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Teaser Understanding of fundamental, characterization, clinical and regulatory aspects of nanomedicines is vital to enhance their translational potential. Hence, challenges and opportunities related to the commercialization of nanomedicines are discussed.

Facilitating the translation of nanomedicines to a clinical product: challenges and opportunities

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There are numerous hurdles hindering the clinical translation of nanomedicines. The major challenges are: reproducible manufacturing and scale-up, availability of appropriate characterization methods, instability under *in vivo* environments, safety issues, poor understanding of the disease heterogeneity and patient preselection strategies, regulatory barriers and inadequate understanding of the biophysical and chemical interactions of nanoformulations. Thus, a better understanding of key physicochemical attributes and their characterization methods, *in vivo* behavior and the *in-vitro–in-vivo* characterization cascade of stability, safety and efficacy testing is needed to accelerate nanomedicine translation. Technologies such as quality-by-design, process analytical techniques and microfluidics could significantly accelerate the translation of nanomedicines. However, these approaches require further learning and an adequate regulatory background. Overall, to achieve an efficient clinical translation, collaboration among academia, industry and regulatory bodies is required to ensure safe and effective nanomedicine products. This review discusses the challenges and opportunities to facilitate the translation of nanomedicines to a commercial product.

Introduction

The recent developments in biomedical sciences have brought numerous advances in the therapy of complex diseases. Nanomedicine products are exploring a variety of novel therapeutic and diagnostic possibilities owing to their specific therapeutic benefits and versatility of applications [1–4]. Nanocarriers are capable of encapsulating small as well as macromolecule drugs, protecting drugs and increasing the drug half-life *in vivo*, enhancing drug payload, providing an opportunity for controlled or stimuli-responsive drug release and enhancing the targeted delivery of therapeutic molecules, among others. In addition, nanomedicines assist in improving drug biodis-

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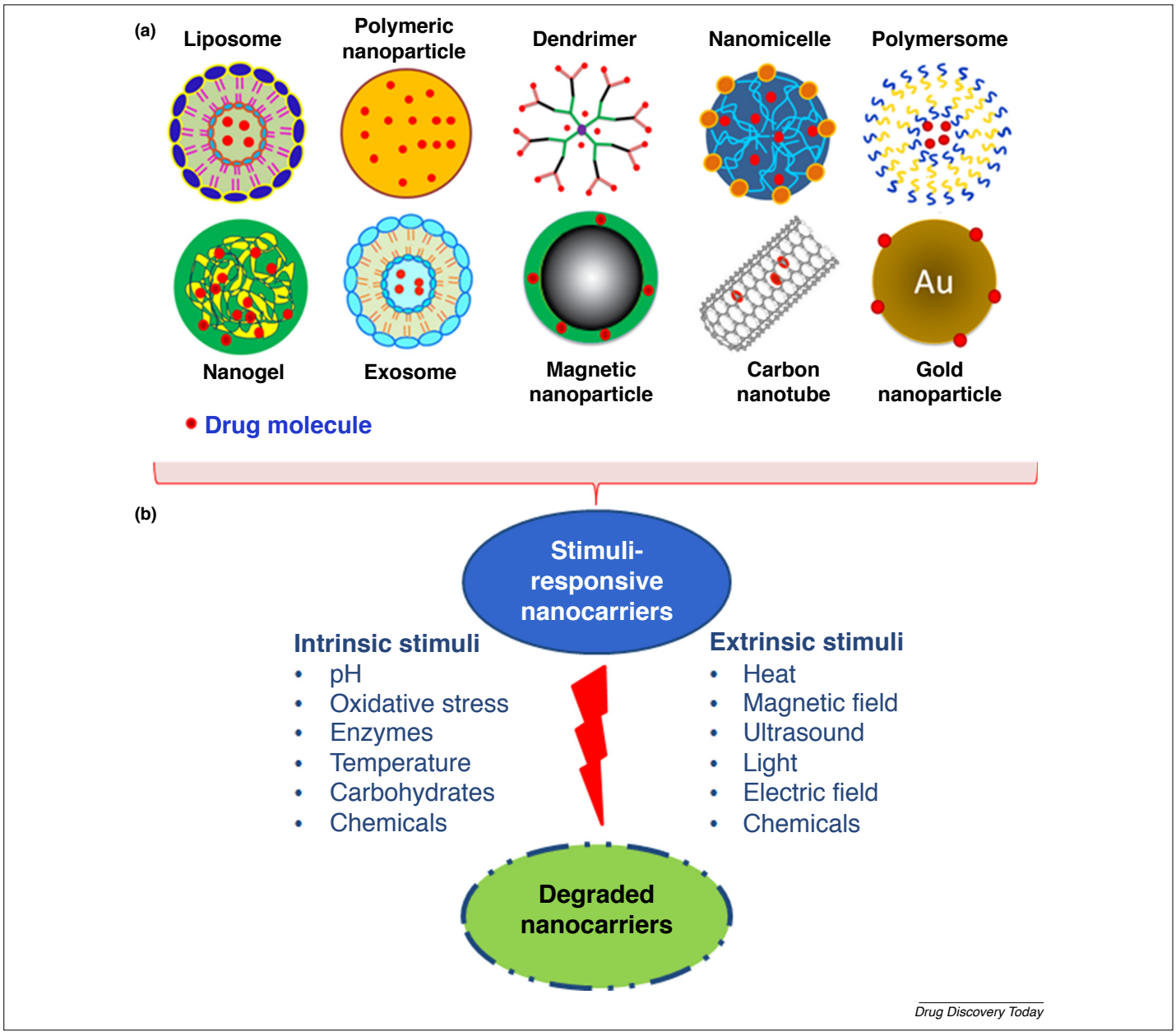

FIGURE 1

(a) Types of nanocarrier systems; reproduced, with permission, from Ref. [4]. (b) Triggered drug release from a nanocarrier in the presence of various external and internal stimuli.

tribution and intracellular delivery. There are several types of nanocarrier systems that have been developed for various indications, as schematically represented in Fig. 1a [4]. Multifunctional nanomaterials have also been developed that combine therapeutic, targeting and imaging capabilities in one system [5,6]. However, these additional functionalities in an individual system can increase the complexity in the development process.

Several therapeutic nanomedicine products have been approved by the FDA and European Medicines Agency (EMA) [2,7–9]. The formulation and evolution over the years of nanomaterials that have made it to the commercial market are summarized in Table 1 [10]. The approval of new nanomedicines is primarily based on improving therapeutic benefits and enhancing the safety profile compared with standard treatments. Doxil®/Caelyx® and Abraxane® anticancer products are two primary examples of success in the clinic [9]. The clinical success of Doxil® and Abraxane® was owing to their ability to focus in tumor cells compared with the conventional chemotherapies with free drugs, which broadly target healthy and cancer cells. Doxil® is a liposomal doxorubicin, which provides a slow drug release in the blood after an intravenous injection. The stealth technology protects liposomes from the immune system and makes Doxil® relatively stable in the body and less toxic compared with standard doxorubicin. Abraxane® is a formulation of paclitaxel bound to albumin nanoparticles (NPs). Abraxane® is more tolerable than conventional paclitaxel (formulated with Kolliphor® EL). The increased tolerance is attributed in part to the absence of toxic solvent which allows Abraxane® to be administered at a significantly higher dose to potentially achieve a better therapeutic efficacy. The success of these nanomedicine products supports the facts that they have a significantly higher therapeutic benefit to the patients

TABLE 1

FDA-approved nanomaterial-based drugs[a]

Name and type of nanomaterial	Year of approval or disease	Nature of nanomaterial	Mechanism of delivery and targeting
Doxil® (liposome)	1995 AIDS/Karposi's sarcoma 2005 ovarian cancer 2008 multiple myeloma	Doxorubicin hydrochloride encapsulated in PEGylated stealth liposome (100 nm)	Accumulation of liposome by passive targeting
Abelcet® (lipid–drug conjugate)	1995 fungal infections	1:1 complex of Amphotericin B with DMPC and DMPG (7:3), ribbon-like structures of a bilayered membrane	Reduce the toxicity of Amphotericin B
DaunoXome® (liposome)	1996 AIDS/Karposi's sarcoma	Liposome encapsulating daunorubicin citrate (45 nm)	Accumulation of liposome by passive targeting and sustained release of daunorubicin
Copaxone® (polymer conjugate)	1996 multiple sclerosis	Random copolymer of l-lysine, l-tyrosine, l-alanine and l-glutamate	Polymer with controlled molecular weight, clearance characteristics and owing to resemblance to myelin it 'decoys' an autoimmune response
AmBisome® (liposome)	1997 systemic fungal infections	Liposome encapsulating Amphotericin B (60–70 nm)	Selective release of the drug from liposome to fungal cell with minimal cellular uptake
DepoCyt® (liposome)	1999, 2007 lymphomatous malignant meningitis	Liposome encapsulating cytarabine	Releases the drug into the cerebral spinal fluid which results in extended half-life and prolonged exposure and drug retention
Visudyne® (liposome)	2000 age-related macular degeneration	Liposome encapsulating verteporfin	Supports the absorption of verteporfin to lipoproteins that carry it to the eyes where it is activated by shining light
Venofer® (magnetic)	2000 iron deficiency in chronic kidney disease	Complex of polynuclear iron (III)-hydroxide in sucrose	Increased and prolonged dosage
Renagel® (polymer conjugate)	2000 chronic kidney disease	Poly(allylamine hydrochloride) crosslinked with epichlorohydrin	Binds to dietary phosphate and prevents its absorption
PegIntron® (polymer conjugate)	2001 hepatitis C	PEG-conjugated IFNα-2β protein	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Pegasys® (polymer conjugate)	2002 hepatitis B and C	PEG-conjugated IFNα-2β protein	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Neulasta® (polymer conjugate)	2002 febrile neutropenia, nonmyeloid malignancies, prophylaxis	PEG-conjugated filgrastim (granulocyte colony-stimulating factor)	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Eligard® (polymer conjugate)	2002 prostate cancer	Leuprolide acetate incorporated in nanoparticles of PLGH copolymer (DL-lactide/glycolide)	Controlled delivery of payload with longer circulation time
Somavert® (polymer conjugate)	2003 acromegaly	PEG-conjugated pegvisomant for injection, an analog of human growth hormone	PEG covalent conjugation increases the stability of GH receptor antagonist
Macugen® (polymer conjugate)	2004 age-related macular and neovascular degeneration	PEG-conjugated antivascular endothelial growth factor aptamer	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
DepoDur® (liposome)	2004 for treatment of chronic pain	Morphine sulfate encapsulated in multivesicular liposomes (~20 μm)	Sustained release post administration in the epidural
Abraxane® (polymer–drug conjugate)	2005 metastatic breast cancer 2012 metastatic non-small-cell lung cancer 2013 metastatic adenocarcinoma of the pancreas	Albumin-conjugated with paclitaxel to form 130 nm particle	Hydrophobic molecules and help endothelial transcytosis of protein-bound and unbound plasma constituents through binding to the cell surface
Mircera® (polymer conjugate)	2007 anemia associated with chronic renal failure in adults	PEG-conjugated erythropoietin receptor activator	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Cimzia® (polymer conjugate)	2008 Crohn's disease 2009 rheumatoid arthritis 2012 psoriatic arthritis 2013 ankylosing spondylitis	PEG-conjugated tumor necrosis factor (TNF)-α inhibitor (certolizumab)	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein

TABLE 1 (*Continued*)

Name and type of nanomaterial	Year of approval or disease	Nature of nanomaterial	Mechanism of delivery and targeting
Feraheme™ (magnetic)	2009 deficiency anemia and iron deficiency in chronic kidney disease	Ferumoxytol SPION with polyglucose sorbitol carboxymethylether	Polymeric coating allows sustained release of Fe^{2+}, decreasing number of doses
Marqibo® (liposome)	2012 acute lymphoblastic leukemia	Liposome encapsulating vincristine sulfate (100 nm)	Enhanced efficacy and reduced toxicity of bare drug
Plegridy® (polymer conjugate)	2014 multple sclerosis	PEG-conjugated IFNβ-1α	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Onivyde® (liposome)	2015 pancreatic cancer	PEG-conjugated liposome nanoparticle encapsulating Irinotecan	Enhanced efficacy, improved circulation time which allows accumulation in tumor site by EPR and reduced toxicity of bare drug
Adynovate® (polymer conjugate)	2015 hemophilia	PEG-conjugated antihemophilic factor (recombinant)	PEG covalent conjugation increases the drug hydrodynamic radius and retention time without effecting the target site of protein
Genexol® PM	Breast cancer, non-small-cell lung cancer, ovarian cancer	Polymeric-micelle-formulated paclitaxel consisting of PEG and poly(D,L-lactic acid) (PDLLA), and free of Cremophor® EL	Stabilization of microtubules, thus preventing cell division
Myocet® (liposome)	2000 (in Europe and Canada) breast neoplasms	Non-PEGylated liposome-encapsulated doxorubicin–citrate complex corresponding to 50 mg doxorubicin hydrochloride	Works by interfering with the DNA within cells, preventing them from making more copies of DNA and making proteins. This means that cancer cells cannot divide and eventually die

[a] Table reproduced and modified, with permission, from Ref. [10].

compared with the conventional drug treatment for clinical success. However, most of the nanomedicine products fail to accomplish a high therapeutic efficacy in clinical trials and never reach the commercial stages.

Compositions of the nanocarrier systems play a vital part in their regulatory approval and can be generally categorized as inorganic, polymeric and liposomes. Polymeric or liposomes have been a major component of nanoformulation-based drugs in clinical translation with the highest rate of FDA approval. However, over the past decade several inorganic nanomaterial-based drugs have also been clinically approved [10]. The distribution of nanomaterials used in drug products from 1973 to 2015 on the basis of their type, indication and the overview of routes of administration is represented in Fig. 2 [11]. Stimuli-responsive nanomedicines are also an attractive area for drug delivery [12]. These innovative systems can trigger drug release in a spatial and temporal manner in response to several stimuli (e.g., pH, temperature, enzymes, oxidative stress, magnetic field, light, ultrasound, heat) (Fig. 1b). This is a promising area to explore further and could have a significant impact to ensure safe and beneficial therapeutic effects. Benefiting from the response to specific stimuli (internal or external), these nanocarriers can reduce the side effects of encapsulated therapeutics, which improves patient compliance [13]. With the introduction of novel stimuli-responsive polymeric systems, we envision an increase in the approval of similar nanomedicine products for human use.

Development in nanomedicines demands sustained clinical translation and commercialization. However, most of the nanomedicine products fail to accomplish a high or improved therapeutic efficacy and/or safety, have a low targeting effect and thus successful clinical translation and commercialization [14]. In most translation failures, nanomedicines that confirmed excellent efficacy in animal models rarely show promise in clinical trials. The major factors that have contributed toward the clinical failure of nanomedicines are challenges in: reproducible and cost-effective manufacturing and scale-up; appropriate regulatory guidelines; availability of characterization methods; safety issues; instability under biological environments; and poor understanding of the disease heterogeneity in the patients [2,9,14–16]. Hence, an understanding of fundamental, characterization, clinical and regulatory aspects of nanomedicines is vital to precisely control the development process and to enhance the translational potential. Primarily, nanomedicine physicochemical attributes, characterization and manufacturing challenges are taken into consideration to achieve an enhanced therapeutic efficacy. However, there has been little focus on designing the nanomedicine products based on disease pathophysiology, patient features, patient preselection strategies and identification of appropriate biomarker profiles to achieve the optimal performance. These features must be defined early in development phases for clinical and cost-effective development of a product. Also, from the start of the project, it is essential to consider the relationship between the disease, patient pathophysiology and physicochemical properties of nanomedicines, to select the appropriate systems. Considering these facts, in this review, current challenges and opportunities to facilitate the translation of nanomedicines to a commercial product are discussed.

Challenges to nanomedicine product development and clinical translation

Scalable and reproducible manufacturing challenges

The scalable, controlled and reproducible manufacturing of nanomedicines under good manufacturing practice (GMP) conditions presents unique challenges [7,14,15,17]. Subtle variations in the manufacturing process of nanomedicines can significantly affect

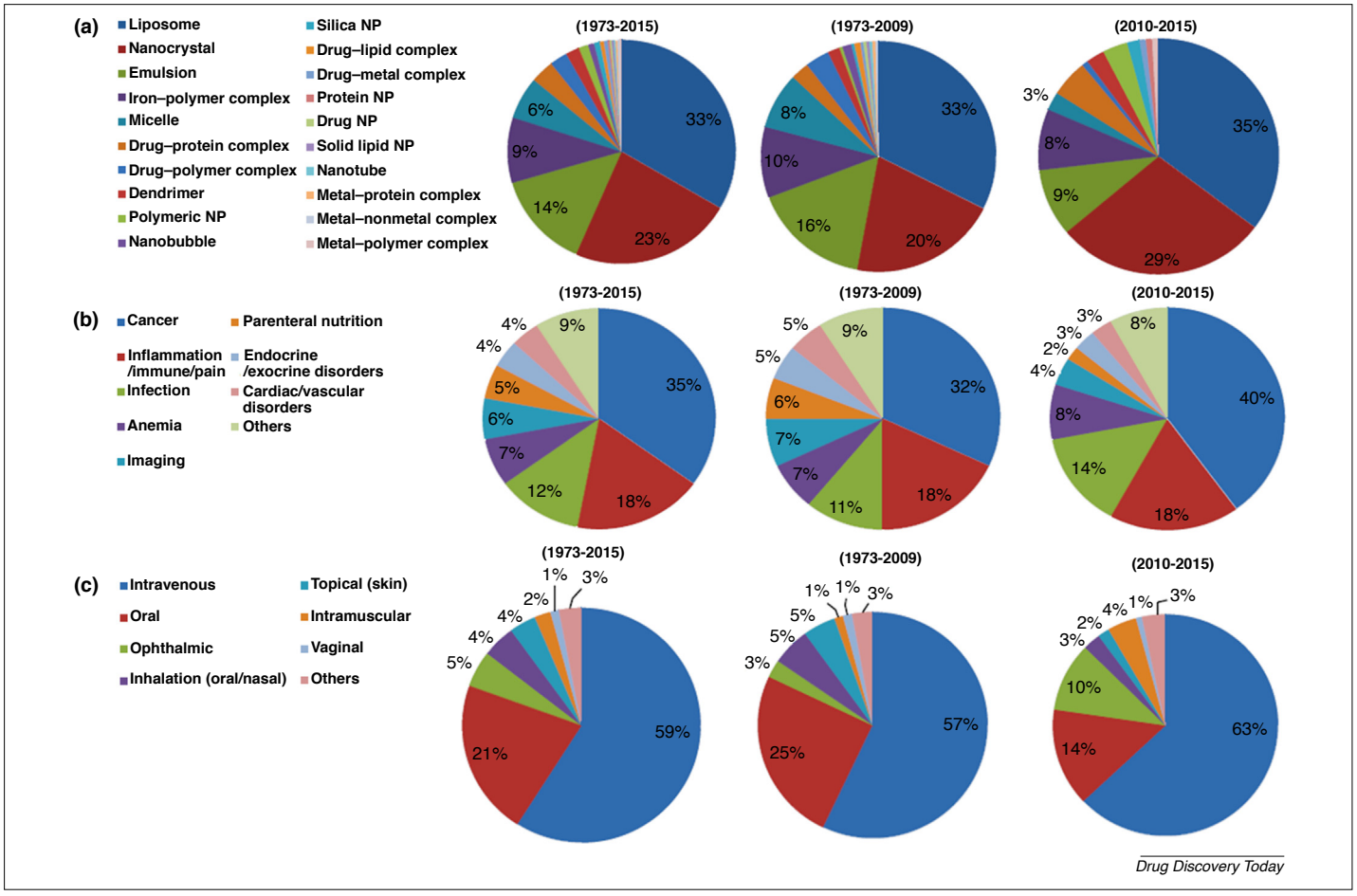

FIGURE 2

Distribution of nanomaterial use in drug products from 1973 to 2015. (a) Breakdown of the types of nanomaterials used in drug products. The nanotechnology terminologies do not represent any implication for Center for Drug Evaluation and Research (CDER) drug product labeling and were used only to describe or interpret the type of nanomaterials in identified drug products for this analysis. (b) Breakdown of the types of indication for drug products containing nanomaterials. The uses (prophylactic, therapeutic, diagnostic) for each product were categorized into nine application areas based on their intended or approved use. (c) Overview of routes of administration for drug products containing nanomaterials. Reproduced, with permission, from Ref. [11]. Abbreviation: NP, nanoparticle.

the physicochemical properties such as size, shape, composition, crystallinity, drug loading, drug release, and surface functionality and chemistry, among others, as summarized in Fig. 3a. The alterations in these properties ultimately influence the therapeutic outcomes of the final product. The nanoformulation process often involves multiple complex steps. At the laboratory scale, the optimization and reproducibility of these steps (e.g., homogenization, centrifugation, extrusion, lyophilization, sterilization, etc.) can be accomplished relatively easily; however, on a large scale it is difficult [15]. Moreover, the steps involved in nanomanufacturing demand an in-depth understanding of the process along with experienced technicians, further enhancing the cost and complexity of the development process.

The lack of data related to the scaling-up of nanoformulations further hinders their commercialization. There are several formulation methods available for the manufacturing of nanomedicines but the two major ones are emulsion-based and nanoprecipitation approaches [18]. In general, formulation methods can be classified as bottom-up (beginning from a dissolved molecule to a precipitate) and top-down (starting from a large drug to a smaller one) processes. However, the bottom-up approach needs the removal of the traces of the remaining solvent, which is very challenging, and there is difficulty in controlling the process, thus it is less popular in industrial manufacturing [19]. Only a few reports are available that are supported by scale-up aspects of the lab-scale development of nanomedicines to industrial manufacturing; for example the scale-up of NPs loaded with ibuprofen was evaluated at the pilot-scale by increasing the lab-batch volume from 60 ml to 1.5 l [20]. The NPs were produced by salting-out, emulsification–diffusion and nanoprecipitation processes. Eudragit® L100-55 and poly(vinyl alcohol) (PVA) were used as polymer and emulsifying agent, respectively. Overall, NP characteristics were reproduced well at lab- and pilot-scales but the scale-up process induced a slight decrease in the size and drug loading. Another study to find the scale-up parameters essential for the formulation of nanocapsules (NCs) was performed [21]. Two polymers: poly-ε-caprolactone (PCL) and Eudragit® E100 were used for the preparation of NCs. The core of the NCs was made of Miglyol® 812 and indomethacin was used as the drug in solution in the oil. Starting from the lab-scale (0.06 l), a pilot-scale (2 l) batch was designed to produce NCs using an emulsification–diffusion technique. Experiments were conducted by varying operating parameters such as the impeller

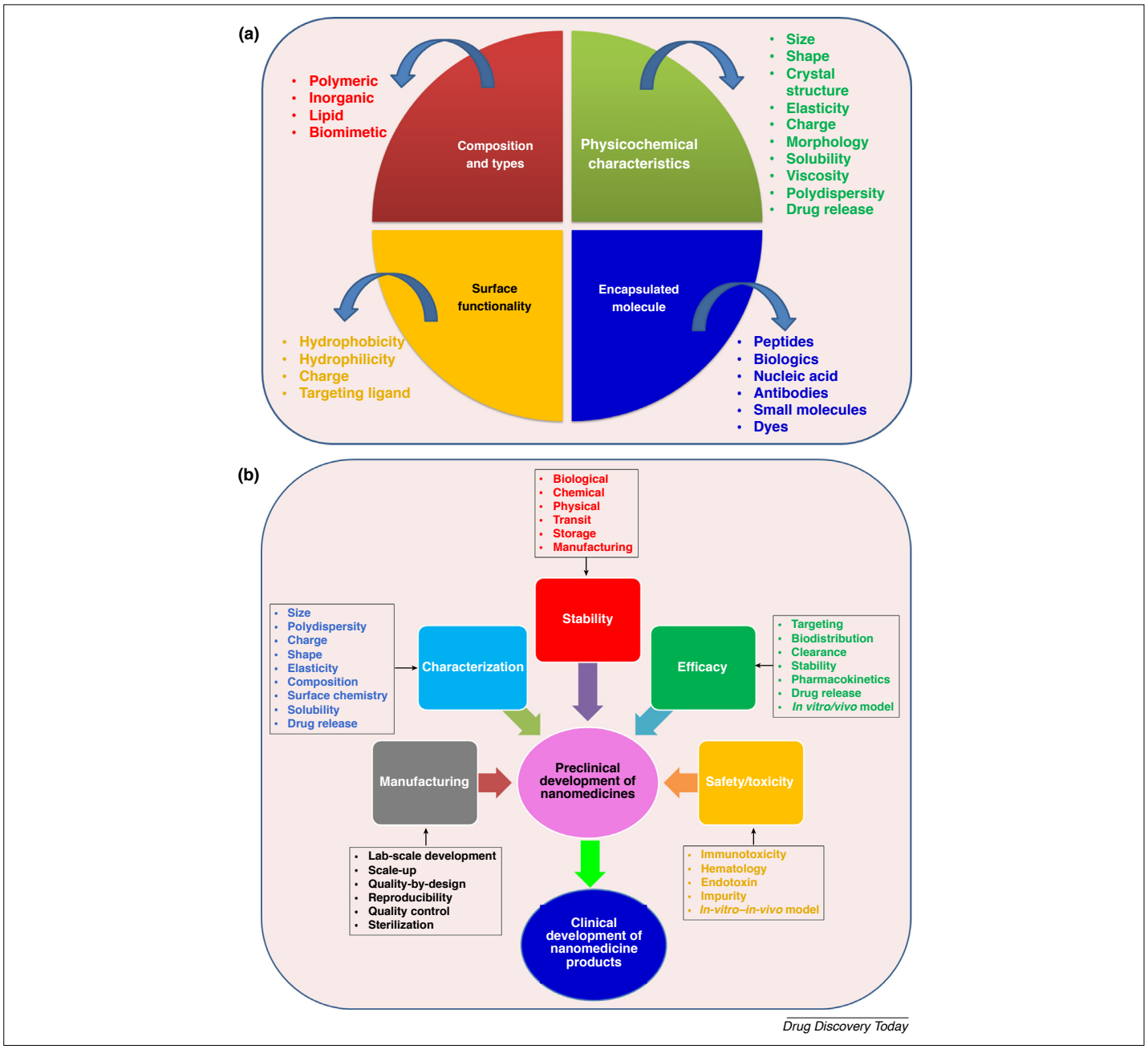

FIGURE 3

(a) An overview of composition and characteristics of nanomedicines. (b) Key areas (manufacturing, characterization, characterization, efficacy and safety or toxicity) in preclinical development of nanomedicines.

speed, agitation time and the reagent concentrations. A lower particle size was noted with an increase in the impeller speed and agitation time although the entrapment efficiency was not affected [21].

There are several components associated with nanomedicine manufacturing and, hence, during the scale-up of lab methods, the desired critical quality attributes (CQAs) of nanomedicines might not be reproduced. Advanced approaches that can reproducibly prepare nanoformulations with desired characteristics in a high-throughput manner are strongly desirable. A careful selection of safe materials, solvents used, manufacturing method, cost-effectiveness and clinical acceptability of the finished product is important from the scale-up point of view. In general, pharmaceutical industries have a well-established set-up for conventional dosage forms (e.g., solid, semisolid, liquid); however, they are not well-equipped for the manufacturing of nanomedicines. Thus, it is important that the nanoformulation process is designed while considering the manufacturing constraints in the industry. One such constraint is in the use of organic solvents due to safety concerns [14,15]. Hence, use of safe or low-toxicity solvents (and, if possible, methods using aqueous solvents) has to occur for industrial manufacturing and development of nanomedicine products [15]. For example, Ranjan *et al.* evaluated the use of safe solvents while performing the scale-up of curcumin-loaded poly (D,L-lactide-co-glycolide) (PLGA) NPs [22].

Nanomedicine products administered to humans must ensure their sterility [23] and thus the manufacturing must be equipped

with an aseptic area [15]. The sterilization process can pose challenges to the stability of nanomedicines. For example, the γ-irradiation methods can degrade or affect particle integrity of NPs [24–26]. Nanoformulations containing biological molecules (e.g. proteins and peptides) require special consideration owing to their high susceptibility to degradation by sterilization techniques [27]. Thus, finding an appropriate sterilization method that can be used without compromising the physicochemical properties and stability of therapeutic molecules is one of the major challenges in nanomedicine development. In one study, injectable NPs were prepared by nanoprecipitation using poly(γ-benzyl-L-glutamate) (PBLG) and analyzed for their particle size, zeta potential and surface properties [28]. The sterilization of the PBLG NP suspension by membrane filtration or autoclaving was evaluated. Sterilization with membrane filtration showed no significant effect on surface properties of NPs. Also, no microbial contamination was seen, indicating that the sterile NP formulations had been achieved after membrane filtration. Recently, the effects of sterilization and depyrogenation on stability and applications of NPs were reviewed [25]. Several techniques were compared for the removal of microbial contamination from NPs. Of these methods, filtration could have potentially removed microbial contamination without changing the physicochemical properties of the NPs, toxicity or functionality. However, it was summarized that no single process can be applied to all NP preparations and each NP–drug system should be validated on a case-by-case basis.

Other manufacturing challenges are mainly associated with the freeze-drying and storage conditions. Freeze-drying should be carefully evaluated to ensure that the physicochemical attributes of nanomedicines are preserved [29]. In one study, PCL NPs were prepared by the emulsification–diffusion method and then were frozen at different freezing rates [30]. The NPs were freeze-dried under different operating conditions and the particle size was analyzed – first after the freezing step and finally after the sublimation step. It was observed that the freezing process broke the NPs and caused the leakage of their contents during the freeze-drying step. The higher the freezing rate the larger the size of NPs during the freezing process [30]. The influence of freeze-drying with different cryoprotectants and γ-irradiation sterilization on the physicochemical properties of ciprofloxacin-HCl-loaded PLGA NPs was evaluated [31]. NPs prepared by emulsification solvent evaporation followed by high-pressure homogenization were freeze-dried in the presence of 5% w/v mannitol, trehalose or glucose, with 5% w/v or 15% w/v dextran as the cryoprotectant. NPs were irradiated at a dose of 25 kGy using a Cobalt-60 source. The freeze-drying process induced a significant increase in particle size when cryoprotectant was added or not added to the formulation (except in the case of mannitol). No significant difference in the particle size was observed, but reconstitution was problematic, and a slower and/or similar drug release was seen after γ-sterilization. Overall, results showed that γ-sterilization should be carefully investigated because it might cause changes in the properties of the drug formulations. In one of the recent studies, the stabilization of flurbiprofen-loaded PCL NPs (FB-PCL-NPs) (prepared by solvent displacement with poloxamer 188 as the stabilizer) under a freeze-drying process was evaluated for commercial development [32]. Freezing and primary drying were optimized, and the design of experiments was used to validate secondary drying conditions and component concentrations. The successful design of the NP system resulted from rational cooperation between a good formulation and the right conditions in the freeze-drying process [32].

An understanding of the effect of storage conditions on the stability and biocompatibility of NPs is of paramount importance for their translation into the clinic and reproducibility in preclinical evaluations. Nanomedicine stability could be reduced over time depending upon the storage conditions. For example, based on the chemical and morphological characteristics of a polymer, it could start degrading after NP formulation in aqueous or organic solvents. This might result in changes in properties and *in vivo* performance of formulations. Also, the storage of NPs in water, PBS, and/or biological fluids could affect the measurement of NP size, surface charge and drug-release profile. To prolong the storage stability of nanoformulations, one common approach is freeze-drying, which presents challenges as explained earlier. Lemoine *et al*. evaluated the stability of NPs prepared with PCL, poly(D,L-lactide) and poly(D,L-lactide-co-glycolide), stored at different temperatures of 16 °C, 4 °C and 37 °C and in different media [33]. Results recommended the suitable storage conditions of NPs at 4 °C and 37 °C. In one study, stability and effect of storage conditions on lipidoid NPs (LNPs) was analyzed [34]. The LNP efficacy in HeLa cells under the influence of pH, temperature and lyophilization was evaluated. Results showed that, under aqueous conditions, LNPs were most stable over 150 days under refrigeration (2 °C) compared with at room temperature or at −20 °C. It was also suggested that LNPs can be stored under physiological conditions (pH 7) [34]. Superparamagnetic iron oxide NPs (SPIONs) with a hybrid coating consisting of lauric acid and albumin were stored over the 12 weeks at temperatures from 4 °C to 45 °C and tested for their physicochemical properties [35]. No denaturation of the protein or colloidal instability was observed; however, the biocompatibility was affected, because cellular uptake of the SPIONs was dependent on the storage conditions.

Screening, quality control and characterization challenges

Identifying the appropriate methods to characterize the physicochemical or biological properties of nanomedicines is challenging from a technical as well as a regulatory standpoint. In general, preclinical characterization includes a comprehensive description of the physicochemical characteristics, manufacturing process, quality, efficacy and safety *in vitro* and in animal models, and stability analysis [7]. The physicochemical characteristics include size-distribution, surface morphology, surface functionality, solubility, drug loading, drug release, among others (Fig. 3b). These characteristics as well as the stability (chemical, physical and microbiological) of nanomedicines under accelerated, normal and *in vivo* environments are desired to ensure the robust performance of the product. In early development, characterization is focused on providing a thorough understanding of the physicochemical aspects of the product and how they are affected by variations in the formulation method and operating and/or storage conditions. Such initial descriptions are valuable to establish the acceptable range of process or formulation parameters and can provide an understanding of the effect of these parameters on the physicochemical properties of nanomedicines [36,37]. However,

lack of reliable and validated techniques to analyze nanomedicine characteristics and stability under a GMP environment are major hurdles in their clinical translation [38] and there are several possible reasons for this, as discussed below.

Industries are usually equipped with analytical techniques best-suited for conventional dosage forms; however, nanomedicine characterization involves advanced approaches or techniques. These techniques include dynamic light scattering (DLS) and NP tracking analysis (NTA) for size-distribution, Zetasizer for surface charge, transmission/scanning electron microscopy (TEM/SEM) for size-distribution and surface morphology, small-angle X-ray diffraction for polymer layer thickness measurement [e.g., poly(ethylene glycol) (PEG) coating on NP surface], X-ray photoelectron or Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy analyses for surface chemistry, liquid chromatography (LC) for drug release and loading evaluation, among others [39]. The analytical techniques commonly used for the evaluation of physicochemical characteristics of nanoformulations, in addition to the strength and limitations of each method, is summarized in Table 2 [39]. Nevertheless, these techniques are expensive and require a team of experts to conduct the analysis and results interpretation [14,15]. This substantially adds to the cost of the nanomanufacturing characterization and development. Moreover, some of these techniques have significant issues for example DLS has size and shape constraints and the electron microscopy methods are intensive and samples must be specially prepared. Hence, when possible, the use of multiple techniques that complement each other to evaluate the same parameter is recommended for example DLS, NTA, TEM/SEM can be used in parallel for size-distribution measurement and LC or NMR can be used for drug release or loading evaluation. In addition, these methods must be sensitive enough to detect potential subtle variations. Another challenge is the characterization of nanomedicine's shape [40,41] and elasticity [42], which affects their circulation time, biodistribution, cell uptake and interactions with cells or tissues. Hence, these parameters also need to be evaluated using appropriate methods.

Nanomedicine characterization is frequently performed under settings that do not effectively reflect the complexity of the biophysical environment of human organs and tissues. Moreover, owing to the complexity of the human body, the *in-vitro–in-vivo* correlations of nanomedicines are difficult to predict precisely, which greatly hinders their clinical translation [43,44]. Therefore, approaches such as microfluidics have emerged as promising tools for creating *in vitro* microenvironments that mimic *in vivo* conditions [44,45]. The development of a reliable *in vivo* model is challenging, owing to the demands on spatial control and regular arrangement of cells. Microfluidics displays structures and networks at relevant physiological length scales, provides flexibility in channel design and incorporates fluid flow and mechanical forces that allow the cell-based assays to mimic the *in vivo* microenvironment. In addition, the requirement of a very small amount of sample in microfluidics enables high throughput screening, thus it is cost-effective. Microfluidics is also a versatile technology in the production and evaluation of nanocarriers [44–46] (Fig. 4). NPs are usually prepared by nanoprecipitation in the microfluidic channels with continuous flows, which ensures the quality and avoids batch-to-batch variability in production. The small nanoliter volume of fluids flowing inside the microfluidics channels can significantly reduce the consumption of reagents [44,45]. The starting materials, for example the copolymers, can assemble into NPs when a change in solvent quality occurs, which can be accomplished by an efficient mixing of the organic and aqueous solvents. The process of assembling copolymers into NPs can be divided into three steps: formation of a nucleus by copolymers during a solvent change; growth of the size of the nucleus by adding more copolymers until the formation of a polymer brush layer on the surface of NPs; the equilibrium between the free and assembled copolymers to keep the size of the NPs stable [44]. The solvent-mixing time affects the particle size distribution of NPs. The accurate manipulation of fluids at the picoliter scale in microchannels results in a precisely controlled mixing process. The homogenous solvent situation facilitates the stabilization of NPs and the formation of small, narrowly distributed NPs. In general, the physicochemical properties of NPs can be precisely controlled by tuning the microfluidic formulation parameters such as the concentrations and types of particle precursors in solvents, flow ratios between the solvents and nonsolvents, and their total flow rates [43,44]. Despite the recent advances, the industrial translation of microfluidics is challenging. One major issue is related to the production rate per day of NPs, which is usually in the milligram range [44]. However, recently developed parallel and stackable systems could continuously produce NPs on the larger scale with similar properties to those seen on a small scale [44]. The advantages, challenges, development stages and the potential impact of microfluidics on different steps in the nanomedicine translation are summarized in Table 3 [45]. The detailed description of microfluidics is out of the scope of this review and can be accessed elsewhere [44,45].

It is essential that manufacturing of nanomedicines include quality control (QC) checks at all the steps to reproducibly produce the batches that meet the CQAs of the product [7,15]. For multicomponent nanomedicines, the amounts of each component and the structure and interactions among them should be quantified and evaluated. Analysis of the individual components over time can reveal degradation products generated during the synthesis or storage. It is also important to analyze the physical state of components that impact functional aspects of nanomedicines such as the drug-release rate and biodegradation [15]. For components with limited shelf-life and stability, the lyophilization and reconstitution methods must be evaluated so that the product characteristics are not altered.

In summary, developing robust characterization methods is one of the most important aspects in the development of nanomedicine products. Toward this goal, the Nanotechnology Characterization Laboratory (NCL) (https://ncl.cancer.gov/) founded in collaboration with the National Cancer Institute (NCI), National Institute of Standards and Technology (NIST) and the FDA supports the preclinical characterization of nanomedicines to accelerate the product development and translation. The goal is to provide robust characterization for the identification of crucial parameters related to the effectiveness and safety of nanomedicines. Other organizations such as the European Nanomedicine Characterization Laboratory (EU-NCL) (http://www.euncl.eu) were also set up to provide a testing infrastructure covering a comprehensive set of preclinical assays (physicochem-

TABLE 2

Analytical modalities for evaluation of the physicochemical characteristics of nanomaterials[a]

Techniques	Physicochemical characteristics analyzed	Strengths	Limitations
Dynamic light scattering (DLS)	• Hydrodynamic size distribution	• Nondestructive/invasive manner • Rapid and more-reproducible measurement • Measures in any liquid media, solvent of interest • Hydrodynamic sizes accurately determined for monodisperse samples • Modest cost of apparatus	• Insensitive correlation of size fractions with a specific composition • Influence of small numbers of large particles • Limit in polydisperse sample measures • Limited size resolution • Assumption of spherical shape samples
Fluorescence correlation spectroscopy (FCS)	• Hydrodynamic dimension • Binding kinetics	• High spatial and temporal resolution • Low sample consumption • Specificity for fluorescent probes • Method for studying chemical kinetics, molecular diffusion, concentration effect and conformation dynamics	• Limit in fluorophore species • Limited applications and inaccuracy owing to lack of appropriate models
Zeta potential	• Stability • Referring to surface charge	• Simultaneous measurement of many particles (using ELS)	• Electro-osmotic effect • Lack of precise and repeatable measurement
Raman scattering (RS) Surface enhanced Raman scattering (SERS) Tip-enhanced Raman spectroscopy (TERS)	• Hydrodynamic size and size distribution (indirect analysis) • Conformation change of protein–metallic-NP conjugate • Structural, chemical and electronic properties	• Complementary data to IR • No requirement of sample preparation • Potential of detecting tissue abnormality • Enhanced RS signal (SERS) • Increased spatial resolution (SERS) • Topological information of nanomaterials (SERS, TERS)	• Relatively weak single compared to Rayleigh scattering • Limited spatial resolution (only to μm) • Extremely small cross-section • Interference of fluorescence • Irreproducible measurement (SERS)
Near-field scanning optical microscopy (NSOM)	• Size and shape of nanomaterials	• Simultaneous fluorescence and spectroscopy measurement • Nanoscaled surface analysis at ambient conditions • Assessment of chemical information and interactions at nanoscaled resolution	• Long scanning time, small specimen area analyzed • Incident light intensity insufficient to excite weak fluorescent molecules • Difficulty in imaging soft materials • Analysis limited to the nanomaterial surface
Circular dichroism (CD)	• Structure and conformational change of biomolecules (e.g., protein and DNA) • Thermal stability	• Nondestructive and prompt technique	• Nonspecificity of residues involved in conformational change • Less sensitive than absorption methods • Weak CD signal for nonchiral chromophores • Challenging for analysis of molecules containing multiple chiral chromophores
Mass spectroscopy (MS)	• Molecular weight • Composition structure • Surface properties (secondary ion MS)	• High accuracy and precision in measurement • High sensitivity to detection (a very small amount of sample required)	• Expensive equipment • Lack of complete databases for identification of molecular species • Limited application to date in studying nanomaterial bioconjugates
Infrared spectroscopy (IR) Attenuated total reflection Fourier transform infrared (ATR-FTIR)	• Structure and conformation of bioconjugate • Surface properties (ATR-FTIR)	• Fast and inexpensive measurement • Minimal or no sample preparation requirement (ATR-FTIR) • Improving reproducibility (ATR-FTIR) • Independence of sample thickness (ATR-FTIR)	• Complicated sample preparation (IR) • Interference and strong absorbance of H_2O (IR) • Relatively low sensitivity in nanoscale analysis
Scanning electron microscopy (SEM) Environmental SEM (ESEM)	• Size and size distribution • Shape • Aggregation • Dispersion	• Direct measurement of the size and size distribution and shape of nanomaterials • High resolution (down to subnanometer) • Images of biomolecules in natural state provided using ESEM	• Conducting sample or coating conductive materials required • Dry samples required, • sample analysis in nonphysiological conditions (except ESEM) • Biased statistics of size distribution in heterogeneous samples • Expensive equipment • Cryogenic method required for most NP bioconjugates • Reduced resolution in ESEM

TABLE 2 (*Continued*)

Techniques	Physicochemical characteristics analyzed	Strengths	Limitations
Transmission electron microscopy (TEM)	• Size and size distribution • Shape heterogeneity • Aggregation • Dispersion	• Direct measurement of the size and size distribution and shape of nanomaterials with higher spatial resolution than SEM • Several analytical methods coupled with TEM for investigation of electronic structure and chemical composition of nanomaterials	• Ultrathin samples in required • Samples in nonphysiological condition • Sample damage or alternation • Poor sampling • Expensive equipment
Scanning tunneling microscopy (STM)	• Size and size distribution • Shape • Structure • Dispersion • Aggregation	• Direct measurement • High spatial resolution at atomic scale	• Conductive surface required • Surface electronic structure and surface topography unnecessarily having a simple connection
Atomic force microscopy (AFM)	• Size and size distribution • Shape • Structure • Sorption • Dispersion • Aggregation • Surface properties (modified AFM)	• 3D sample surface mapping • Subnanoscaled topographic resolution • Direct measurement of samples in dry, aqueous or ambient environment	• Overestimation of lateral dimensions • Poor sampling and time consuming • Analysis in general limited to the exterior of nanomaterials
Nuclear magnetic resonance (NMR)	• Size (indirect analysis) • Structure • Composition • Purity • Conformational change	• Nondestructive or noninvasive method • Little sample preparation	• Low sensitivity • Time consuming • Relatively large amount of sample required • Only certain nuclei NMR active
X-ray diffraction (XRD)	• Size, shape and structure for crystalline materials	• Well-established technique • High spatial resolution at atomic scale	• Limited applications in crystalline materials • Only single conformation/binding state of sample accessible • Low intensity compared to electron diffraction
Small-angle X-ray scattering (SAXS)	• Size and size distribution • Shape • Structure	• Nondestructive method, simplification of sample preparation • Amorphous materials and sample in solution accessible	• Relatively low resolution

[a] Table reproduced, with permission, from Ref. [39].

ical, *in-vitro–in-vivo* biological testing). These allow researchers to fully understand the ADME (absorption, distribution, metabolism, and excretion), safety and immunological effects of nanomedicines.

Therapeutic efficacy and pharmacokinetics evaluation challenges

One of the essential requirements for any product to become commercially successful is establishing its benefits over the existing products especially in terms of efficacy and safety. The development of a new drug or nanomedicine product starts with preclinical testing followed by the submission of an investigational new drug (IND) application to begin the clinical trial. The clinical trials consist of a stepwise evaluation of the safety and therapeutic efficacy and are divided in Phases I, II and III [47]. Following IND approval, nanomedicine systems are evaluated clinically using the same process or parameters as for the small-molecule drugs. The general pipeline for the development of nanomedicines is represented in Fig. 5 [23]. Normally, preclinical and clinical studies support the development, clinical use, safety and understanding of the therapeutic differences of nanomedicines from existing formulations. Determining ADME in animal models is also part of the preclinical evaluation. The *in vitro* and *in vivo* evaluations are aimed at characterizing the interactions of the product with biologic systems. *In vivo* tests offer the most vital information including efficacy and toxicity, but they are expensive. Hence, *in vitro* alternatives that can recreate *in vivo* environments are useful, but it is extremely difficult to recreate *in vivo* conditions given the complexities associated with human organs, tissues and diseases. Thus, the translation of nanomedicines could be greatly improved by the development of animal models that mimic the heterogeneity and anatomical histology of humans. Moreover, the conventional *in vitro* cell culture models lack the complexity of biological tissues and control over the fluid flow. For example, under standard cell culture methods, NPs often settle on the surface of the cells; however, blood and interstitial fluid flow directly impact NP–cell interactions *in vivo*. Microfluidic devices mimicking the biological environments can capture the NP–cell interactions under physiological flow conditions [44–46].

During scale-up, small deviations in the formulation and manufacturing process produce subtle changes in the physicochemical properties of nanomedicines, which can result in altered therapeutic efficacy and safety profiling. There are several characteristics of NPs that significantly impact the ADME and safety profile. Out of these, particle-size-distribution, surface charge and shape are some of the important ones. Owing to their small size

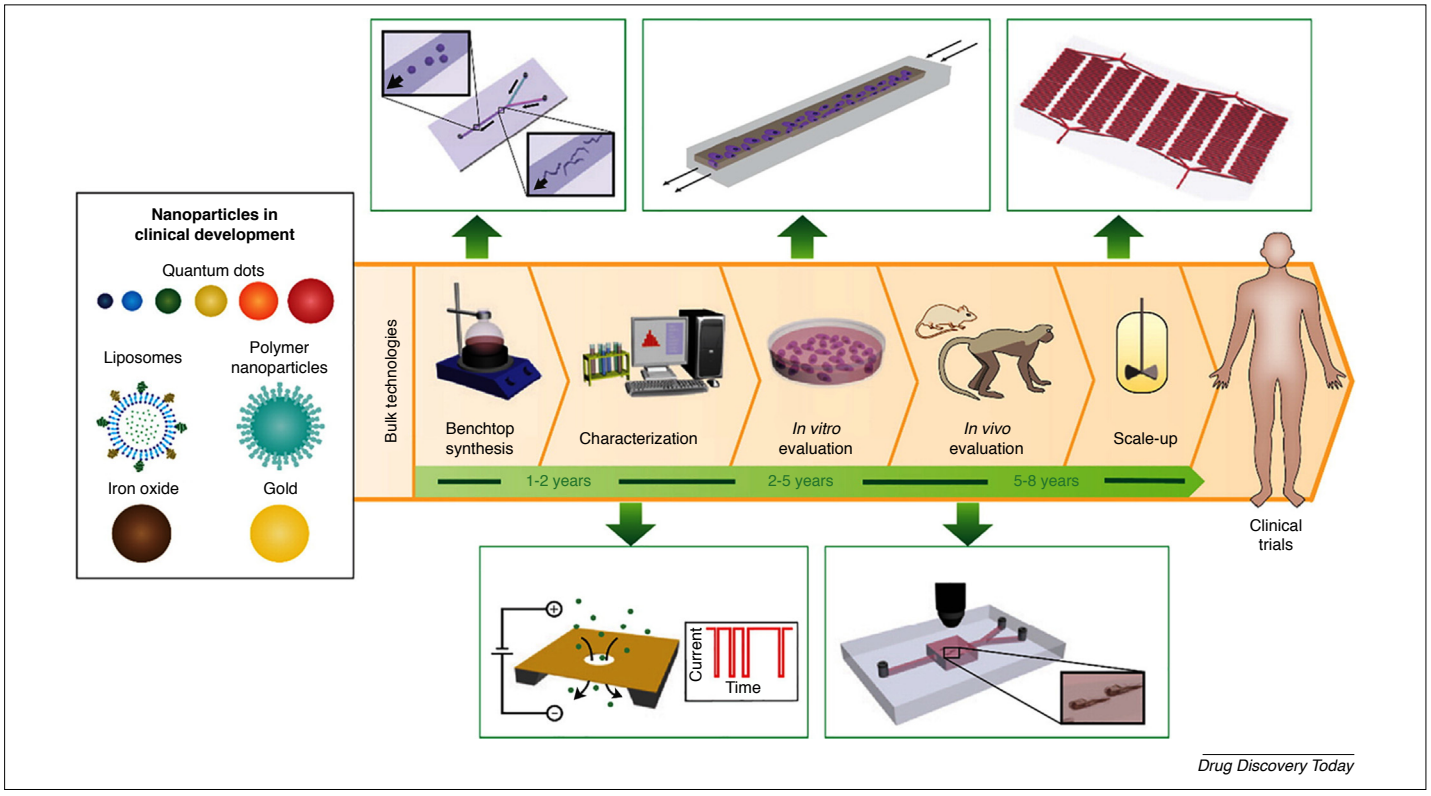

FIGURE 4

Nanoparticles in clinical development, steps for their translation (with average timescales) and microfluidic methods (green boxes) that could improve or complement current technologies. Synthesis is carried out in large reaction flasks, whereas microfluidic synthesis is carried out at micro- and nano-scales that enable improved control over reaction conditions. Characterization often involves taking a small sample of nanoparticles and measuring their properties offline, whereas nanopores embedded in microfluidic devices enable real-time, in-line characterization. *In vitro* evaluation in plate wells produces a microenvironment far from that of the *in vivo* situation, whereas continuous flow in microfluidic systems results in conditions closer to *in vivo* conditions. *In vivo* evaluation in large animals is helpful for estimating the pharmacology of nanoparticles. To complement these studies microfluidic systems could enable real-time tracking of nanoparticles in large numbers of small organisms. Scale-up is generally carried out in reactor vessels several times larger than benchtop flasks, whereas parallelization of microfluidic channels can increase the production rate of nanoparticles with properties identical to those at the bench scale. Reproduced, with permission, from Ref. [45].

and thus high surface:volume ratios, nanoformulations are reactive and their physical interactions with biological surfaces can alter the therapeutic effect [37,48]. After administration, small NPs were eliminated by renal excretion, larger NPs were rapidly taken up by the mononuclear phagocytic system (MPS) cells present in the liver, spleen and, to a lesser extent, in the bone marrow [49]. NPs of 150–300 nm were found primarily in the liver and spleen, whereas NPs of 30–150 nm were found in the bone marrow, heart, kidney and stomach [49]. NP size, shape and surface charge dictate biodistribution among the different organs including the lungs, liver, spleen and kidneys [50]. Therefore, the NP size-distribution needs to be carefully controlled during the manufacturing process. NP surface properties (charge, hydrophobicity, functionality, etc.) are crucial for their interaction with cells and the opsonization process [51,52]. Various blood components (e.g., albumin, fibrinogen, IgG) coat the NPs in the opsonization process, which targets the particles to be cleared by macrophages. Polymers such as PEG protect NPs from opsonization by providing a hydrophilic surface [52]. PEG-oligocholic-acid-based micellar NPs with high positive or negative surface charge were efficiently taken up by RAW 264.7 murine macrophages after opsonization [53]. A high liver uptake was observed for highly positively or negatively charged NPs, probably owing to active phagocytosis by macrophage Kupffer cells. By contrast, a low liver uptake, but very high tumor uptake, was noted when the surface charge of NPs was slightly negative [53]. The density and type of active targeting moieties (ligand) on the NP surface can also alter biodistribution, targeting toxicity and cellular uptake [54,55]. For instance, variation of the density of surface-targeting ligands can potentially elicit complement activation and the immune responses of nanocarriers [56,57].

Under the physiological environment, the nanoformulation interaction with biological components forms a protein corona that is primarily composed of proteins [58]. The protein corona can be considered unique for each given nanoformulation and significantly depends on the physicochemical properties such as size, shape, and surface chemistry. The characteristics of the biological environment, for example the type and physiological state of the plasma, incubation time, temperature, and pH, play an important part in the formation of protein corona. The formation of protein corona can trigger an immune response and impact nanoformulation properties, toxicity, targeting capabilities, cell uptake, accumulation, biodegradation and clearance [58]. Hence, an in-depth understanding of the protein corona is important in the design and to achieve the desired therapeutic outcomes of nanoformulations.

TABLE 3

Advantages, disadvantages/challenges, stage of development and potential impact of microfluidic systems on different steps in the clinical translation of nanoparticles (reproduced with permission from Ref. [45])

	Advantages	Disadvantages/challenges	Stage of development	Potential impact
Synthesis	• Tunable nanoparticle size • Narrower size distribution • Reproducible synthesis • Potential for high-throughput synthesis and optimization of nanoparticles	• Solvent and high-temperature incompatibility for low-cost polydimethylsiloxane microchannels • Higher costs and complexities in the fabrication of glass and silicon microdevices	*****	Rapid combinatorial, controlled and reproducible synthesis of libraries of distinct nanoparticles for a specific application, and/or reference nanoparticles for toxicology studies
Characterization	• Label-free characterization • Potential for feedback control and real-time nanoparticle optimization	• Current methods are not applicable to all classes of nanoparticles • Not all properties can be characterized, such as drug encapsulation and release, and signal-to-noise ratio	*	In-line rapid characterization and optimization of nanoparticles
In vitro	• Biological conditions closer to *in vivo* microenvironments • Potential for high-throughput screening of a large number of nanoparticles at different concentrations	• Higher costs and complexities in the fabrication and operation compared with well plates • Might not be reusable and if reusable, it would be difficult to keep sterile	****	High-throughput studies of nanoparticle toxicity, efficacy, tumor penetration and organ distribution, using 'organ-on- a-chip' systems
In vivo	• Large number of organisms could be used for a single measurement • High-throughput evaluation of toxicity for a large number of nanoparticles	• Lack of methods to translate data from small-scale organisms to other species • Pharmacokinetics or biodistribution cannot be determined	**	Real-time tracking of the distribution or toxicity of nanoparticles on small-scale organisms
Large-scale synthesis	• Continuous synthesis • Bench-scale to clinical-scale reproducibility • Parallelization allows for tuning scale of production	• Difficult to build systems at low-cost that are comparable to a batch reactor able to prepare grams or kilograms of nanoparticles	***	Synthesis of nanoparticles for human administration using stackable parallel microfluidic units

(*****) Rank: Most advanced in development; (*) to least advanced in development, based on the amount of research carried out on each category, as well as the potential ease of adoption by industry.

The shape of the nanocarrier affects their biodistribution, circulation time and uptake by cells [40,41]. A comparative study of elongated, nonspherical and spherical microparticles (MPs; 2 μm) and NPs (150 nm) with and without PEGylation was carried out to target two phagocytosis-inhibiting techniques [59]. The uptake into murine macrophage (J774.A1) cells was significantly reduced upon PEGylation or elongated particle geometry. A combination of elongated shape and PEGylation showed the strongest phagocytosis-inhibiting effect for NPs [59]. *In vitro* cell uptake studies of differently shaped convex NPs, such as spheres, rods, cubes or disks, were performed [60]. Comparing identical NP surface area, ligand–receptor interaction strength and grafting density of the PEG, it was observed that the cellular uptake of NPs was in the order of sphere > cube > rod > disk. The NP shape effect was mainly induced by the different membrane-bending energies during endocytosis. Overall, the spherical shape was more promising for improving the efficacy of the cargo [60]. Results from one study showed that rod-shaped and spherical particles improved the lung-targeting after 30 min in mice when coated with anti-intercellular adhesion molecule (ICAM)-1 antibody [61]. Also, both particles showed enhanced uptake in liver and spleen when coated with IgG. The strategy offered a combination of novel chemical, physical and biological approaches to maximize the tissue targeting [61]. The circulation time and cell uptake of NPs can also be affected by their elasticity (stiffness) [42]. To investigate the role of particle elasticity on *in vivo* performances, PEG-based hydrogel NPs of 200 nm with elastic moduli ranging from 0.255 to 3000 kPa have been synthesized [62]. The softer NPs (10 kPa) exhibit a prolonged circulation time and subsequently enhanced targeting compared with harder NPs (3000 kPa). Furthermore, softer NPs exhibited significantly lower cellular uptake in immune, endothelial and cancer cells *in vitro*. Hence, identifying the optimal NP parameters for the intended indication is crucial. Currently, there are no appropriate *in vivo* models to predict the diverse behaviors of nanomedicines, so their development with desirable properties must rely on preclinical animal testing. The lack of reliable screening methods that can evaluate the efficacy and safety of nanomedicines with a good *in vivo* correlation is a substantial barrier [7,14].

Safety evaluation challenges

Nanomedicines exhibit significantly varied properties (e.g., high surface-area:volume ratio) compared with the same material at the larger scales. This could subsequently alter their interaction with cells and biomolecules, as well as their biodistribution, and thereby their safety profile is significantly affected. There are currently

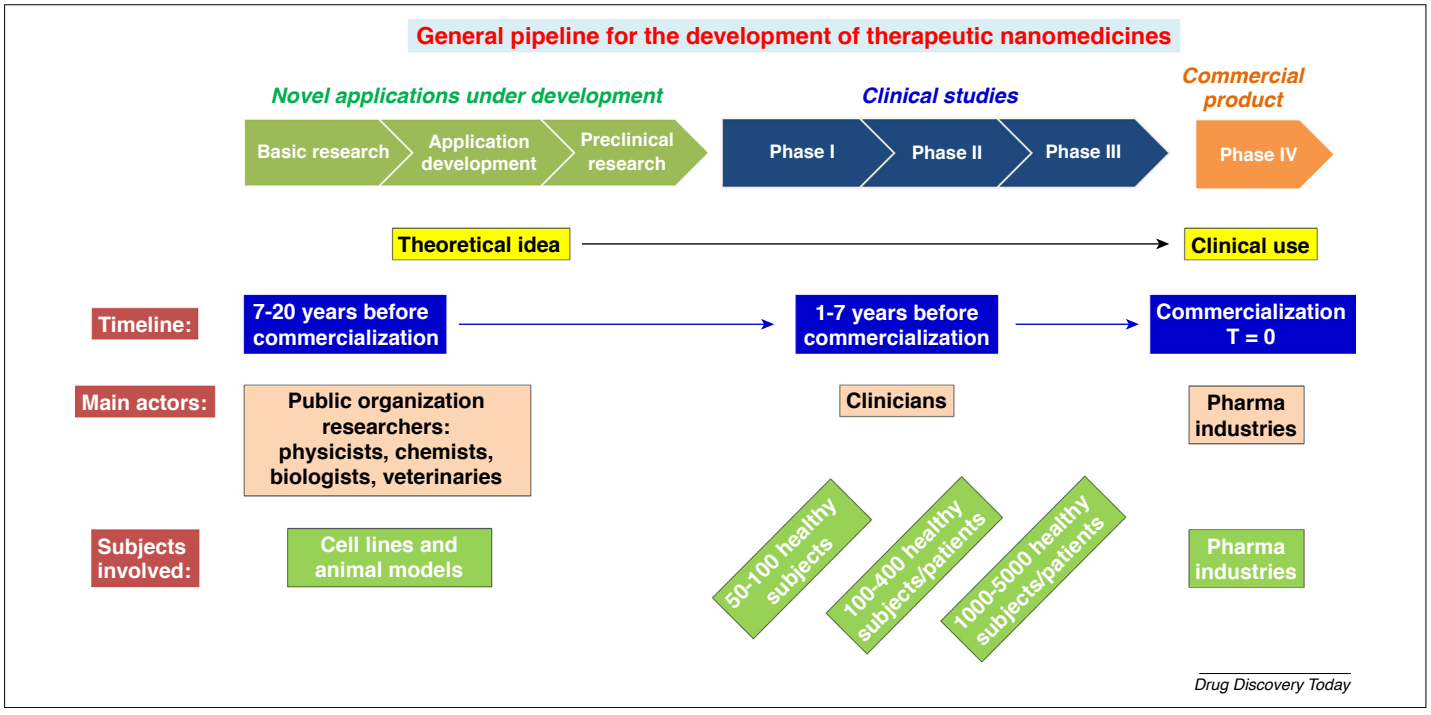

FIGURE 5

Schematic representation of the process of nanomedicine development. Adapted, with permission, from Ref. [23].

no specific requirements from the regulatory agencies for the preclinical and clinical testing of nanoformulations and the preclinical tests for small-molecule drugs are considered adequate to assess toxicity, efficacy and ADME of nanomedicine products [7]. However, preclinical data, especially immunotoxicity, cannot accurately predict the safety of nanomedicines and the data obtained cannot always be extrapolated to humans [7,15]. Moreover, *in vitro* toxicity tests are mostly carried out using cell culture models in monolayers (2D). However, uptake of nanoformulations into cells and tissues is influenced by interactions among their own physicochemical characteristics (e.g., surface charge, shape, functionality and material composition). Therefore, 3D cell systems might provide better results [63,64]. Recently, microfluidics technologies have emerged as promising tools for creating *in vitro* environments that mimic *in vivo* conditions. However, accesses to cultivated cells and sampling for assays are difficult and raise issues with microfluidics-based 3D culture systems. In addition, the development of cost-effective and easy-to-use systems is challenging. Hence, efforts are needed to promise reproducibility and high-throughput analysis to establish validated 3D cell culture models. Although the 3D cell culture models can mimic the *in vivo* setting better than 2D cell culture, they still cannot efficiently predict toxicity *in vivo*.

Nanomaterials can be easily contaminated with endotoxin (lipopolysaccharides) during production or handling owing to the highly reactive nature of their surface. Endotoxin can lead to serious health issues owing to its inherent stability and presence in biological systems [65,66]. Moreover, because of the potent inflammatory activity of endotoxin, contaminated nanomedicines can be inflammatory or toxic, which can misidentify their real biological effects [67]. More than 30% of all nanoformulations fail in early preclinical development owing to endotoxin contamination [65,66]. Thus, the endotoxin level of nanomedicines must be carefully evaluated using appropriate methods [66,68]. According to the FDA, the endotoxin limit is 0.5 EU/ml (Guidance for Industry: Pyrogen and Endotoxins Testing: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm314718.htm). The *in vivo* rabbit pyrogen test (RPT) and the *in vitro* Limulus amoebocyte lysate (LAL) assay are the most common endotoxin detection methods approved by FDA and EMA [66,67]. Alternative and sensitive *in vitro* bioassays such as the human peripheral blood mononuclear cell (PBMC) activation assay and the human monocyte activation test (MAT) are also approved by the European Centre for the Validation of Alternative Methods (ECVAM) for assessing pyrogens [66,67]. However, RPT, PBMC and monocyte assays do not specifically measure endotoxin but do detect inflammatory responses from all types of inflammation-inducing agents. Thus, the LAL assay is recommended to specifically detect the endotoxin level in nanomaterials. The advantages, disadvantages, and comparison of different test methods to detect endotoxin in nanomaterials are described elsewhere [66–68]. In 2012, the FDA published a guideline that discussed two new methods as alternative LAL methods: the recombinant horseshoe crab factor C assay (specific for endotoxin, not recognizing β-glucan) and the macrophage activation-type pyrogen test (sensitive but not specific for endotoxin) (https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm314718.htm). However, the FDA recommends validating the alternative methods for individual products. Also, to eliminate the problem of NP interference with the LAL assay, an endotoxin extraction method is stated in the ISO 29701:2010 regulation (https://www.iso.org/obp/ui/#iso:std:iso:29701:ed-1:v1:en).

A range of physiochemical characteristics such as size-distribution, morphology, shape, surface area, sedimentation and aggregation can significantly affect biophysicochemical interactions of

nanomedicines. Nanoformulation sedimentation during *in vitro* testing can significantly influence the rate and/or extent of drug uptake by cells, and thus influences the pharmacological and/or toxicological responses [69,70]. This is because NPs can sediment and their concentration on the cell surface can be higher, which could lead to increased uptake by cells. Moreover, if NPs are aggregated, their sedimentation and uptake rates would be higher than that of individual NPs. Hence, careful examination of NP stability under *in vivo* conditions and their tendency toward sedimentation in biological fluids is essential. Results from one study showed that the cellular uptake of gold NPs depended on the sedimentation and diffusion velocities of the NPs and was independent of size, shape, density, surface coating and initial NP concentration [69]. Data suggested that sedimentation must be considered when performing *in vitro* cellular uptake studies with large and/or heavy NPs. The correlation among the hydrodynamic size, sedimentation stability, and cellular toxicity of alumina NPs was investigated for a concentration range of 25–200 μg/ml and incubation time of 0–72 h using floating (THP-1) and adherent (J774A.1, A549 and 293) cells [71]. A decrease of the viability was found in the cells in a dose-dependent manner. However, the time-dependent decrease in adherent cell viability was predominantly related to the sedimentation of NPs in culture medium. Hence, sedimentation and aggregation properties should be evaluated to elucidate the interaction of NPs with organelles, cells and tissues. A thorough understanding of the fundamental mechanisms of potential NP–cellular interactions will help to generate criteria for the design of NPs that can be used *in vivo* [14].

Engineered nanoformulations are small and have a high specific surface area and reactivity, leading to the production of higher levels of reactive oxygen species (ROS), resulting in cytotoxicity and genotoxicity [72]. Owing to their large specific surface area, nanoformulations can potentially absorb transition metals onto the surface, which can catalyze several reactions to generate hydroxyl radicals [73]. Limbach *et al*. found that nanosilica doped with transition metals in A549 cells generated high levels of ROS [74]. Another issue with nanomedicines is environmental safety during the manufacturing process. The handling of a nano-sized powder demands special caution and adequate protection because such particles are capable of skin penetration and can also lead to pulmonary toxicity [15,75]. The relationship between a workers' group presenting with mysterious symptomatic findings and their NP exposure has been evaluated [76]. The cases stimulate concern that long-term exposure to some NPs without protective measures can relate to serious damage to human lungs [76]. In this respect, the formulation of NPs entirely within a liquid environment could have significantly lower environmental impact. Hence, there is urgent need to reduce the knowledge gap between the physicochemical properties of nanomedicines and their influence on the manifestation of toxicity issues. Accordingly, physicochemical properties and safety of nanomedicines in biological systems should be systematically investigated before the extensive introduction of these products.

Inadequate regulation and challenges in industry, physicians and socioeconomic acceptance

Nanomedicine is a diverse and complex arena and there are challenges in getting a clear definition, as well as effective regulation, of these products. Although a significant numbers of approved nanomedicine products have appeared, the lack of specific regulatory guidelines for development and characterization of these products at biophysical levels has hampered their clinical potential [7,8,14]. One of the obstacles underlying the regulation of nanomedicines is that the clinical use of these complex therapeutics is strongly dependent on their physicochemical properties. These properties can be easily altered by slight changes in raw materials and also by small modifications in the manufacturing processes, which can significantly affect the biological and the safety profile of nanomedicines. Hence, there is a crucial need for the regulatory organizations to develop a comprehensive list of tests that cover the characterization, efficacy, biodistribution and toxicity aspects of nanomedicines. A fundamental regulatory question for nanomedicine translation is whether the product meets the standards of a scientific and acceptable definition of nanomedicine (https://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm). Moreover, there are challenges in addressing the aggregates and agglomerates of nanomedicines. Aggregates and agglomerates do not reflect individual particles and thus the expected safety or health risk based on the results of the nanoformulation diameter could be misleading. Overall, owing to the complexity of nanomedicine products, it is apparent that the regulatory pathways face several hurdles. To simplify or shorten the approval process, evaluation of key physicochemical parameters for manufacturing, efficacy and safety, and scientifically acceptable definition of nanomedicines, is needed. The regulatory perspective and challenges of nanomedicines has been reviewed in detail by other authors [8,77].

For nanomedicine products to be commercially successful, the development methods need to be followed by the industry. In this regard, the nanomedicine products face numerous challenges. Commercialization in the field of nanomedicine is currently driven by small-to-medium-sized companies [78,79]. However, these companies are rarely successful in commercializing any new product without the support of larger companies because of the high development costs [79]. Therefore, collaboration with larger multinational companies is crucial. In addition, initiatives must be taken to bridge the gap between the lab and the large-scale industrial manufacturing of nanomedicines to facilitate commercialization. The commercialization of nanomedicine products is also dependent on their reputation and acceptance within the community. Unfortunately, the public is relatively ignorant of nanomedicine benefits in addition to the associated safety issues because of the limited knowledge of nanotechnology [14]. However, they might only become aware of the potential of nanomedicine products if these formulations perform well in the clinics. One of the major driving forces for the development of novel products such as nanomedicines is the need for newer therapeutic options by physicians. However, physicians have significant concerns regarding the safety and efficacy of nanomedicines [14]. Despite all the issues, innovations in healthcare are expected to bring new nanomedicine products to the market and the investment in the market is predicted to rise [14]. According to Grand View Research, the global nanomedicine market is anticipated to reach US$350.8 billion by 2025 (http://www.grandviewresearch.com/press-release/global-nanomedicine-market). The nanomedicine approach is

anticipated to drive R&D developments, subsequently resulting in revenue generation in the coming years. It is expected that nanomedicine will revolutionize current therapies but, to achieve successful commercialization, it is essential to demonstrate the physicians' and patients' acceptance and socioeconomic added values. Also, there has been little focus on designing the nanomedicine products based on disease pathophysiology of the patients and patient-selection criteria. Hence, from the start of the project, it is essential to consider the patient pathophysiology, and physicochemical properties of nanomedicines to select the appropriate systems. Aspects of a patient's acceptance of the nanomedicine therapy should be considered from the early stages of the product design for a successful commercialization of these novel products. A key consideration when adopting nanomedicine or other innovative therapies is the cost:benefit ratio compared to available conventional treatments.

Concluding remarks and future perspectives on nanomedicine translation and commercialization

The application of nanomedicines in healthcare is changing current diagnosis and therapy concepts. Despite their therapeutic significance, only a few products have reached the market. A comprehensive preclinical assessment of nanomedicines includes physicochemical characterization, efficacy, pharmacology, and toxicology evaluations (Fig. 3b) [65]. In summary: (i) the challenges in physicochemical characterization include the unavailability of appropriate and sensitive methods; (ii) the challenges in determining the efficacy include selection of the appropriate models, drug encapsulation and release, stability, and evaluation of biological activity; (iii) the challenges in pharmacology and toxicology evaluations are related to the drug biodistribution, availability of relevant animal models, determining the mechanisms of toxicity and the *in-vitro–in-vivo* correlation between toxicity assays. Other technical challenges include sterilization and endotoxin removal of nanomedicines. Thus, a better understanding of crucial physicochemical characteristics, *in vivo* behavior as well as the *in-vitro–in-vivo* characterization cascade of safety and efficacy testing is needed to accelerate nanomedicine translation [65,80].

The development of a nanomedicines requires that the product quality must satisfy manufacturing, industry, the patient or customer and the regulatory demands. In this regard, the implementation of a robust QC system is the key to ensuring successful manufacturing and quality of nanomedicines [81–84]. Identification of the product CQAs helps in determining whether a batch meets or fails the standard requirements. Thus, identifying the essential process conditions is crucial to attain key attributes of a product. Incorporating a quality-by-design (QbD) approach in product development can contribute to gaining thorough product and process knowledge and enabling cost-effective manufacturing [81–84]. The QbD concept is strongly recommended by regulatory agencies to ensure a high-quality product. In the QbD approach, the formulation and process are designed to consistently deliver a product that meets the CQAs necessary for clinical performance. This necessitates the understanding of the influence of raw materials and process parameters on the product quality. In pharmaceutical manufacturing, QbD identifies CQAs and investigates the effects of factors based on scientific design and risk assessment. In addition, QbD helps construct a comprehensive understanding of relations between manufacturing conditions and final product characteristics to facilitate the scale-up of the nanomanufacturing process. Although promising, more systemic studies employing the QbD concept need to be conducted. Training programs are needed for the scientists for a better understanding of the QbD terminologies such as design space, CQAs, among others, and application software.

Nanomedicine manufacturing and its characteristics are difficult to predict or measure because the formulation processes are sensitive to raw material attributes and any subtle changes in the processing conditions. Hence, the development of process analytical technology (PAT) is encouraging to monitor the product quality [81,83,85]. The FDA has encouraged the use of PAT to obtain process data in real-time and build quality assurance into the manufacturing process (https://www.fda.gov/downloads/drugs/guidances/ucm070305.pdf). PAT techniques can provide valuable insight and understanding for process scale-up/optimization and help accommodate the inherent process variability and improve control [81,83,85]. PAT provides information of CQAs with the goal of improving the final product quality as well as reducing the manufacturing cost [81,83,85]. The commonly used PAT tools include near-infrared, infrared, Raman, UV and MS, and real-time imaging techniques, among others. Among these, near-infrared spectroscopy has been extensively applied in industry. There is a potential that nanomedicines will accomplish wide clinical application under the influence of QbD and PAT concepts. However, efforts are needed to develop the real-time monitoring methods for a better process understanding and control. Instead of using nanomedicines to develop a formulation for clinically effective drugs, engineering specific features into the drug itself to make it compatible for encapsulation, loading and conjugation with nanoformulations is also a viable option.

It remains difficult to reproducibly manufacture the NP batches with identical properties. Furthermore, knowledge on the *in vivo* fate and biophysical and chemical interactions of NPs remains limited and there are few platforms that can evaluate the biological behavior of NPs *in vitro*, and can be correlated with the *in vivo* performances. Thus, there is a need for high-throughput methods for evaluating the interactions of NPs with cells, plasma proteins and the complement system. It is expected that technologies such as microfluidics undertaking some of these challenges could significantly accelerate the discovery and clinical translation of nanomedicines. In addition to the advantages in fabricating reproducible NPs on an industrial scale, microfluidics also contribute significantly to the cost-effective and *in vivo* mimetic screening of NPs. Although still being evaluated, microfluidics systems have the potential to become broadly implemented owing to their economics, reproducibility and capability of easy modifications and integration with other technologies [44–46]. Another exciting prospect is the integration of multiple steps of nanomedicine development into a single system through the incorporation of microfluidics, robotics or automation technologies. This can significantly reduce the time and cost of nanoformulation development. But, to accelerate the progress of microfluidics-based NP screening technology, the collaboration among scientists from

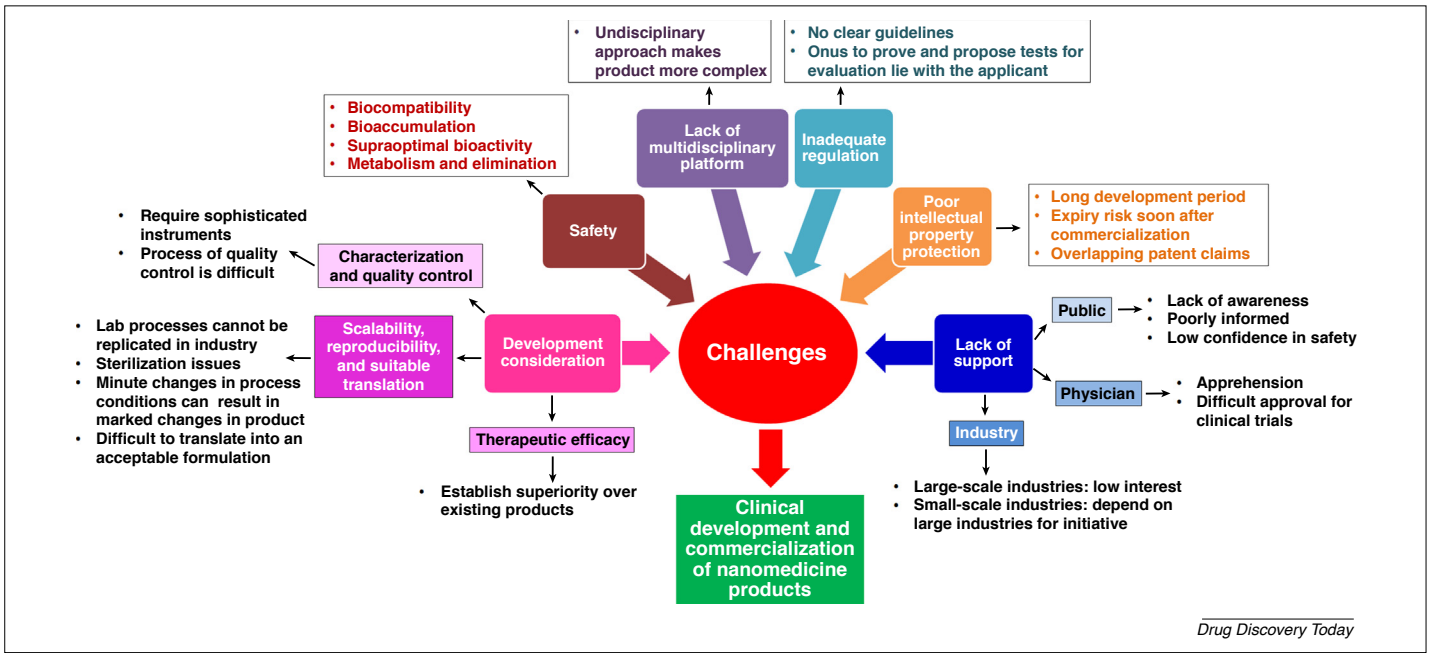

FIGURE 6

A summary of overall challenges to the development and commercialization of nanomedicine products. Adapted, with permission, from Ref. [14].

materials, formulations, microfluidics and biomedical engineering backgrounds is desirable.

Stimuli-responsive nanomedicines are also an attractive and promising area to explore that could have a significant impact on ensuring safe and beneficial therapeutic effects. However, the obstacles for these smart nanosystems in potential clinical applications are their adequate evaluation, development, optimization and scale-up for industrial production. Moreover, there is a variability in stimuli such as pH and enzyme levels from one patient to another, which is difficult to control in clinical applications. A successful commercialization of nanomedicines also needs consumer confidence, which requires learning, risk assessments and an adequate regulatory background. The several hurdles hampering the clinical translation of nanoformulations are discussed in this review and summarized in Fig. 6 [14]. In summary, the strategies that could significantly enhance the therapeutic efficacy of nanomedicine products can include:

- Defining key physicochemical parameters influencing the drug efficacy and safety.
- Understanding robust characterization methods.
- Application of QbD, PAT and microfluidics approaches in the manufacturing, scale up and evaluation.
- Development of adequate *in vitro*, *ex vivo* and *in vivo* models.
- Understanding product interactions with the biological environment.
- Development of validated stability, safety and efficacy assays.
- Development of specific regulatory guidelines for manufacturing and characterization.
- Focus on selecting the right patients and patient preselection criteria to develop strategies on patient-focused product design.
- Identifying a suitable biomarker profile that is predictive of therapeutic outcomes.
- Employing clinically applicable imaging techniques that can be correlated to the fate of the drug and delivery system *in vivo*.
- Clinical trials focused on well-defined outcomes and understanding disease- and patient-specific pathophysiology

Overall, to bridge the gap of nanomedicine's lab research to industrial development, effective collaboration among academics, scientists, industry, regulatory agencies, consortia, investors, and clinicians is required to develop comprehensive approaches to ensure reproducibility and precise control for an effective, safe and cost-effective nanomedicine product.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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