin, *J. Am. Chem. Soc.* **1989**, *111*, 4138.
[7] M. Nishizawa, V. P. Menon, C. R. Martin, *Science* **1995**, *268*, 700.
[8] C. A. Foss, G. L. Hornyak, J. A. Stockert, C. R. Martin, *Adv. Mater.* **1993**, *5*, 135.
[9] C. J. Brumlik, C. R. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 3174.
[10] R. V. Parthasarathy, K. L. N. Phani, C. R. Martin, *Adv. Mater.* **1995**, *7*, 896.
[11] P. Kohli, *Science* **2004**, *305*, 984.
[12] C. R. Martin, *Science* **1994**, *266*, 1961.
[13] J. L. Perry, P. Guo, S. K. Johnson, H. Mukaibo, J. D. Stewart, C. R. Martin, *Nanomedicine* **2010**, *5*, 1151.
[14] H. Mukaibo, L. P. Horne, D. Park, C. R. Martin, *Small* **2009**, *5*, 2474.
[15] F. Xu, J. E. Wharton, C. R. Martin, *Small* **2007**, *3*, 1718.
[16] H. Hillebrenner, F. Buyukserin, M. Kang, M. O. Mota, J. D. Stewart, C. R. Martin, *J. Am. Chem. Soc.* **2006**, *128*, 4236.
[17] B. B. Lakshmi, P. K. Dorhout, C. R. Martin, *Chem. Mater.* **1997**, *9*, 857.
[18] J. D. Klein, R. D. Herrick, D. Palmer, M. J. Sailor, C. J. Brumlik, C. R. Martin, *Chem. Mater.* **1993**, *5*, 902.
[19] S. Hou, J. Wang, C. R. Martin, *J. Am. Chem. Soc.* **2005**, *127*, 8586.
[20] S. Hou, J. Wang, C. R. Martin, *Nano Lett.* **2005**, *5*, 231.
[21] P. Gao, A. Hunter, S. Benavides, M. J. Summe, F. Gao, W. A. Phillip, *ACS Appl. Mater. Interfaces* **2016**, *8*, 3386.
[22] J. C. Hulteen, C. R. Martin, *J. Mater. Chem.* **1997**, *7*, 1075.
[23] C. R. Martin, *Acc. Chem. Res.* **1995**, *28*, 61.
[24] S. K. Chakarvarti, J. Vetter, *Radiat. Meas.* **1998**, *29*, 149.
[25] B. B. Lakshmi, C. J. Patrissi, C. R. Martin, *Chem. Mater.* **1997**, *9*, 2544.
[26] Y. Wang, A. S. Angelatos, F. Caruso, *Chem. Mater.* **2008**, *20*, 848.
[27] R. L. Fleischer, P. B. Price, R. M. Walker, *Nuclear Tracks in Solids: Principles and Applications*, University Of California Press, Berkeley, CA, USA **1975**.
[28] R. L. Fleischer, H. W. Alter, S. C. Furman, P. B. Price, R. M. Walker, *Science* **1972**, *178*, 255.
[29] E. Ferain, R. Legras, *Nucl. Instrum. Methods Phys. Res., Sect. B* **1994**, *84*, 331.
[30] C. P. Bean, M. V. Doyle, G. Entine, *J. Appl. Phys.* **1970**, *41*, 1454.
[31] I. Vlassiouk, P. Y. Apel, S. N. Dmitriev, K. Healy, Z. S. Siwy, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21039.
[32] R. Xie, L. Chu, W. Chen, W. Xiao, H. Wang, J. Qu, *J. Membr. Sci.* **2005**, *258*, 157.
[33] J. E. Wharton, P. Jin, L. T. Sexton, L. P. Horne, S. A. Sherrill, W. K. Mino, C. R. Martin, *Small* **2007**, *3*, 1424.
[34] C. C. Harrell, Z. S. Siwy, C. R. Martin, *Small* **2006**, *2*, 194.
[35] B. Zhang, Y. Zhang, H. S. White, *Anal. Chem.* **2004**, *76*, 6229.
[36] J.-H. Choi, S.-K. Park, H.-Y. Ng, *Sep. Purif. Technol.* **2009**, *65*, 184.
[37] C.-C. Ho, A. L. Zydney, *J. Membr. Sci.* **1999**, *155*, 261.
[38] F. Nasirpouri, P. Southern, M. Ghorbani, A. Iraji zad, W. Schwarzacher, *J. Magn. Magn. Mater.* **2007**, *308*, 35.
[39] D. Thassu, M. Deleers, Y. Pathak, *Nanoparticulate Drug Delivery Systems*, Informa Healthcare, NY **2007**.

[40] *Applications of Ionizing Radiation in Materials Processing* (Eds: Y. Sun, A. G. Chmielewski), Institute Of Nuclear Chemistry And Technology, Warszawa, Poland **2017**.
[41] J. Tringe, N. Ileri, S. Létant, P. Stroeve, M. Shirk, S. Zaidi, R. Balhorn, C. Siders, (Lawrence Livermore Natl. Lab. LLNL), *LLNL-TR-401188*, **2008**.
[42] A. Despić, V. P. Parkhutik, in *Mod. Asp. Electrochem. No 20* (Eds: J. O. Bockris, R. E. White, B. E. Conway), Springer US, Boston, MA **1989**, pp. 401–503.
[43] K. A. Khan, J. K. Kasi, N. Afzulpurkar, E. Bohez, A. Tuantranont, B. Mahaisavariya, in *Communications and Electronics (ICCE), 2010 Third International Conference*, IEEE, **2010**, pp. 98–101.
[44] J. J. Kasianowicz, M. S. Z. Kellermayer, D. W. Deamer, *Structure and Dynamics of Confined Polymers: Proceedings of the NATO Advanced Research Workshop on Biological, Biophysical & Theoretical Aspects of Polymer Structure and Transport Bikal, Hungary 20–25 June 1999*, SpringerNetherlands, Dordrecht **2002**.
[45] C. A. Foss, G. L. Hornyak, J. A. Stockert, C. R. Martin, *J. Phys. Chem.* **1994**, *98*, 2963.
[46] C. A. Foss, G. L. Hornyak, J. A. Stockert, C. R. Martin, *J. Phys. Chem.* **1992**, *96*, 7497.
[47] E. J. Bae, W. B. Choi, K. S. Jeong, J. U. Chu, G.-S. Park, S. Song, I. K. Yoo, *Adv. Mater.* **2002**, *14*, 277.
[48] J. J. Hill, N. Schwartz, G. Chester, Google Patents, **2016**.
[49] D. I. Petukhov, D. A. Buldakov, A. A. Tishkin, A. V. Lukashin, A. A. Eliseev, *Beilstein J. Nanotechnol.* **2017**, *8*, 561.
[50] T. Nakashima, M. Shimizu, M. Kukizaki, *Adv. Drug Delivery Rev.* **2000**, *45*, 47.
[51] T. Nakashima, M. Shimizu, M. Kukizaki, *J. Ceram. Soc. Jpn.* **1992**, *100*, 1411.
[52] T. Nakashima, M. Shimizu, *J. Ceram. Soc. Jpn.* **1993**, *101*, 528.
[53] T. Nakashima, M. Shimizu, M. Kukizaki, *Key Eng. Mater.* **1992**, *61–62*, 513.
[54] M. Kukuzaki, K. Fujimoto, S. Kai, K. Ohe, T. Oshima, Y. Baba, *Sep. Purif. Technol.* **2010**, *72*, 347.
[55] C. Charcosset, I. Limayem, H. Fessi, *J. Chem. Technol. Biotechnol.* **2004**, *79*, 209.
[56] Q.-Z. Zhou, G.-H. Ma, Z.-G. Su, *J. Membr. Sci.* **2009**, *326*, 694.
[57] S. Nagashima, M. Koide, S. Ando, K. Makino, T. Tsukamoto, H. Ohshima, *Colloids Surf. A* **1999**, *153*, 221.
[58] M. Kukizaki, M. Goto, *Colloids Surf. A* **2007**, *293*, 87.
[59] Y. Zhang, G. Lian, S. Zhu, L. Wang, W. Wei, G. Ma, *Ind. Eng. Chem. Res.* **2008**, *47*, 6412.
[60] F. Szoka, D. Papahadjopoulos, *Annu. Rev. Biophys. Bioeng.* **1980**, *9*, 467.
[61] F. Szoka, D. Papahadjopoulos, *Annu. Rev. Biophys. Bioeng.* **1980**, *9*, 467.
[62] A. Samad, Y. Sultana, M. Aqil, *Curr. Drug Delivery* **2007**, *4*, 297.
[63] S. C. Semple, R. Leone, J. Wang, E. C. Leng, S. K. Klimuk, M. L. Eisenhardt, Z.-N. Yuan, K. Edwards, N. Maurer, M. J. Hope, P. R. Cullis, Q.-F. Ahkong, *J. Pharm. Sci.* **2005**, *94*, 1024.
[64] F. Olson, C. A. Hunt, F. C. Szoka, W. J. Vail, D. Papahadjopoulos, *Biochim. Biophys. Acta, Biomembr.* **1979**, *557*, 9.
[65] S. Farrell, K. K. Sirkar, *J. Membr. Sci.* **1997**, *127*, 223.
[66] R. C. MacDonald, R. I. MacDonald, B. P. M. Menco, K. Takeshita, N. K. Subbarao, L. Hu, *Biochim. Biophys. Acta, Biomembr.* **1991**, *1061*, 297.
[67] F. Szoka, F. Olson, T. Heath, W. Vail, E. Mayhew, D. Papahadjopoulos, *Biochim. Biophys. Acta, Biomembr.* **1980**, *601*, 559.
[68] F. Olson, E. Mayhew, D. Maslow, Y. Rustum, F. Szoka, *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 167.
[69] D. G. Hunter, B. J. Frisken, *Biophys. J.* **1998**, *74*, 2996.
[70] P. J. Patty, B. J. Frisken, *Biophys. J.* **2003**, *85*, 996.
[71] S. Ong, M. Chitneni, K. Lee, L. Ming, K. Yuen, *Pharmaceutics* **2016**, *8*, 36.
[72] B. J. Frisken, C. Asman, P. J. Patty, *Langmuir* **2000**, *16*, 928.
[73] T. Lian, R. J. Y. Ho, *J. Pharm. Sci.* **2001**, *90*, 667.
[74] A. Sharma, *Int. J. Pharm.* **1997**, *154*, 123.
[75] T. M. Allen, *Science* **2004**, *303*, 1818.
[76] T. M. Allen, P. R. Cullis, *Adv. Drug Delivery Rev.* **2013**, *65*, 36.
[77] P. Guo, J.-O. You, J. Yang, M. A. Moses, D. T. Auguste, *Biomaterials* **2012**, *33*, 8104.
[78] P. Guo, J. Yang, D. R. Bielenberg, D. Dillon, D. Zurakowski, M. A. Moses, D. T. Auguste, *J. Controlled Release* **2017**, *10*, 57.
[79] R. van der Meel, S. Oliveira, I. Altintas, R. Haselberg, J. van der Veeken, R. C. Roovers, P. M. P. van Bergen en Henegouwen, G. Storm, W. E. Hennink, R. M. Schiffelers, R. J. Kok, *J. Controlled Release* **2012**, *159*, 281.
[80] V. Gupta, N. Gupta, I. H. Shaik, R. Mehvar, I. F. McMurtry, M. Oka, E. Nozik-Grayck, M. Komatsu, F. Ahsan, *J. Controlled Release* **2013**, *167*, 189.
[81] P. Guo, J.-O. You, J. Yang, D. Jia, M. A. Moses, D. T. Auguste, *Mol. Pharmaceutics* **2014**, *11*, 755.
[82] P. Guo, J. Yang, D. Jia, M. A. Moses, D. T. Auguste, *Theranostics* **2016**, *6*, 1.
[83] I. Koltover, *Science* **1998**, *281*, 78.
[84] V. P. Torchilin, T. S. Levchenko, R. Rammohan, N. Volodina, B. Papahadjopoulos-Sternberg, G. G. M. D'Souza, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1972.
[85] M. Rao, C. R. Alving, *Adv. Drug Delivery Rev.* **2000**, *41*, 171.
[86] C. R. Alving, V. Koulchin, G. M. Glenn, M. Rao, *Immunol. Rev.* **1995**, *145*, 5.
[87] J. A. Zuris, D. B. Thompson, Y. Shu, J. P. Guilinger, J. L. Bessen, J. H. Hu, M. L. Maeder, J. K. Joung, Z.-Y. Chen, D. R. Liu, *Nat. Biotechnol.* **2015**, *33*, 73.
[88] S. Zhen, Y. Takahashi, S. Narita, Y.-C. Yang, X. Li, *Oncotarget* **2016**, *8*, 9375.
[89] L. Wang, F. Li, L. Dang, C. Liang, C. Wang, B. He, J. Liu, D. Li, X. Wu, X. Xu, A. Lu, G. Zhang, *Int. J. Mol. Sci.* **2016**, *17*, 626.
[90] M. L. Immordino, F. Dosio, L. Cattel, *Nanomedicine* **2006**, *1*, 297.
[91] T. M. Allen, C. Hansen, F. Martin, C. Redemann, A. Yau-Young, *Biochim. Biophys. Acta, Biomembr.* **1991**, *1066*, 29.
[92] P. Milla, F. Dosio, L. Cattel, *Curr. Drug Metab.* **2012**, *13*, 105.
[93] T. Heath, R. Fraley, D. Papahdjopoulos, *Science* **1980**, *210*, 539.
[94] S. Sofou, G. Sgouros, *Expert Opin. Drug Delivery* **2008**, *5*, 189.
[95] P. Sapra, T. M. Allen, *Prog. Lipid Res.* **2003**, *42*, 439.
[96] E. Forssen, M. Willis, *Adv. Drug Delivery Rev.* **1998**, *29*, 249.
[97] M.-K. Yeh, H.-I. Chang, M.-Y. Cheng, *Int. J. Nanomed.* **2011**, *7*, 49.
[98] Y. (Chezy) Barenholz, *J. Controlled Release* **2012**, *160*, 117.
[99] M. Theodoulou, C. Hudis, *Cancer* **2004**, *100*, 2052.
[100] A. Wang-Gillam, C.-P. Li, G. Bodoky, A. Dean, Y.-S. Shan, G. Jameson, T. Macarulla, K.-H. Lee, D. Cunningham, J. F. Blanc, R. A. Hubner, C.-F. Chiu, G. Schwartsmann, J. T. Siveke, F. Braiteh, V. Moyo, B. Belanger, N. Dhindsa, E. Bayever, D. D. Von Hoff, L.-T. Chen, *Lancet* **2016**, *387*, 545.
[101] I. Garrido-Laguna, M. Hidalgo, *Nat. Rev. Clin. Oncol.* **2015**, *12*, 319.
[102] P. Michl, T. M. Gress, *Gut* **2013**, *62*, 317.
[103] Y. Guo, H. Yui, H. Minamikawa, B. Yang, M. Masuda, K. Ito, T. Shimizu, *Chem. Mater.* **2006**, *18*, 1577.
[104] Q. Chen, H. Zhao, T. Ming, J. Wang, C. Wu, *J. Am. Chem. Soc.* **2009**, *131*, 16650.
[105] Q. Chen, J. Wang, L. Shao, *Macromol. Rapid Commun.* **2013**, *34*, 1850.
[106] D. Liu, A. Mori, L. Huang, *Biochim. Biophys. Acta, Biomembr.* **1992**, *1104*, 95.
[107] G. T. Vladisavljević R. A. Williams, *Adv. Colloid Interface Sci.* **2005**, *113*, 1.
[108] M. Shima, Y. Kobayashi, T. Fujii, M. Tanaka, Y. Kimura, S. Adachi, R. Matsuno, *Food Hydrocolloids* **2004**, *18*, 61.
[109] K. Suzuki, I. Shuto, Y. Hagura, *Food Sci. Technol. Int.* **1996**, *2*, 43.

[110] X. Wang, G. Wang, W. Li, B. Zhao, B. Xing, Y. Leng, H. Dou, K. Sun, L. Shen, X. Yuan, J. Li, K. Sun, J. Han, H. Xiao, Y. Li, P. Huang, X. Chen, *Small* **2013**, *9*, 3327.
[111] Z. Chen, S. Zhang, Z. Li, G. Ma, Z. Su, *Artif. Cells, Nanomed. Biotechnol.* **2017**, *45*, 897.
[112] S. Vandergraaf, C. Schroen, R. Boom, *J. Membr. Sci.* **2005**, *251*, 7.
[113] W. Liu, X.-L. Yang, W. S. Winston Ho, *J. Pharm. Sci.* **2011**, *100*, 75.
[114] E. van der Zwan, K. Schroën, K. van Dijke, R. Boom, *Colloids Surf. A* **2006**, *277*, 223.
[115] V. Schröder, H. Schubert, *Colloids Surf. A* **1999**, *152*, 103.
[116] A. J. Abrahamse, A. van der Padt, R. M. Boom, W. B. C. de Heij, *AIChE J.* **2001**, *47*, 1285.
[117] L.-Y. Chu, R. Xie, J.-H. Zhu, W.-M. Chen, T. Yamaguchi, S. Nakao, *J. Colloid Interface Sci.* **2003**, *265*, 187.
[118] D.-X. Hao, F.-L. Gong, G.-H. Hu, Y.-J. Zhao, G.-P. Lian, G.-H. Ma, Z. Su, *Ind. Eng. Chem. Res.* **2008**, *47*, 6418.
[119] Q. Yuan, R. A. Williams, S. Biggs, *Colloids Surf. A* **2009**, *347*, 97.
[120] T. Yanagishita, Y. Tomabechi, K. Nishio, H. Masuda, *Langmuir* **2004**, *20*, 554.
[121] S.-H. Park, T. Yamaguchi, S. Nakao, *Chem. Eng. Sci.* **2001**, *56*, 3539.
[122] I. Kobayashi, T. Takano, R. Maeda, Y. Wada, K. Uemura, M. Nakajima, *Microfluid. Nanofluid.* **2008**, *4*, 167.
[123] C. Lovelyn, A. A. Attama, *J. Biomater. Nanobiotechnol.* **2011**, *02*, 626.
[124] M. J. Lawrence, G. D. Rees, *Adv. Drug Delivery Rev.* **2000**, *45*, 89.
[125] F. Buyukozturk, J. C. Benneyan, R. L. Carrier, *J. Controlled Release* **2010**, *142*, 22.
[126] L. M. Hansen, B. S. Hardie, J. Hidalgo, *Ann. Surg.* **1976**, *184*, 80.
[127] P. Nandivada, S. J. Carlson, M. I. Chang, E. Cowan, K. M. Gura, M. Puder, *Adv. Nutr.* **2013**, *4*, 711.
[128] P. C. Calder, G. L. Jensen, B. V. Koletzko, P. Singer, G. J. A. Wanten, *Intensive Care Med.* **2010**, *36*, 735.
[129] H. Yodono, K. Matsuo, A. Shinohara, *Anti-Cancer Drugs* **2011**, *22*, 277.
[130] S. Sahara, N. Kawai, M. Sato, H. Minamiguchi, M. Nakai, I. Takasaka, K. Nakata, A. Ikoma, N. Sawa, T. Sonomura, S. Shirai, *Jpn. J. Radiol.* **2010**, *28*, 362.
[131] S. Bhattacharya, R. Novell, G. M. Dusheiko, A. J. Hilson, R. Dick, K. E. Hobbs, *Cancer* **1995**, *76*, 2202.
[132] J.-M. Chang, W.-S. Tzeng, H.-B. Pan, C.-F. Yang, K.-H. Lai, *Cancer* **1994**, *74*, 2449.
[133] R. Katoh, Y. Asano, A. Furuya, K. Sotoyama, M. Tomita, *J. Membr. Sci.* **1996**, *113*, 131.
[134] C. Charcosset, *J. Food Eng.* **2009**, *92*, 241.
[135] S. M. Joscelyne, G. Trägårdh, *J. Food Eng.* **1999**, *39*, 59.
[136] M. N. Yukuyama, D. D. M. Ghisleni, T. J. A. Pinto, N. A. Bou-Chacra, *Int. J. Cosmet. Sci.* **2016**, *38*, 13.
[137] M. Gallarate, M. E. Carlotti, M. Trotta, S. Bovo, *Int. J. Pharm.* **1999**, *188*, 233.
[138] A. Gijsbertsen-Abrahamse, *J. Membr. Sci.* **2004**, *230*, 149.
[139] Q. Wei, W. Wei, B. Lai, L.-Y. Wang, Y. Wang, Z.-G. Su, G.-H. Ma, *Int. J. Pharm.* **2008**, *359*, 294.
[140] V. Lassalle, M. L. Ferreira, *Macromol. Biosci.* **2007**, *7*, 767.
[141] H.-J. Krause, A. Schwarz, P. Rohdewald, *Int. J. Pharm.* **1985**, *27*, 145.
[142] J. M. Anderson, M. S. Shive, *Adv. Drug Delivery Rev.* **2012**, *64*, 72.
[143] D. D. Von Hoff, M. M. Mita, R. K. Ramanathan, G. J. Weiss, A. C. Mita, P. M. LoRusso, H. A. Burris, L. L. Hart, S. C. Low, D. M. Parsons, S. E. Zale, J. M. Summa, H. Youssoufian, J. C. Sachdev, *Clin. Cancer Res.* **2016**, *22*, 3157.
[144] K. A. Autio, J. A. Garcia, A. S. Alva, L. L. Hart, M. I. Milowsky, E. M. Posadas, C. J. Ryan, J. M. Summa, H. Youssoufian, H. I. Scher, R. Dreicer, *J. Clin. Oncol.* **2016**, *34*, 233.
[145] C. Charcosset, A. El-Harati, H. Fessi, *J. Controlled Release* **2005**, *108*, 112.
[146] E. Kakazu, T. Murakami, K. Akamatsu, T. Sugawara, R. Kikuchi, S. Nakao, *J. Membr. Sci.* **2010**, *354*, 1.
[147] I. Limayem Blouza, C. Charcosset, S. Sfar, H. Fessi, *Int. J. Pharm.* **2006**, *325*, 124.
[148] M. Kukizaki, M. Goto, *J. Membr. Sci.* **2006**, *281*, 386.
[149] S. Schultz, G. Wagner, K. Urban, J. Ulrich, *Chem. Eng. Technol.* **2004**, *27*, 361.
[150] S. M. Jafari, Y. He, B. Bhandari, *J. Food Eng.* **2007**, *82*, 478.
[151] J. M. Perrier-Cornet, P. Marie, P. Gervais, *J. Food Eng.* **2005**, *66*, 211.
[152] P. Guo, C. R. Martin, Y. Zhao, J. Ge, R. N. Zare, *Nano Lett.* **2010**, *10*, 2202.
[153] M. Maas, P. Guo, M. Keeney, F. Yang, T. M. Hsu, G. G. Fuller, C. R. Martin, R. N. Zare, *Nano Lett.* **2011**, *11*, 1383.
[154] P. Guo, T. M. Hsu, Y. Zhao, C. R. Martin, R. N. Zare, *Nanomedicine* **2013**, *8*, 333.
[155] M. Raoufi, T. Das, I. Schoen, V. Vogel, D. Brüggemann, J. P. Spatz, *Nano Lett.* **2015**, *15*, 6357.
[156] M. Raoufi, N. Aslankoohi, C. Mollenhauer, H. Boehm, J. P. Spatz, D. Brüggemann, *Integr. Biol.* **2016**, *8*, 1059.
[157] H. Uehara, M. Ishizuka, H. Tanaka, M. Kano, T. Yamanobe, *RSC Adv.* **2016**, *6*, 13971.
[158] M. J. Olszta, X. Cheng, S. S. Jee, R. Kumar, Y.-Y. Kim, M. J. Kaufman, E. P. Douglas, L. B. Gower, *Mater. Sci. Eng. R: Rep.* **2007**, *58*, 77.
[159] E. DiMasi, M. J. Olszta, V. M. Patel, L. B. Gower, *CrystEngComm* **2003**, *5*, 346.
[160] L. B. Gower, D. J. Odom, *J. Cryst. Growth* **2000**, *210*, 719.
[161] M. J. Olszta, E. P. Douglas, L. B. Gower, *Calcif. Tissue Int.* **2003**, *72*, 583.
[162] L. Gower, D. Tirrell, *J. Cryst. Growth* **1998**, *191*, 153.
[163] M. R. Powell, M. Sullivan, I. Vlassiouk, D. Constantin, O. Sudre, C. C. Martens, R. S. Eisenberg, Z. S. Siwy, *Nat. Nanotechnol.* **2008**, *3*, 51.
[164] L. Innes, M. R. Powell, I. Vlassiouk, C. Martens, Z. S. Siwy, *J. Phys. Chem. C* **2010**, *114*, 8126.
[165] M.-T. Wolfram, M. Burger, Z. S. Siwy, *J. Phys.: Condens. Matter* **2010**, *22*, 454101.
[166] E. R. Cruz-Chu, K. Schulten, *ACS Nano* **2010**, *4*, 4463.
[167] B. Hyland, Z. S. Siwy, C. C. Martens, *J. Phys. Chem. Lett.* **2015**, *6*, 1800.
[168] E. C. Yusko, Y. N. Billeh, M. Mayer, *J. Phys.: Condens. Matter* **2010**, *22*, 454127.
[169] K. M. Pustulka, A. R. Wohl, H. S. Lee, A. R. Michel, J. Han, T. R. Hoye, A. V. McCormick, J. Panyam, C. W. Macosko, *Mol. Pharmaceutics* **2013**, *10*, 4367.
[170] B. K. Johnson, R. K. Prud'homme, *Aust. J. Chem.* **2003**, *56*, 1021.
[171] W. S. Saad, R. K. Prud'homme, *Nano Today* **2016**, *11*, 212.
[172] R. H. Fang, Y. Jiang, J. C. Fang, L. Zhang, *Biomaterials* **2017**, *128*, 69.
[173] A. K. A. Silva, R. Di Corato, T. Pellegrino, S. Chat, G. Pugliese, N. Luciani, F. Gazeau, C. Wilhelm, *Nanoscale* **2013**, *5*, 11374.
[174] S. Tan, T. Wu, D. Zhang, Z. Zhang, *Theranostics* **2015**, *5*, 863.
[175] J. P. K. Armstrong, M. N. Holme, M. M. Stevens, *ACS Nano* **2017**, *11*, 69.
[176] C.-M. J. Hu, L. Zhang, S. Aryal, C. Cheung, R. H. Fang, L. Zhang, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 10980.
[177] C.-M. J. Hu, R. H. Fang, B. T. Luk, L. Zhang, *Nat. Nanotechnol.* **2013**, *8*, 933.
[178] C.-M. J. Hu, R. H. Fang, J. Copp, B. T. Luk, L. Zhang, *Nat. Nanotechnol.* **2013**, *8*, 336.
[179] C.-M. J. Hu, R. H. Fang, K.-C. Wang, B. T. Luk, S. Thamphiwatana, D. Dehaini, P. Nguyen, P. Angsantikul, C. H. Wen, A. V. Kroll, C. Carpenter, M. Ramesh, V. Qu, S. H. Patel, J. Zhu, W. Shi, F. M. Hofman, T. C. Chen, W. Gao, K. Zhang, S. Chien, L. Zhang, *Nature* **2015**, *526*, 118.
[180] B. T. Luk, C.-M. Jack Hu, R. H. Fang, D. Dehaini, C. Carpenter, W. Gao, L. Zhang, *Nanoscale* **2013**, *6*, 2730.
[181] R. Shingles, R. E. McCarty, *Anal. Biochem.* **1995**, *229*, 92.

[182] R. H. Fang, C.-M. J. Hu, B. T. Luk, W. Gao, J. A. Copp, Y. Tai, D. E. O'Connor, L. Zhang, *Nano Lett.* **2014**, *14*, 2181.
[183] T. Kang, Q. Zhu, D. Wei, J. Feng, J. Yao, T. Jiang, Q. Song, X. Wei, H. Chen, X. Gao, J. Chen, *ACS Nano* **2017**, *11*, 1397.
[184] D. Dehaini, X. Wei, R. H. Fang, S. Masson, P. Angsantikul, B. T. Luk, Y. Zhang, M. Ying, Y. Jiang, A. V. Kroll, W. Gao, L. Zhang, *Adv. Mater.* **2017**, *29*, 1606209.
[185] S. C. Jang, O. Y. Kim, C. M. Yoon, D.-S. Choi, T.-Y. Roh, J. Park, J. Nilsson, J. Lötvall, Y.-K. Kim, Y. S. Gho, *ACS Nano* **2013**, *7*, 7698.
[186] B. György, T. G. Szabó, M. Pásztói, Z. Pál, P. Misják, B. Aradi, V. László, É. Pállinger, E. Pap, Á. Kittel, G. Nagy, A. Falus, E. I. Buzás, *Cell. Mol. Life Sci.* **2011**, *68*, 2667.
[187] M. G. Palmgren, P. Askerlund, K. Fredrikson, S. Widell, M. Sommarin, C. Larsson, *Plant Physiol.* **1990**, *92*, 871.
[188] N.-J. Cho, L. Hwang, J. Solandt, C. Frank, *Materials* **2013**, *6*, 3294.
[189] K. Byrappa, S. Ohara, T. Adschiri, *Adv. Drug Delivery Rev.* **2008**, *60*, 299.
[190] E. Reverchon, R. Adami, *J. Supercrit. Fluids* **2006**, *37*, 1.
[191] K. P. Johnston, *Science* **2004**, *303*, 482.
[192] G. B. Jacobson, E. Gonzalez-Gonzalez, R. Spitler, R. Shinde, D. Leake, R. L. Kaspar, C. H. Contag, R. N. Zare, *J. Pharm. Sci.* **2010**, *99*, 4261.
[193] J. Ge, G. B. Jacobson, T. Lobovkina, K. Holmberg, R. N. Zare, *Chem. Commun.* **2010**, *46*, 9034.
[194] G. B. Jacobson, R. Shinde, C. H. Contag, R. N. Zare, *Angew. Chem., Int. Ed.* **2008**, *47*, 7880.
[195] G. B. Jacobson, R. Shinde, R. L. McCullough, N. J. Cheng, A. Creasman, A. Beyene, R. P. Hickerson, C. Quan, C. Turner, R. L. Kaspar, C. H. Contag, R. N. Zare, *J. Pharm. Sci.* **2010**, *99*, 2750.
[196] D. Hu, C. Lin, L. Liu, S. Li, Y. Zhao, *J. Food Eng.* **2012**, *109*, 545.
[197] F. Xia, D. Hu, H. Jin, Y. Zhao, J. Liang, *Food Hydrocolloids* **2012**, *26*, 456.
[198] H. Jin, F. Xia, C. Jiang, Y. Zhao, L. He, *Chin. J. Chem. Eng.* **2009**, *17*, 672.
[199] F. Zabihi, N. Xin, S. Li, J. Jia, T. Cheng, Y. Zhao, *J. Supercrit. Fluids* **2014**, *89*, 99.
[200] W. Wang, Y. Wang, Y. Gao, Y. Zhao, *J. Supercrit. Fluids* **2014**, *85*, 95.
[201] Y. Gao, W. Shi, W. Wang, Y. Wang, Y. Zhao, Z. Lei, R. Miao, *Ind. Eng. Chem. Res.* **2014**, *53*, 2839.
[202] H. Jin, S. Li, D. Hu, Y. Zhao, *Powder Technol.* **2012**, *227*, 17.
[203] F. Zabihi, M. Yang, Y. Leng, Y. Zhao, *J. Supercrit. Fluids* **2015**, *99*, 15.
[204] G. Yang, Y. Zhao, Y. Zhang, B. Dang, Y. Liu, N. Feng, *Int. J. Nanomed.* **2015**, 6633.
[205] F. Zabihi, N. Xin, J. Jia, T. Chen, Y. Zhao, *Ind. Eng. Chem. Res.* **2014**, *53*, 6569.
[206] R. C. Petersen, D. W. Matson, R. D. Smith, *Polym. Eng. Sci.* **1987**, *27*, 1693.
[207] M.-S. Kim, S.-J. Jin, J.-S. Kim, H. J. Park, H.-S. Song, R. H. H. Neubert, S.-J. Hwang, *Eur. J. Pharm. Biopharm.* **2008**, *69*, 454.
[208] R. Roy, J. Yang, M. A. Moses, *J. Clin. Oncol.* **2009**, *27*, 5287.
[209] R. Roy, G. Louis, K. R. Loughlin, D. Wiederschain, S. M. Kilroy, C. C. Lamb, D. Zurakowski, M. A. Moses, *Clin. Cancer Res.* **2008**, *14*, 6610.
[210] R. Roy, U. M. Wewer, D. Zurakowski, S. E. Pories, M. A. Moses, *J. Biol. Chem.* **2004**, *279*, 51323.
[211] R. Roy, A. Dagher, C. Butterfield, M. A. Moses, *Mol. Cancer Res.* **2017**, *15*, 1608.
[212] J. Yang, D. R. Bielenberg, S. J. Rodig, R. Doiron, M. C. Clifton, A. L. Kung, R. K. Strong, D. Zurakowski, M. A. Moses, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3913.
[213] C. Bettegowda, M. Sausen, R. J. Leary, I. Kinde, Y. Wang, N. Agrawal, B. R. Bartlett, H. Wang, B. Luber, R. M. Alani, E. S. Antonarakis, N. S. Azad, A. Bardelli, H. Brem, J. L. Cameron, C. C. Lee, L. A. Fecher, G. L. Gallia, P. Gibbs, D. Le, R. L. Giuntoli, M. Goggins, M. D. Hogarty, M. Holdhoff, S.-M. Hong, Y. Jiao, H. H. Juhl, J. J. Kim, G. Siravegna, D. A. Laheru, C. Lauricella, M. Lim, E. J. Lipson, S. K. N. Marie, G. J. Netto, K. S. Oliner, A. Olivi, L. Olsson, G. J. Riggins, A. Sartore-Bianchi, K. Schmidt, I.-M. Shih, S. M. Oba-Shinjo, S. Siena, D. Theodorescu, J. Tie, T. T. Harkins, S. Veronese, T.-L. Wang, J. D. Weingart, C. L. Wolfgang, L. D. Wood, D. Xing, R. H. Hruban, J. Wu, P. J. Allen, C. M. Schmidt, M. A. Choti, V. E. Velculescu, K. W. Kinzler, B. Vogelstein, N. Papadopoulos, L. A. Diaz, *Sci. Transl. Med.* **2014**, *6*, 224ra24.
[214] S.-J. Dawson, D. W. Y. Tsui, M. Murtaza, H. Biggs, O. M. Rueda, S.-F. Chin, M. J. Dunning, D. Gale, T. Forshew, B. Mahler-Araujo, S. Rajan, S. Humphray, J. Becq, D. Halsall, M. Wallis, D. Bentley, C. Caldas, N. Rosenfeld, *N. Engl. J. Med.* **2013**, *368*, 1199.
[215] A. M. Newman, S. V. Bratman, J. To, J. F. Wynne, N. C. W. Eclov, L. A. Modlin, C. L. Liu, J. W. Neal, H. A. Wakelee, R. E. Merritt, J. B. Shrager, B. W. Loo, A. A. Alizadeh, M. Diehn, *Nat. Med.* **2014**, *20*, 548.
[216] D. M. Rissin, C. W. Kan, T. G. Campbell, S. C. Howes, D. R. Fournier, L. Song, T. Piech, P. P. Patel, L. Chang, A. J. Rivnak, E. P. Ferrell, J. D. Randall, G. K. Provuncher, D. R. Walt, D. C. Duffy, *Nat. Biotechnol.* **2010**, *28*, 595.
[217] S. R. Quake, *Science* **2000**, *290*, 1536.
[218] Y. Song, J. Hormes, C. S. S. R. Kumar, *Small* **2008**, *4*, 698.
[219] L. Capretto, D. Carugo, S. Mazzitelli, C. Nastruzzi, X. Zhang, *Adv. Drug Delivery Rev.* **2013**, *65*, 1496.
[220] T. K. F. Yung, K. C. A. Chan, T. S. K. Mok, J. Tong, K.-F. To, Y. M. D. Lo, *Clin. Cancer Res.* **2009**, *15*, 2076.
[221] E. T. Lagally, I. Medintz, R. A. Mathies, *Anal. Chem.* **2001**, *73*, 565.
[222] A. Huebner, M. Srisa-Art, D. Holt, C. Abell, F. Hollfelder, A. J. deMello, J. B. Edel, *Chem. Commun.* **2007**, 1218.
[223] M. P. Landry, H. Ando, A. Y. Chen, J. Cao, V. I. Kottadiel, L. Chio, D. Yang, J. Dong, T. K. Lu, M. S. Strano, *Nat. Nanotechnol.* **2017**, *12*, 368.
[224] Y. Song, C. S. S. R. Kumar, J. Hormes, *J. Nanosci. Nanotechnol.* **2004**, *4*, 788.
[225] J. B. Edel, R. Fortt, J. C. deMello, A. J. deMello, *Chem. Commun.* **2002**, 1136.
[226] C.-X. Zhao, A. P. J. Middelberg, in *Handb. Nanoparticles* (Ed: M. Aliofkhazraei), Springer International Publishing, Cham, Switzerland **2016**, pp. 455–473.
[227] A. Jahn, W. N. Vreeland, M. Gaitan, L. E. Locascio, *J. Am. Chem. Soc.* **2004**, *126*, 2674.
[228] A. Jahn, W. N. Vreeland, D. L. DeVoe, L. E. Locascio, M. Gaitan, *Langmuir* **2007**, *23*, 6289.
[229] L. Martín-Banderas, M. Flores-Mosquera, P. Riesco-Chueca, A. Rodríguez-Gil, Á. Cebolla, S. Chávez, A. M. Gañán-Calvo, *Small* **2005**, *1*, 688.
[230] R. Karnik, F. Gu, P. Basto, C. Cannizzaro, L. Dean, W. Kyei-Manu, R. Langer, O. C. Farokhzad, *Nano Lett.* **2008**, *8*, 2906.
[231] A. Jahn, S. M. Stavis, J. S. Hong, W. N. Vreeland, D. L. DeVoe, M. Gaitan, *ACS Nano* **2010**, *4*, 2077.
[232] L. Capretto, D. Carugo, S. Mazzitelli, C. Nastruzzi, X. Zhang, *Adv. Drug Delivery Rev.* **2013**, *65*, 1496.
[233] J. C. Stachowiak, D. L. Richmond, T. H. Li, A. P. Liu, S. H. Parekh, D. A. Fletcher, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 4697.
[234] S. Ota, S. Yoshizawa, S. Takeuchi, *Angew. Chem., Int. Ed.* **2009**, *48*, 6533.
[235] T. Kurakazu, S. Takeuchi, in *Micro Electro Mechanical Systems (MEMS), 2010 IEEE 23rd International Conference*, IEEE, **2010**, pp. 1115–1118.
[236] S. P. Nunes, *Macromolecules* **2016**, *49*, 2905.
[237] E. A. Jackson, M. A. Hillmyer, *ACS Nano* **2010**, *4*, 3548.
[238] M. Gopinadhan, P. Deshmukh, Y. Choo, P. W. Majewski, O. Bakajin, M. Elimelech, R. M. Kasi, C. O. Osuji, *Adv. Mater.* **2014**, *26*, 5148.

[239] X. Feng, M. E. Tousley, M. G. Cowan, B. R. Wiesenauer, S. Nejati, Y. Choo, R. D. Noble, M. Elimelech, D. L. Gin, C. O. Osuji, *ACS Nano* **2014**, *8*, 11977.
[240] X. Feng, S. Nejati, M. G. Cowan, M. E. Tousley, B. R. Wiesenauer, R. D. Noble, M. Elimelech, D. L. Gin, C. O. Osuji, *ACS Nano* **2016**, *10*, 150.
[241] S. Qu, T. Dilenschneider, W. A. Phillip, *ACS Appl. Mater. Interfaces* **2015**, *7*, 19746.
[242] Y. Zhang, J. L. Sargent, B. W. Boudouris, W. A. Phillip, *J. Appl. Polym. Sci.* **2015**, *132*, n/a.
[243] X. Feng, K. Kawabata, G. Kaufman, M. Elimelech, C. O. Osuji, *ACS Nano* **2017**, *11*, 3911.