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Review

PLGA-based nanomedicines manufacturing: Technologies overview and challenges in industrial scale-up

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ABSTRACT

Nanomedicines based on poly(lactic-co-glycolic acid) (PLGA) carriers offer tremendous opportunities for biomedical research. Although several PLGA-based systems have already been approved by both the Food and Drug Administration (FDA) and the European Medicine Agency (EMA), and are widely used in the clinics for the treatment or diagnosis of diseases, no PLGA nanomedicine formulation is currently available on the global market. One of the most impeding barriers is the development of a manufacturing technique that allows for the transfer of nanomedicine production from the laboratory to an industrial scale with proper characterization and quality control methods. This review provides a comprehensive overview of the technologies currently available for the manufacturing and analysis of polymeric nanomedicines based on PLGA nanoparticles, the scale-up challenges that hinder their industrial applicability, and the issues associated with their successful translation into clinical practice.

1. Introduction

Nanotechnology is among the most promising Key Enabling Technologies (KETs) that can provide innovative and radical solutions to the unmet needs of society (Soares et al., 2018; Tinkle et al., 2014). Today, nanotechnology touches every aspect of human life, including medicine, giving rise to one of the most important emerging areas of medical health research: nanomedicine (European-Commission, 2020). Nanomedicine involves the use of nanomaterials and nanotechnologies to address challenges in diagnosis, monitoring, control, prevention and treatment of diseases (Agrahari and Hiremath, 2017; Patra et al., 2018; Soares et al., 2018; Tinkle et al., 2014). Nanomedicines formulated as drug delivery systems typically involve active pharmaceutical ingredients that are either encapsulated within or conjugated to nano-size carrier matrices (Murthy, 2007). The carrier material can be based on inorganic (e.g., metal nanoparticles, semi-conductor quantum dots of various sizes and shapes (Biju et al., 2008; Tagit et al., 2015; Tagit et al., 2017; Tagit et al., 2011)) or organic nanostructures, including (and not limited to) polymers (Kumari et al., 2010), dendrimers (Gillies and Frechet, 2005), micelles (Kataoka et al., 2012), liposomes (Alavi et al., 2017), solid lipid nanoparticles (Mukherjee et al., 2009) and polymer-active pharmaceutical ingredient (API) conjugates (Larson and

Abbreviations: API, Active pharmaceutical ingredient; CDMOs, Contract and development manufacturing organizations; CDSP, Continuous downstream processes; CE, Cellulose ester; CIJM, Confined impinging jets mixer; CMAs, Critical material attributes; CMOs, Contract manufacturing organizations; CPPs, Critical process parameters; CQAs, critical quality attributes; CROs, Contract research organizations; DOE, Design of Experiments; EMA, European Medicine Agency; FBMR, Focused beam reflectance measurement; FDA, Food and Drug Administration; GMP, Good Manufacturing Practice; HPH, High pressure homogenization; HSM, High shear mixing; KETs, Key Enabling Technologies; ME, Mixed cellulose; MIVM, Multi-inlet vortex mixer; mPES, Modified polyethersulfone; O/W, Oil-in-water; PEEK, Polyether ethyl ketone; PES, Polyethersulfone; PLGA, Poly(lactic-co-glycolic acid); PS, Polysulfone; PSMA, Prostate-specific membrane antigen; QbD, Quality by Design; QC, Quality control; QTPP, Quality target product profile; RC, Regenerated cellulose; Re, Reynolds number; RES, Reticuloendothelial system; SC-CO_2, Supercritical carbon dioxide; SR-DLS, Spatially resolved dynamic light scattering; TFF, Tangential flow filtration; W/O/W, Water-in-oil-in-water; W/O, Water-in-oil.

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Ghandehari, 2012). These nanomedicines are finely engineered at the nanoscale to introduce various benefits such as protection of therapeutic agents from degradation, increased solubility and bioavailability, improved pharmacokinetics, reduced toxicity, enhanced therapeutic efficacy, decreased API immunogenicity, targeted delivery, and simultaneous diagnostics and treatment options with a single system (Agrahari and Hiremath, 2017; Patra et al., 2018).

To achieve the desired therapeutic efficacy, the nanocarrier should: *i)* hold the API firmly during transport in the blood compartments, but *ii)* be able to effectively release the API once it has reached the desired target to exert its pharmaceutical action. In addition, the transporter should *iii)* be "stealthy" in the blood compartments to effectively evade reticuloendothelial system (RES) screening, but *iv)* make contact and penetrate the right cells at the target action site (Sun et al., 2012). In this regard, nanocarriers based on poly(lactic-co-glycolic acid) (PLGA) offer tremendous opportunities in terms of design and performance thanks to the various properties of PLGA that make it an ideal nanocarrier (Han et al., 2016; Makadia and Siegel, 2011; Singh et al., 2014). PLGA is a biodegradable and biocompatible polymer with a wide range of degradation times that can be tuned by its molecular weight and copolymer ratio. PLGA is soluble in common solvents including acetone, chlorinated solvents and ethyl acetate, can be processed into almost any shape and size, and can encapsulate molecules of virtually any size (Gentile et al., 2014; Makadia and Siegel, 2011; Södergård and Stolt, 2002). Thus, PLGA polymers have been largely tested as delivery vehicles for drugs, proteins and various other macromolecules such as DNA, RNA and peptides (Jain, 2000; Makadia and Siegel, 2011). In addition to chemical composition and molecular weight of the polymer, the physical properties of the PLGA nanocarrier, such as size, shape, surface area-to-volume ratio, etc., can be 'tuned' to obtain the desired release profile (Gentile et al., 2014; Makadia and Siegel, 2011).

With its excellent biocompatibility, tunable degradation and release characteristics, and high versatility, PLGA has been approved for several biomedical applications. In fact, albeit not nano, over 60 PLGA-based drug products with varying properties (i.e., size, shape, etc.) are currently available on the market. Some of the best known PLGA formulations are based on microparticle depot preparations such as Decapeptyl® (the first drug product on the market, based on triptorelin), Lupron Depot® (leuprolide acetate), Nutropin Depot® (somatropin), Suprecur® MP (buserelin acetate), Sandostatin® LAR Depot (octreotide acetate), Somatuline® LA (lanreotide acetate), Trelstar™ Depot (triptorelin pamoate), Vivitrol® (naltrexone) and Risperdal® Consta™ (risperidone). In addition, PLGA-based implants (e.g., Zoladex®, Ozurdex®, Profact® Depot, Durysta™, etc. based on goserelin acetate, dexamethasone, buserelin and bimatoprost, respectively) and even in situ forming implants based on Atrigel® system, i.e. Eligard® (leuprolide acetate), are available (Schwendeman et al., 2014). The already substantial presence of PLGA-based products on the market indicates a promising future also for PLGA-based nanomedicine formulations.

As any new pharmaceutical product, the launch of a PLGA-based nanomedicine formulation comprises a complex pathway (Fig. 1) from design, laboratory-scale development to scale-up manufacturing. The therapeutic efficacy and safety profile of nanomedicines need to be characterized through extensive (pre)clinical pharmacodynamics (i.e. efficacy and toxicity) and pharmacokinetics (i.e. biodistribution) studies in order to support the design and optimization of the nanomedicines (Havel et al., 2016).

In addition to therapeutic efficacy and safety, production scalability is another key requirement for clinical and commercial development of PLGA nanomedicines, which is closely connected with the applied manufacturing technology (Agrahari and Agrahari, 2018; Agrahari and Hiremath, 2017; Paliwal et al., 2014). However, challenges arise when moving from benchtop to large-scale production because, unlike the conventional drug products, the efficacy and safety as well as the unique drug delivery characteristics of each individual nanomedicine formulation are a direct consequence of the physicochemical properties of the

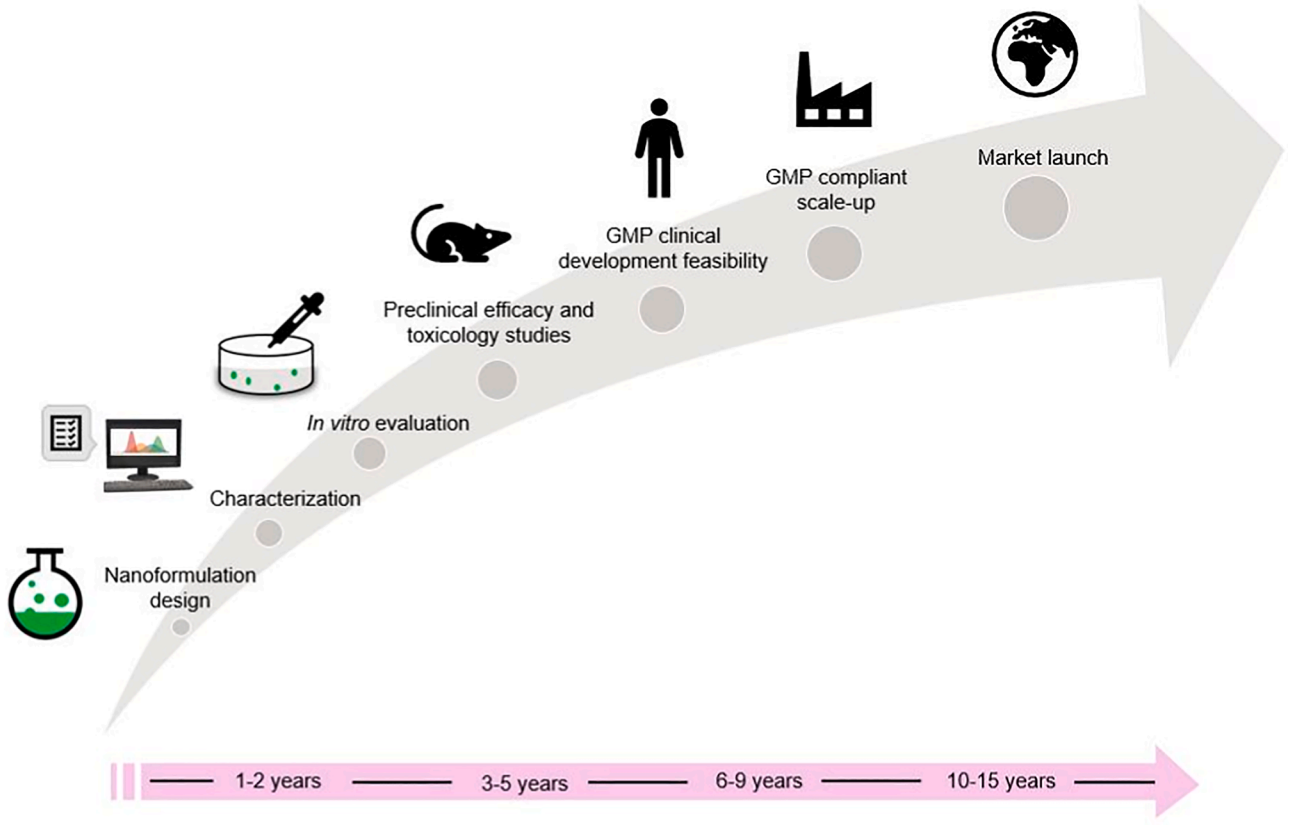

Fig. 1. Steps and average timelines for clinical development of nanomedicine formulations.

nanoparticles that carry the API (Metselaar and Lammers, 2020), which can be altered when adopting a larger scale production process. Therefore, in addition to establishing large-scale processes for Good Manufacturing Practice- (GMP) compliant production (Sun et al., 2012), adequate quality controls (QC) of nanocarriers using various characterization techniques are needed to determine whether scale-up directly or indirectly affects the clinical performance of the nanomedicines (Hua et al., 2018).

This study provides an overview of the technologies available for the production and analysis of PLGA nanoparticles, the scale-up challenges that hamper their industrial applicability, and the issues associated with their successful translation into clinic.

2. PLGA nanoparticle preparation routes

Several different techniques have been reported for lab-scale preparation of PLGA nanoparticles via both bottom-up and top-down approaches (Fig. 2). PLGA nanoparticles can be chemically synthesized using lactide and glycolide monomers through bottom-up techniques such as precipitation polymerization, emulsion polymerization and interfacial polymerization. In so-called top-down techniques such as emulsion solvent evaporation (single- or multiple-phase emulsions), solvent displacement (nanoprecipitation), dialysis, salting out and supercritical carbon dioxide (SC-CO_2), the nanoparticles are physically formed using previously synthesized polymers chains (i.e., PLGA) (Astete and Sabliov, 2006; Krishnaswamy and Orsat, 2017; Nagavarma et al., 2012; Rao and Geckeler, 2011; Vauthier and Bouchemal, 2009; Wang et al., 2016). Bottom-up techniques tend to be readily scalable to large batches but typically offer limited control over size, size distribution and particle shape (Merkel et al., 2010). The particles obtained by this method are found in suspension together with many other impurities (e.g., excess of surfactant in micellar form, initiators, unreacted monomers), which require laborious and expensive purification steps to reach the desired final grade of purity (Merkel et al., 2010). Moreover, APIs with reactive groups may display undesired cross-reactivity during the polymerization process (Becker and Wurm, 2018). Therefore, top-down particle manufacturing methods are usually preferred over bottom-up techniques, particularly for larger-scale production, as they offer a better control over the formulation characteristics (Merkel et al., 2010).

Emulsion-based approaches are among the most widely used top-down techniques due to the rapidity and simplicity of operation, adaptability to the encapsulation of APIs with different physicochemical properties, and low cost of equipment. Depending on the aqueous solubility of the API, oil-in-water (O/W) single emulsions, or water-in-oil-in-water (W/O/W) double emulsion systems can be used. For the encapsulation of hydrophobic compounds, PLGA and the API are dissolved together in the organic phase and then emulsified with an aqueous solution containing the surfactant (O/W) (Astete and Sabliov, 2006; Dinarvand et al., 2011). Encapsulation of a hydrophilic API requires an initial formation of a water-in-oil (W/O) emulsion, in which the API is in the aqueous phase and the polymer is in the organic phase. Subsequently, the W/O emulsion is mixed together with a second aqueous solution containing the surfactant, creating a W/O/W system (Dinarvand et al., 2011). PLGA nanoparticles are formed upon the removal of the organic solvent through diffusion, evaporation, or salting-out (McCarron et al., 2006). As the emulsion-based methods involve two immiscible phases, a substantial energy needs to be applied in the form of high shear forces, which can damage sensitive, thermolabile APIs and lead to the degradation of the PLGA chains itself. Alternatively, hydrophobic APIs can be encapsulated using nanoprecipitation method, in which the water-miscible organic phase containing PLGA and API is injected into an aqueous phase. In this technique, nanoparticles are rapidly formed by the quick solvent diffusion and interfacial deposition of PLGA (Dinarvand et al., 2011). Additional niche methods for the preparation of PLGA nanoparticles involve e.g. the use of SC-CO_2 as a solvent, as an antisolvent, or as an extractant (Gangapurwala et al., 2020); or dialysis techniques, in which the solvent and antisolvent are separated by a dialysis membrane of appropriate molecular weight cutoff (Errico et al., 2009). Overall, the high versatility

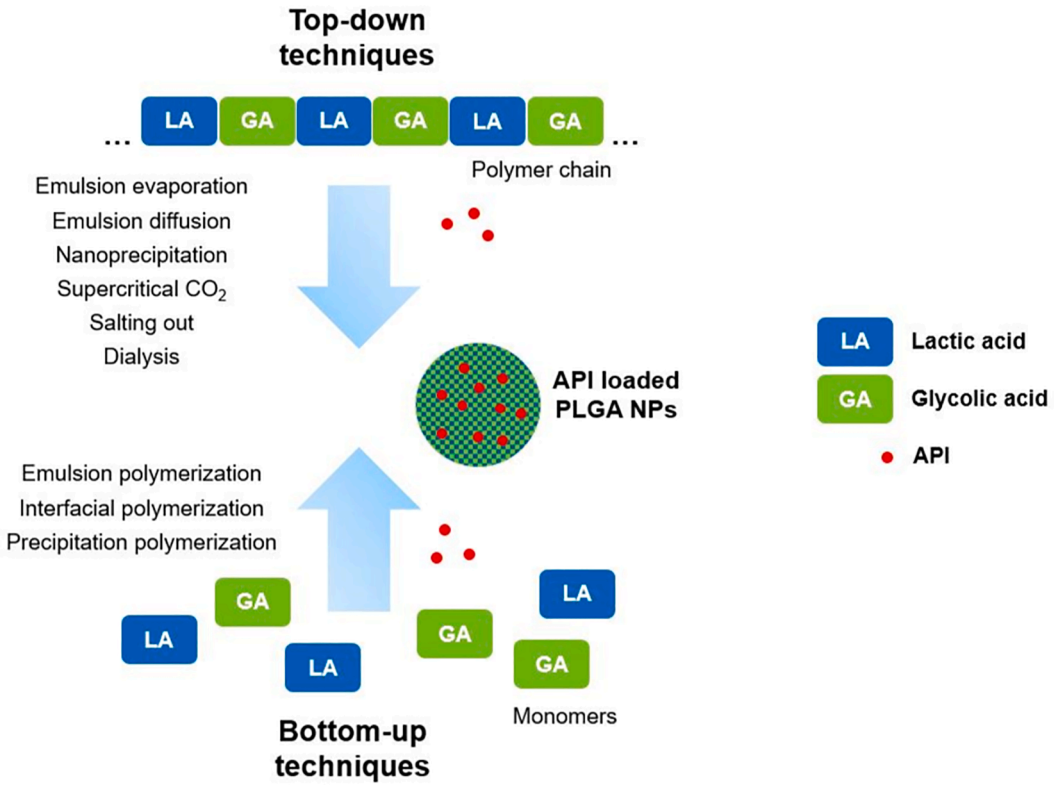

Fig. 2. Top-down and bottom-up approaches for PLGA nanoparticle preparation.

of PLGA allows for a broad selection of solvents and API encapsulation strategies through various particle formation mechanisms.

2.1. PLGA nanoparticle manufacturing technologies

As described by numerous researchers, several technologies and devices can be used for the production of PLGA nanoparticles via emulsion- or nanoprecipitation-based methods (Castro et al., 2020; Qi et al., 2019). The most common approaches involve simple mechanical stirring, sonication, high shear mixing (HSM), high pressure homogenization (HPH), and microfluidics.

Simple mechanical stirring and probe sonication are straightforward and broadly exploited techniques due to their versatility and ease of operation (Hernández-Giottonini et al., 2020; Huang and Zhang, 2018; Operti et al., 2018; Schiller et al., 2015). PLGA nanoparticles can be produced via nanoprecipitation, salting out and dialysis using mechanical stirring, and via emulsion solvent evaporation/diffusion operating probe sonication. In a recent study, Hernández-Giottonini et al. (Hernández-Giottonini et al., 2020) have extensively demonstrated that nanoparticle physicochemical characteristics can be adjusted precisely by varying the formulation parameters to obtain customized nanoparticle characteristics. However, since these technologies are used only for small batch preparations, scaling up the production can alter the formulation characteristics, which is a main drawback. In addition, the direct contact of the immersed probe with the sample can lead to cross-contamination (e.g. heavy metals) in probe sonication. This problem can be avoided by employing indirect sonication strategies, in which the energy is distributed to the sample through the sample container itself (Freitas et al., 2006; Schiller et al., 2015). Furthermore, harsh homogenization conditions and high temperature generated by the high voltage input might influence sensitive APIs (Schiller et al., 2015). Nevertheless, high-energy emulsification devices, such as HSM, HPH, and microfluidizer (also an HPH technique) are commonly used in pharmaceutical, food, chemical, and cosmetic industries for the production of emulsion formulations. In these techniques, cavitation, high shear and impact forces drive the formation of fine emulsions (Cheaburu-Yilmaz et al., 2019). Although the three techniques are commonly used for the production of micron size particles, several studies highlight the possibility to reach nano-size scale by adjusting the process and formulation parameters (Dong and Feng, 2007; Operti et al., 2018; Sani et al., 2009; Tukulula et al., 2018). HSM, HPH, and microfluidization are simple and easy-to-reproduce processes with a broad range of achievable particle sizes and the possibility of inline operation. However, during the scale-up, the high temperature created in the devices due to longer process times plus the harsh homogenization forces may affect sensitive materials. Moreover, a thorough cleaning of the equipment, along with cleaning validation, is often challenging due to the complex geometry of the rotor blades of the shear mixers and the tiny channels present in the reaction chambers of microfluidizers and pressure homogenizers. Eventually, high shear speed might create disturbance in flow rates (e.g. sucking effects) when connecting the system inline to other equipment, while homogenizers and microfluidizers can suffer from potential blockage of the small channels if the produced PLGA particles precipitate or the obtained suspension is too viscous (Operti et al., 2018).

Although some decades have passed since microfluidic systems have entered the scene for the production of nanomedicines (Convery and Gadegaard, 2019), there is a constant evolution of this technology. The versatility in terms of mixing geometry and microchip design makes microfluidics technology very attractive and popular for nanoparticle manufacturing (Rezvantalab and Moraveji, 2019). Among others, microfluidics offers great control over particle size and size distribution, low sample and reagent consumption, and a small physical and economic footprint. However, the extreme sensitivity of these devices to streamline disturbances, microchannel clogging and fouling phenomena, as well as the necessity of specialized microfabrication facilities can create a barrier for their industrial utilization. While low-throughput due to slow flow (typically in the μL/min range) and device compatibility with organic solvents have been regarded as main limitations (Lim et al., 2014; Min et al., 2014; Ortiz de Solorzano et al., 2016), recent studies have achieved faster production rates using robust devices fabricated out of durable materials. For instance, a polyimide-based, 3D hydrodynamic flow focusing microfluidic reactor with 8 identical microchannels operating in parallel enabled the production of PLGA nanoparticles in ~10 g/h scale via nanoprecipitation (Min et al., 2014). Unlike the 2D flow focusing, 3D hydrodynamic flow focusing enabled the reproducible mass production of nanoparticles without aggregation under flash-flow conditions with high Reynolds number (Re 400). A similar throughput (~10 g/h) has been achieved using a 3D co-axial flow capillary device fabricated out of glass capillaries (Liu et al., 2015). Regardless of the type of organic solvent and surfactant, highly monodisperse nanoparticles were obtained via nanoprecipitation in quantities as large as ~240 g/day (Liu et al., 2015). As highlighted in these examples, large scale production of PLGA nanoparticles is commonly achieved with nanoprecipitation-based approaches, for which the range of suitable solvents and APIs suitable is limited. In this respect, interdigital micromixers that can produce monodisperse droplets without ultrasonic or mechanical shear forces through microchannel emulsification process offer clear advantages such that organic solvents immiscible with water and labile APIs can be used (Ortiz de Solorzano et al., 2016). For instance, the production of drug-loaded PLGA nanoparticles has been achieved using an interdigital micromixer at ~10 g/h scale through microchannel emulsification (Ortiz de Solorzano et al., 2016). Furthermore, by coupling two interdigital micromixers, PLGA nanoparticles loaded with gold nanoparticles were produced at ~10 g/h scale (168 mg/min) via a double emulsion (W/O/W) process (Larrea et al., 2017), which is comparable to throughput obtained with nanoprecipitation-based methods.

Recently, millifluidic systems that can manipulate fluids in ~1 mm channels have been shown to be suitable for large-scale production of PLGA nanoparticles. These devices also include confined impinging jets mixers (CIJMs) and multi-inlet vortex mixers (MIVMs) and exploit the flash nanoprecipitation technique (Lim et al., 2014; Pagels and Prud'homme, 2017; Saad and Prud'homme, 2016; Sun et al., 2017; Turino et al., 2018). Such technique uses rapid micromixing, on the order of milliseconds, to establish homogeneous supersaturation conditions and controlled precipitation. In a recent study, Turino et al. (2018) used CIJM technology to produce florfenicol-loaded PLGA nanoparticles at a flow rate of up to 120 mL/min. In addition, Lim et al. (2014) have developed reactors based on coaxial turbulent jet mixers and reported a throughput of 131 g/h. The simple reactor design that involves inserting a blunt syringe needle into a "T" tube fitting without the need for specialized equipment and microfabrication facilities renders this technique appealing from an industrial standpoint.

Spray-drying and electrospraying are both atomization-based technologies that enable the production of PLGA particles (Bohr et al., 2014). These methods are commonly used for microparticle generation, however, nanometer-sized particles can also be achieved as demonstrated by Pamujula et al. (2004)) and Zarchi et al. (2015). Spray drying is a rapid and scalable technique already established in pharmaceutical manufacturing for generating amorphous solid dispersion by uniformly dispersing APIs in a polymer matrix. Unfortunately, typically, a low final yield is obtained due to the loss of adhered product along the walls of the drying chamber. In addition, the production of particles at the nanometer scale is often limited due to insufficient liquid atomization forces (pressure and centrifugal) (Bohr et al., 2014; Pamujula et al., 2004). Electrospraying has the advantage of offering high encapsulation efficiency and narrow particles size distribution, less agglomeration compared to other conventional atomizers, and offers various setup configurations for diverse purposes (e.g., single, coaxial dual or tricapillary nozzle). However, given the large number of adjustable parameters, its use can make formulation optimization, and thus its

applications, challenging (Nguyen et al., 2016).

The summary of advantages and limitations of each mentioned technology is shown in Table 1.

Other interesting technologies, although less known or applied for the preparation of PLGA nanocarriers, are supercritical fluid technologies (Asandei et al., 2004; Campardelli and Reverchon, 2017; Dalvi et al., 2013; Gangapurwala et al., 2020; Ghaderi et al., 1999; Kluge et al., 2009; Zabihi et al., 2014), membrane-based techniques (Albisa et al., 2017; Astete and Sabliov, 2006; Gasparini et al., 2008; Hu et al., 2014; Khayata et al., 2012; Wang et al., 2019), and nanoimprint lithography (Bowerman et al., 2017; Zhang et al., 2020). The advantages and limitations of these technologies are summarized in Supplementary Information Table S1.

The achievable API loading is an important parameter to consider for large-scale production of PLGA nanoparticles. The applied manufacturing technology alone is not sufficient to decree the encapsulation efficiency (EE) because many formulation and process parameters are equally influential on the efficiency of API loading. The technology adopted for large scale production should not alter the EE as well as other properties of PLGA nanoparticles upon scaling-up. In this respect, continuous manufacturing technologies can be preferred over batch methods as the production at the desired target scale can be achieved without changing the process or formulation parameters, but the operation time (Operti et al., 2018).

2.2. Continuous versus batch processes

Although the technologies listed in Table 1 are primarily designed for batch processing, technologies such as HSM, sonication (direct or indirect), HPH, microfluidization, microfluidics and millifluidics can be adapted for inline operation. This flexibility of configuration makes them more attractive from an industrial standpoint as the manufacturing can be implemented in either modes of operation (i.e., batch or continuous).

Indeed, batch versus continuous flow manufacturing is one of the major topics of debate in the pharmaceutical industry (Kinematics). The traditional pharmaceutical batch processing involves the emplacement of various components of a formulation together through a step-by-step manner. As materials move from one step to the next, the batch in production must terminate before proceeding to the following one (Kinematics). In contrast, the continuous flow processes involve moving one work unit at a time between each process step without interruption in time, sequence, substance or extension (General-Kinematics, 2019). The main advantage of batch production is that the setup costs are initially less expensive. Also, each batch can be easily customized to be unique. Using batch production, specific quantities of a certain product can be adjusted based on changing market demands (Kinematics). However, a complete and thorough cleaning of the equipment after each produced batch must be carried out, which takes time, demands challenging documentation and validation methods, and affects production costs (FDA, 2014). Conversely, continuous pharmaceutical manufacturing helps increase productivity as it is more time-efficient with reduced energy requirements, required operation space, and amount of overall waste, calling for cleaning procedures only when the production is stopped. Therefore, for most applications continuous flow can save time, energy, and costs when implemented correctly. Continuous production has already been used in the chemical industry for many years for large-scale mass production of chemicals in single-use plants (Baumann et al., 2020). In recent decades, continuous manufacturing processes have gained attention of pharmaceutical industries as well as some academic research centers for the production of API formulations (Adamo et al., 2016; Cole et al., 2017; McWilliams et al., 2018; Nepveux et al., 2015). Continuous processes are more suitable for the scale-up manufacturing of nanomedicines as they offer the advantage of preferential termination of the production at the desired target scale without changing the process or formulation parameters. Also, the risk of human error is reduced because continuous processing requires the involvement of fewer people in the manufacturing process from start to finish (Kinematics). However, implementation of continuous processing requires substantial capital investments in new equipment, as the lab and manufacturing infrastructure in pharmaceutical organizations are both designed predominantly for batch chemistry (McWilliams et al., 2018). Besides, in certain cases technologies must be adapted in response to applicable engineering norms regarding contamination risk and/or qualification. Additionally, continuous pharmaceutical manufacturing processes need high-tech control systems to ensure the high quality of the end products (Myerson et al., 2015). In this respect, it is particularly important that manufacturing operators are trained well to support the design, development, and implementation of continuous manufacturing (Baumann et al., 2020).

Overall, adopting a continuous manufacturing with an integrated comprehensive quality control approach can ultimately facilitate large scale production of PLGA nanomedicines.

2.3. Continuous downstream processes

The key objectives of downstream processing are purification of the process stream, removal of process and product-related impurities and, finally, prevention of product degradation so as to achieve high recovery and satisfactory product quality profile (Rathore et al., 2015). Typically, the choice of downstream processes depends on the upstream processes and the recovery requirements, such as upper impurity levels and final product concentrations. Unlike upstream manufacturing, development of downstream processes are often mistakenly sidelined, whereas, as it is a fundamental part of the production chain, it should be considered from the outset when a nanomedicine product is to be placed on the market (Holzer, 2017; Jungbauer, 2013).

2.3.1. PLGA nanoparticle purification methods

In the early stages of development, the purification of the particles is usually carried out using centrifugation and dialysis at the laboratory scale. However, these processes are run in batch mode with the output of each step collected in a vessel. Such approaches often requires manual handling, which can be challenging to achieve reproducibility particularly when increasing the scale to larger volumes (Dalwadi et al., 2005). In order to perform the entire process in the continuous mode, all the unit operations need to be integrated with the capability of recycling of streams and purging of impurities as required (Rathore et al., 2015). To date, in the panorama of continuous downstream process (CDSP) technologies for nanoparticle purification, the foremost exploited technologies are membrane separation techniques (tangential- or cross-flow filtration) and continuous flow centrifugation (Dalwadi et al., 2005; Holzer, 2017; Łącki et al., 2018).

Tangential flow filtration (TFF) is a type of filtration that can be run continuously where the fluid flows along the surface of a filter membrane rather than passing through it. The main advantage is that the filter cake does not settle on the filter during washing, but rather is flushed away during the process, increasing the length of time a filter unit can be operational (Łącki et al., 2018). For instance, Dalwadi et al. (Dalwadi et al., 2005) demonstrated a linear relationship between filtrate volume and time in TFF purification of PLGA nanoparticles. The method could efficiently remove approximately 91% of the stabilizer molecules with a low impact on yield, size and stability of the final product. Scale-up of a TFF stage is possible and is generally considered easy since the membrane cartridges (cassettes or hollow fibers) are linearly scalable. This linear scalability is achieved by the geometric similarity of the membrane cartridges at different scales. Geometric similarity ensures that scaling up, and scaling down for process validation purposes, can be achieved by keeping the processed volume per membrane area constant at different scales without changing process performance (Łącki et al., 2018). However, it should be noted that when

Table 1
List of the main top-down technologies utilized for manufacturing PLGA nanoparticle formulations along with their advantages and considerations for scale-up.

Technology	Method	Process type	Advantages	Possible limitations towards industrial applicability
Simple mechanical stirring (Errico et al., 2009; Hernández-Giottonini et al., 2020; Huang and Zhang, 2018)	Nanoprecipitation (or solvent displacement) Salting out Dialysis Phase separation (coacervation)	Batch	Simple and inexpensive set-up Reduced energy usage Moderate conditions suitable to process sensitive APIs	Only for small batch preparations Change of properties upon scaling up the production
Sonication (Feczkó et al., 2011; Freitas et al., 2006; Hernández-Giottonini et al., 2020; Lee and Chang, 2017; McCall and Sirianni, 2013; McCarron et al., 2006; Operti et al., 2018; Schiller et al., 2015)	Emulsification solvent evaporation Emulsification solvent diffusion/extraction Salting out	Direct, Batch	Easy and fast process Different probe size available to afford different batch sizes Disposable material	Only for small batch preparations Change of formulation properties upon scaling up the production Direct sample contact with probe Cooling jacket necessary
		Direct, Inline	Different probe size available to afford different batch sizes Disposable material Continuous processing possible Scalable	Direct sample contact with probe Cooling jacket necessary
		Indirect, Batch	Indirect sonication avoids contamination risks from probe Different probe size available to afford different batch sizes	Only for small batch preparations Change of formulation properties upon scaling up the production Cooling jacket necessary
		Indirect, Inline	Indirect sonication avoids contamination risks from probe Disposable material Continuous processing possible Scalable	Cooling jacket necessary
High Shear Mixing (Kumar et al., 2004; Lee et al., 2005; Operti et al., 2018; RaviKumar et al., 2004; Zweers et al., 2004)	Emulsification solvent evaporation Emulsification solvent diffusion/extraction Salting out	Batch	Easy and fast process Different probes available to afford different effects	Only for small batch preparations Harsh shear forces and high temperature might influence sensitive APIs
		Inline	Continuous processing Scalable	Cooling jacket necessary High shear speed might create disturbance in flow rates Equipment cleaning challenging
High Pressure Homogenizing AND Microfluidization (Dong and Feng, 2007; Operti et al., 2018; Sani et al., 2009)	Emulsification solvent evaporation Emulsification solvent diffusion	Batch/ Inline	Simple and easy to reproduce process Different particle size achievable Continuous processing possible	Labor-intensive handling and continuity challenging Potential blockage of small channels Equipment cleaning challenging Harsh homogenization might influence sensitive APIs
Microfluidics (Gdowski et al., 2018; Operti et al., 2019; Operti et al., 2018; Rezvantalab and Moraveji, 2019)	Nanoprecipitation Emulsification solvent evaporation Emulsification solvent diffusion	Batch/ Inline	Low sample and reagent consumption Small physical and economic footprint Low energy consumption Parallelization and high throughput experimentation Different chips can be applied for different purposes Narrow size distribution Moderate conditions suitable to process sensitive APIs Continuous processing possible	High sensitivity to streamlines disturbances Microchannel clogging and fouling Solvent compatibility with chip material
Millifluidics (Lim et al., 2014; Pagels and Prud'homme, 2017; Prud'homme et al., 2020; Sun et al., 2017; Turino et al., 2018)	Flash nanoprecipitation Inverse flash nanoprecipitation	Batch/ Inline	Fast processing Simple equipment Moderate conditions suitable to process sensitive APIs Continuous processing possible Scalable	APIs and solvents limited Channel clogging and fouling
Spray drying (Pamujula et al., 2004)	Convection drying	Batch	Rapid Scalable Conditions relatively moderate suitable to process sensitive APIs	Low final yield at laboratory scale Low separation capacity of cyclone to separate fine particles

(*continued on next page*)

Table 1 (*continued*)

Technology	Method	Process type	Advantages	Possible limitations towards industrial applicability
Electrospraying (Nguyen et al., 2016; Zarchi et al., 2015)	Electrohydrodynamic atomization	Batch	Narrow size distribution Less agglomeration compared to other conventional mechanical atomizers Conditions relatively moderate suitable to process sensitive APIs Various setup configurations	Limited production of particles at the nanometer scale Large number of parameters to be used can complicate its optimization

highly viscous flows need to be processed, the concept of linear scaling can be challenging. An increase in the viscosity of the permeate may cause the reduction of transmembrane pressure (the pressure difference between the two sides of the membrane) and diminish the filtration efficiency, which can be time consuming for large scale processes (Łącki et al., 2018).

TFF cartridges are typically made of membranes based on regenerated cellulose (RC), cellulose ester (CE), mixed cellulose (ME), polysulfone (PS), polyethersulfone (PES). The problems associated with adsorbing particles as well as biological elements present in the treated specimens have led to the development of new modified PES (mPES) materials, which are more hydrophilic and can reduce fouling phenomena (Raghunath et al., 2012).

Since PLGA needs to be dissolved in organic solvents such as dichloromethane, ethyl acetate, dimethyl sulfoxide, etc., solvent compatibility with membrane filters must be evaluated carefully. For instance, RC cartridges are recommended for the purification of nanoformulations containing dichloromethane. Nevertheless, appropriate dilutions prior to running the TFF process along with pre-assessment trials are highly necessary since variations in temperature, concentrations, durations of exposure and other factors may affect the performance of the membranes. In addition, when choosing an appropriate fiber filter, the molecular cut-off of the membrane as well as the membrane area should be considered depending on the size of the particles and the sample volume to be processed, respectively (Raghunath et al., 2012).

Centrifugation is probably the commonly used unit operation for solid–liquid separation and is frequently exploited for nanoparticle purification. Of the two most commonly used designs, namely tubular centrifuge and disc-stack centrifuge, the disc-stack centrifuge can be run in continuous and batch configurations (Tarleton and Wakeman, 2007). Centrifugal forces (usually up to 14000g) cause particles to accumulate on the underside of the centrifuge. At the underside of the centrifuge, particles slide to the outer periphery of the bowl automatically, sometimes intermittently, through nozzles located on the outer periphery of the bowl in a continuous manner (Rathore et al., 2015; Tarleton and Wakeman, 2007). While disc centrifuges are capable of accepting a wide range of feeds, they are mechanically complex and expensive. In addition, the complex geometry of the centrifuge means that mechanical cleaning is not always possible and chemical cleaning must be used, which requires a more demanding QC process (Tarleton and Wakeman, 2007).

2.3.2. PLGA nanoparticle storage techniques

In addition to the degree of purity, the physicochemical integrity of nanomedicine must be preserved over time throughout its shelf-life. During storage, nanomedicine can be subjected to several degradation pathways mediated by light, air (oxygen), heat and water, which can lead to lower potency or even toxicity of the formulation. It has been shown that hydrolytic degradation of PLGA particles in aqueous media can take place as early as 15 days depending on the PLGA molecular weight, lactide:glycolide ratio and particle morphology (Keles et al., 2015; Swider et al., 2019). Therefore, an efficient removal of water content is particularly needed to improve the nanomedicine stability, as well as to facilitate handling, reduce storage space and transportation costs (Lyophilizationworld, 2020). In this respect, lyophilization and spray drying are the most commonly used techniques to improve the stability of nanoformulations. Continuous aseptic spray-drying (Adali et al., 2020), and continuous freeze-drying (Van Bockstal et al., 2017a; Van Bockstal et al., 2017b) can both potentially be exploited for future PLGA nanomedicine continuous manufacturing. Continuous freeze-drying, which utilizes infrared radiation for drying the frozen samples in spinning vials, can improve process efficiency and product quality (uniformity) compared to conventional batch freeze-drying processes.

2.3.3. PLGA nanoparticle sterilization approaches

Lastly, for systems intended for parenteral administration, finding an appropriate sterilization method is a crucial final step in manufacturing. Each preparation must be validated on a case-by-case basis as nanoparticles can be affected differently by the sterilization method, depending on their components, formulation and/or method of preparation (Ragelle et al., 2017). Sterilization procedures such as gamma irradiation or autoclaving can be detrimental to sensitive APIs, cause PLGA chain alteration, and affect the overall characteristics of the nanoformulation itself (Bozdag et al., 2005; Fernández et al., 2016; Keles et al., 2015). In this case an alternative method can be sterile filtration of the final product. Nevertheless, this process only works for nanoformulations with a size distribution smaller than 0.22 μm. Finally, aseptic manufacturing can be implemented. However, aseptic processes can be costly and difficult to perform particularly for multi-step processes (Kaur et al., 2014; Ragelle et al., 2017). Among the described production technologies (Table 1), aseptic manufacturing is the one that best lends itself to continuous production processes, where production is totally isolated from the surrounding environment and operators. Once established, the conditions needed for sterile continuous production can be maintained indefinitely, for days, weeks, even months (Arnum, 2008).

2.4. Scale-up challenges in nanomedicine manufacturing

Scale-up manufacturing of nanomedicines under regulated GMP conditions present unique challenges (Agrahari and Hiremath, 2017; Desai, 2012; Kaur et al., 2014; Souto et al., 2020; Sun et al., 2012). The GMP guidelines describe the minimum standards that manufacturers must meet in their production processes to ensure that the formulations are consistently of high quality from batch-to-batch, the final product is appropriate for its intended use, all steps in the manufacturing process are well documented and the medicine meets the requirements of the marketing or clinical trial authorizations (EMA, 2018a).

Since a plethora of technologies and multiple unit operations are often involved in nanomedicine production and downstream processes, the process optimization and reproducibility can be achieved relatively easily at small scale. Well-characterized nanoparticles obtained at small scales can be used in pre-clinical studies and early clinical trials, which

usually require a relatively small amount of product (Souto et al., 2020). In contrast, large-scale poses challenges to achieve a robust manufacturing process for controlling the size and shape polydispersity of the nanoparticles, as well as maintaining the stability of the physicochemical properties of the product with minimal batch-to-batch variations (Agrahari and Hiremath, 2017; Desai, 2012; Wu et al., 2020). Potentially, all the variances can limit the clinical and/or commercial translation of every specific nanoformulation. The preparations that are not optimal can only be improved with a high degree of trial and error. This approach may seem logical for a laboratory setting. However, for large-scale production it is necessary to consider the cost of raw materials and the additional need for an infinite number of multistep production processes, which make the industrial manufacturing of nanomedicines very costly in terms of time and money (Agrahari and Hiremath, 2017; Desai, 2012; Kaur et al., 2014; Wu et al., 2020). In order to overcome these problems and save time, it is useful to consider early at the lab scale which approach might be suitable if the product were to be scaled up considering the manufacturing constraints in the industry. In this respect, quality by design (QbD) approach is recommended (FDA, 2009), which involves establishment of predefined objectives regarding the quality target product profile (QTPP) and the critical quality attributes (CQAs) of the formulation in order to achieve its safety and efficacy goals (Beg et al., 2019; Colombo et al., 2018; Troiano et al., 2016). The identification of QTPP and CQAs, such as particle size, size distribution, charge and morphology, and drug loading and release, impurity levels, etc., early in formulation design will help establish standards and determine whether or not a manufacturing process can provide batches that meet the release criteria. Focusing on the manufacturing aspect, the QbD model integrates the impact of critical process parameters (CPPs) and critical material attributes (CMAs) on CQAs to ensure the quality of the final product (Colombo et al., 2018). To achieve high product quality, the manufacturer must act at every step of the development including the selection of the raw materials, the adoption of the best production method that can preserve the functional and physical characteristics of the product, screening the formulation development, scale-up and optimization of manufacturing processes both upstream and downstream (Namjoshi et al., 2020). When evaluating all these tasks, it often happens that multiple input variables (formulation and process parameters) can affect one or more output variables (responses). In this respect, in order to estimate the interaction of these parameters and better understand the process, the Design of Experiments (DOE) approach is often exploited in industrial practice for product development. DOE is a data collection and analysis tool that determines the relationship between factors affecting a process and the results of that process, helping with their subsequent optimization with respect to the final product requirements. DOE can provide a useful process insight, testing whether variables are independent or interact influencing outputs (Cun et al., 2011). Therefore, DOE can help to evaluate the effects of CMAs and CPPs on the CQAs of the final pharmaceutical form, providing maximal information from a minimal number of experiments (Cun et al., 2011; Namjoshi et al., 2020). CMAs and CPPs for PLGA nanomedicine manufacturing involve (but are not limited to) PLGA type, polymer mixture and monomers ratio in the copolymer composition, APIs, type of organic solvent, emulsifier/stabilizer, oil-to-water phase ratio, temperature, pressure, pH, process running time and process volume. Since each production technology has different operating parameters as well as each API and PLGA type have their own physicochemical peculiarities, it is therefore not possible to apply a single generic process to all nanoparticle preparations, and each nanosystem should be validated on a case-by-case basis (Agrahari and Agrahari, 2018). In this regard, analysis and characterization methods are becoming more and more essential to validate the quality of the manufactured batches and to ensure that the CQAs are within the normal characteristic ranges and in acceptance limits (Myerson et al., 2015).

2.5. Analysis and characterization

Since the *in vitro* and *in vivo* performance of nanoparticles depend on their key physicochemical properties, appropriate characterization tools are necessary to precisely detect the size, size distribution, morphology, surface charge, surface functionality, particle interactions/behavior, porosity, drug loading, solubility, pH, viscosity, stability (e.g. aggregation, API leakage, etc.) and toxicity of nanoparticles, among other nanoparticle properties. In addition, each type of manufacturing process may lead to an altered chemical structure of the active ingredient and other components, as well as to a substantial presence of different impurities in the final product (Desai, 2012). As a result, subtle changes in the process may negatively influence the complex structure of the nanoproduct with adverse consequences for its therapeutic outcomes (Agrahari and Hiremath, 2017; Desai, 2012). Therefore, robust nanomedicine characterization techniques are required for both manufacturing and regulatory aspects (Ragelle et al., 2017). The main techniques used for the characterization of PLGA nanoparticles along with basic detection principles are shown in supplementary information Table S2.

The analysis of nanoparticles is usually conducted offline under controlled conditions. The offline approach offers a reliable method for ensuring the consistency and quality of particle products; however, it can introduce delays and sampling errors that make process optimization difficult. Recently, characterization tools suitable for inline monitoring in real-time have been developed. Such devices allow for the fast detection of short-term fluctuations in the production processes and thereby enable the direct adjustment of process parameters during production. In addition, sampling is not needed for these devices as they can be integrated into the process (Besseling et al., 2019; TOLEDO; Zidan et al., 2010). One example of such a device is NanoFlowSizer, which can be integrated into the process by flow-through cells and allows for continuous, real-time monitoring of particle sizes in 0.01 to 1 μm range through spatially resolved dynamic light scattering (SR-DLS) (Besseling et al., 2019). Inline particle size measurement can also be achieved using laser diffraction techniques for particles with typical dimensions between 0.1 and 1000 μm for dry, wet and spray samples (Medendorp et al., 2015). Focused beam reflectance measurement (FBRM) is another example of characterization tool that has been used for in-process measurement of particles (TOLEDO; Zidan et al., 2010), however, the actual available model has constraints in monitoring particles of size diameter below 0.5 μm, rendering the application in the nanoparticle field still limited (Zidan et al., 2010).

Despite increasingly advanced machineries, most production facilities are generally equipped with analytical apparatus suitable for analyzing conventional dosage forms. Most of the analytical techniques used for nanomedicine characterization are complex and require specific and expensive equipment. Furthermore, due to both the complexity of the behavior of nanomaterials and the competence requirements in cutting-edge methods, a team of interdisciplinary experts is needed to perform data analysis and interpretation, which substantially increases the already exorbitant costs of nanomedicine manufacturing (Agrahari and Hiremath, 2017; Landesman-Milo and Peer, 2016).

3. Road to clinically approved PLGA nanomedicines

Notwithstanding the fact that PLGA has been approved since several years for the production of medical devices, along with its long history of being an excipient for long acting injectables, there are still no nanoformulations based on this polymer on the market today (Kim et al., 2019; Rezvantalab et al., 2018). Some of the reasons besides the difficulty in manufacturing scale-up could be related to poor drug loading capacity of certain APIs, high initial burst release of API, generation of acidic products after polymer biodegradation and nanotoxicology (Kim et al., 2019). Nevertheless, there have been attempts for clinical translation of PLGA-based nanomedicine formulations under the name

ACCURINS®. The ACCURINS® platform was designed by means of a QbD approach to target the prostate-specific membrane antigen (PSMA), which is expressed on prostate cancer cells and the blood vessels of other types of solid tumors, for the delivery of docetaxel in a PEG-PLGA/PLA-PEG nanoparticle (BIND-014) (Troiano et al., 2016). Although positive results were obtained in a Phase II study in late 2014 (Anselmo and Mitragotri, 2016; Hrkach et al., 2012), the development of the formulation was halted (Biotech, 2017). Another remarkable milestone in clinical translation of polymeric nanomedicines has been achieved for paclitaxel-loaded PEG-PLA micelles (Werner et al., 2013). This injectable formulation is designed to treat patients with refractory ovarian cancer, metastatic breast cancer, advanced small-cell-lung and gastric cancer. Following a successful market launch in South Korea, India and Indonesia under the name Genexol® PM, it is currently in Phase III clinical trials in EU and US.

Although not based on PLGA and not available in the global market yet, the successful commercialization of Genexol® PM shows the possibility of clinical and commercial development of polymeric nanomedicines despite the aforementioned challenges. Indeed, for an efficient clinical translation, a more rational design of PLGA nanoparticles is necessary to close the existing gap between material research, preclinical experimentation, clinical requirements, and regulatory aspects (Rezvantalab et al., 2018). In order to assist the pharmaceutical industry with the required documentation for market authorization of nanomedicine formulations and to clarify certain scientific doubts, EMA has released the so-called "reflection papers" (EMA, 2018b; Soares et al., 2018). Similarly, the FDA issued a guidance document entitled "Drug Products, Including Biological Products, that Contain Nanomaterials - Guidance for Industry", providing regulatory directions and recommendations for applicants and investigational sponsors for pre-market and post-market submissions for nanoproducts (FDA, 2017).

Currently, an increasing number of contract manufacturing organizations (CMOs), also called contract and development manufacturing organizations (CDMOs), and contract research organizations (CROs) are emerging to provide drug development and production services on a contract basis (Mendenhall and Kontny, 2010). Some of them offer formulation services for commercial development of PLGA nanoparticle preparations which are suitable for a wide range of applications in drug targeting and delivery, imaging, immunoassays, and medical devices that can help fundamental research improve.

4. Concluding remarks

Despite the efficacy and potential therapeutic benefits that PLGA nanoformulations can bring, challenges related to large-scale production hamper their clinical and commercial development. Batch-to-batch variations and lack of product consistency in scale-up manufacturing can be avoided by employing continuous process technologies. Parallel advancements in manufacturing and characterization technologies, and emergence of dedicated research and manufacturing organizations can collectively pave the way to clinical and commercial development of PLGA-based nanomedicines. Given the complexity of these nanoformulations, several interdisciplinary experts should be brought together to address the acquisition and interpretation of data throughout all the design processes. For these reasons, it is essential to promote successful cooperation between large pharmaceutical industries, biotechnology companies, small enterprises and academia, for example through integrated consortia, in order to capitalize on the unique strengths of each partner. In this perspective, it is foreseeable that in the near future outsourcing will be increasingly sought by biopharmaceutical companies through CMOs and CROs which, with their expertise, will become more and more strategic and enabling partners.

CRediT authorship contribution statement

Maria Camilla Operti: Conceptualization, Visualization, Writing - original draft. **Alexander Bernhardt:** Supervision, Writing - review & editing. **Silko Grimm:** Funding acquisition, Project administration, Writing - review & editing. **Andrea Engel:** Supervision, Writing - review & editing. **Carl Gustav Figdor:** Funding acquisition, Project administration, Writing - review & editing. **Oya Tagit:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

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