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CHAPTER 2

Challenges in nonparenteral nanomedicine therapy

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1. Therapeutic and theranostic nanomedicines: Introduction

Nanomedicine and its therapeutic potential are new but evolving science field, where nanoscale materials are employed as a tool for disease diagnosis or targeted drug delivery in a very precise manner. Targeted delivery of chemotherapeutic, immunotherapeutic, and biologic agents in treating numerous diseases is an outstanding application of nanomedicine. Therapeutics based on nanoparticles have great potential to influence the treatment of various human diseases, but instability and early release from nanoparticles decrease the bioavailability of drugs, which impedes its clinical translation. All nanoparticles must rely on control at the nano-size scale, which means small variations may cause significant changes to the nanoformulation [1]. Drug delivery with nanotechnology can offer greater control over the biodistribution of therapeutic agents and thus improve the overall therapeutic index. Researchers are focusing on customized nanoparticles at sizes and shapes complimentary to the biological entities that may act precisely during the cargo and on the actuation process. When it is optimized, such a method should greatly reduce the adversities and side effects, particularly that of chemotherapy, imparting to a patient's healthy cells. The goal of most nano-based strategies for drug delivery is to enhance the therapeutic effectiveness of the active pharmaceutical ingredient (API) and also to reduce the adverse effects [2]. The specificity of measuring API that was previously considered unimportant has now gained much importance due to the understanding of specific pharmacokinetic profiles and dose-limiting toxicity that are critical efficacy determining factors for viable therapies using nano-based delivery strategies. The past two decades have witnessed the unprecedented growth of nanomedicines that are translated into clinics as well. As nanomedicines evolved, techniques to properly evaluate their safety and efficacy are also evolved. Characterization methods for imaging and analysis of nano-based materials are also evolved in demand of the nanomedicine developers and regulators. Pharmacokinetic characteristics

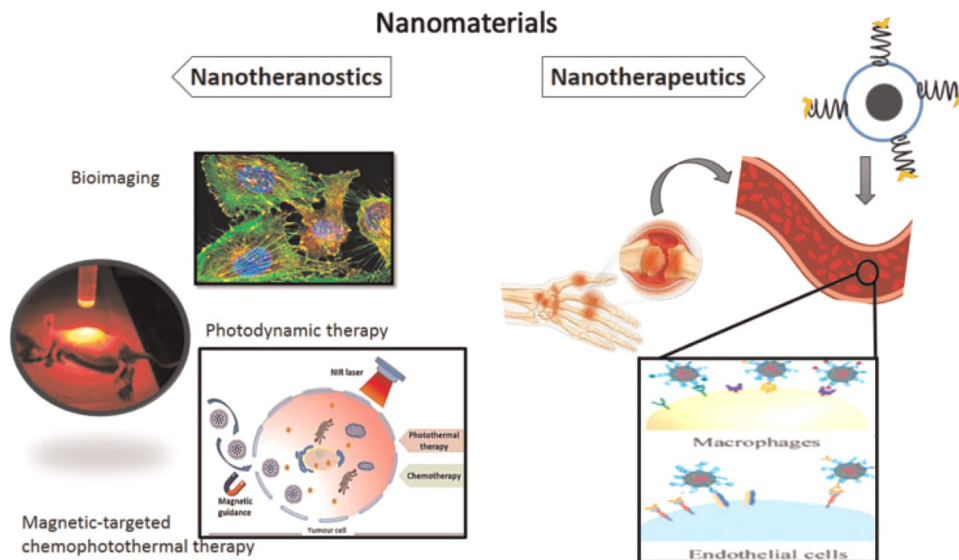


Fig. 1 An illustration of theranostic and therapeutic applications of nanomaterials. (Adapted with permission from Naila Qamar, et al., *Nanomedicine: an emerging era of theranostics and therapeutics for rheumatoid arthritis*, *Rheumatology* 58 (10) 1715–1721. Copyright 2019 © Oxford University Press).

of various nanomedicines with different formulations are determined by particle size, shape (chemical structure), and surface chemical characteristics [3]. The aim of regulating particle size in nanomedicines is to increase their retention in target tissues and to remove them rapidly when distributed to nontarget tissues. Nanomedicines with particle size less than 10 nm are removed by kidneys whereas those with particle size more than 10 nm are sometimes elongated and removed by the liver and/or the mononuclear-phagocyte system (MPS). The physicochemical properties of nanoparticles assist the binding of cellular, blood, and protein components that ease their interactions with immune cells eliciting the immune response [4]. Some developments were also made to synthesize conjugated nanomedicines like that are attached to physiological membranes (by the fusing immune cell membranes to polymeric cores) and thus have immense promise to suppress synovial inflammation, deactivate pro-inflammatory cytokines and provide strong chondro-protection against inflamed joints (Fig. 1).

1.1 History and advancement of nano-therapeutics

The first synthesis of therapeutic nanoparticles can be traced back to the 1950s when polyvinyl-pyrrolidone-mescaline conjugate was developed by incorporating a short peptide spacer between the drug and the polymer [5]. Another early influential event occurred in the mid-1960s when liposomes were discovered [6]. These discoveries mark the birth of the field of nano-therapeutics and during the recent past, relative innovations

of nanocarriers represent most of the highlighted therapeutics and continue to be investigated extensively. Nanoparticle targeting based on chemical properties of nanoparticles and surface coatings comprises active and passive targeting [7]. Passive targeting is defined as nonspecific accumulation in disease tissue (usually cancer tissue). Specific or active targeting is defined as selective transport of nanomedicines containing protein, antibody, or small molecule only to specific tissues and/or specific cells [8]. This may occur via homing to overexpressed cell-surface receptors (Fig. 2).

1.2 History and advancement of nanotheranostics

Theranostics, the coupling of therapeutic products with diagnostic agents, can provide feedback through imaging results or other diagnostic probes about the efficacy of treatment. This may help in optimization and personalization of treatment more efficiently than the current standard of care [9]. Theranostics usually refers to a combinatory scheme of diagnostic therapy for individual patients, testing them for possible reactions when taking a new medication and tailoring their treatment based on personalized test results. By adding the prefix nano, the term nanotheranostics appears where the role of nanomaterials in treatment delivery is dominant [10]. Nanotheranostic is a unique and unconventional treatment approach that carries immense potential to influence our health-care systems. From the last few years, various theranostic systems have been widely used for imaging, therapy, and development of targeted drug delivery systems toward various diseases and disorders [1, 11]. Besides imaging and therapy, nanotheranostic systems are being used to monitor pharmacokinetics, distribution of the particles in the tissue, and accumulation of drug at the target site, etc. However, a lot of factors limit their applications especially in the usage of such formulations as contrast agents and drugs because of not capable of entering the brain due to blood-brain barrier (BBB), which pose a major challenge in the path of development of an efficacious and safe theranostic system [12]. NPs which exhibit low toxicity profile hold great opportunities to be developed as nanotheranostic systems. A major challenge, in nanotheranostics, for the 21st century, is to be able to detect disease biomarkers noninvasively at an early stage of disease progression and its usage as personalized medicine for genetic and phenotypic disorders [13] (Fig. 3).

2. Designing nanomedicines for nonparental administration

Nanomedicines have evolved into various forms including dendrimers, nanocrystals, emulsions, liposomes, solid lipid nanoparticles, micelles, and polymeric nanoparticles since their first launch in the market [14, 15]. The creation of “smart” nanoparticles is an emerging trend in nanomedicine. To facilitate pharmacokinetic and biodistribution analysis, and to thereby improve drug targeting to pathological sites, it would be highly useful if the circulation time and the organ accumulation of nanomedicine formulations could be visualized noninvasively in real-time [16]. To achieve this goal, many different

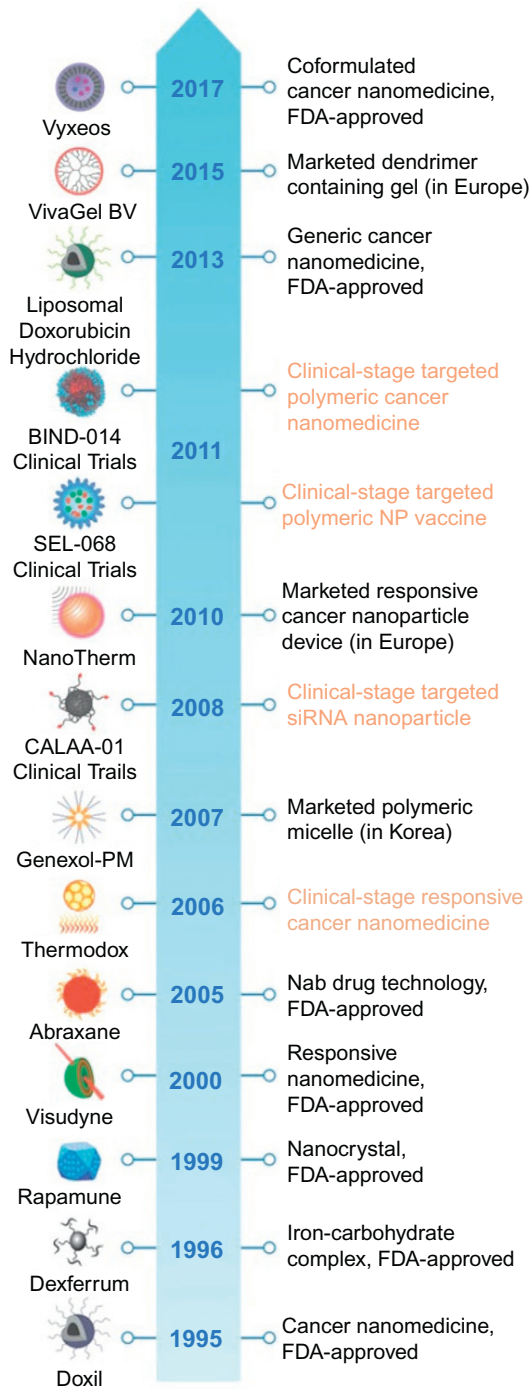


Fig. 2 The evolution of nanomedicines demonstrated with examples that are in clinical trials or reached the market. (Adapted with permission from Swierczewska, et al., *Characterization of nanoparticles intended for drug delivery*, in: *Methods in Molecular Biology*, vol. 1682, 2018. Copyright 2018 © Springer).

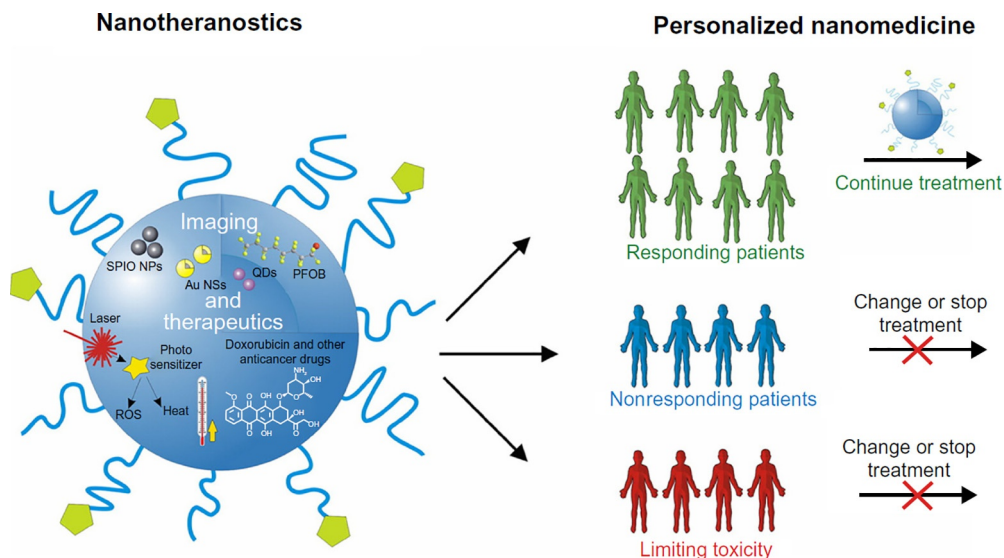


Fig. 3 Potential of nanotheranostic systems in personalizing treatment and improving therapeutic outcomes. (Adapted with permission from S. Mura, P. Couvreur, *Nanotheranostics for personalized medicine*, *Adv. Drug Deliv. Rev.* 64 (2012) (13) 1394–1416. Copyright © Elsevier).

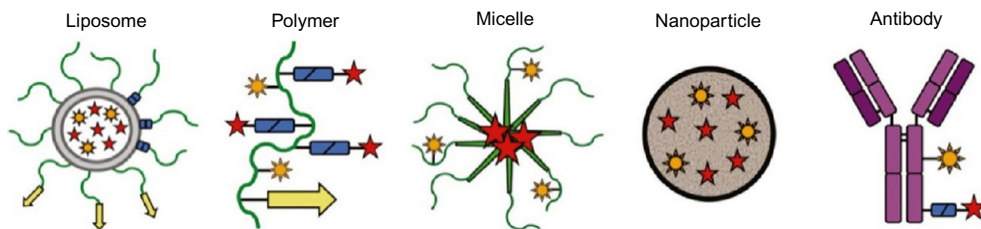


Fig. 4 Classical designs of nanomedicines conjugated or entrapped pharmacologically active agents. (Adapted with permission from Lammers, et al., *Theranostic nanomedicines and image-guided drug delivery*, *Acc. Chem. Res.* 44 (2011) (10) 1029–1038).

types of nanomedicines have been coloaded both with drugs and imaging agents. By delivering pharmacologically active agents more effectively and more selectively to the pathological site (site-specific drug delivery) and/or by guiding them away from potentially endangered healthy tissues (site-avoidance drug delivery), nanomedicines aim to improve the balance between the efficacy and the toxicity of systemic (chemo) therapeutic interventions [17, 18] (Fig. 4).

Due to the wide range use of polymeric biomaterials, a single, ideal polymer or polymeric family does not exist. Instead, a library of materials is available to researchers that

can be synthesized and engineered to best match the specifications of the material's desired biomedical function [19]. Since drug release patterns greatly vary from batch to batch of nanomedicine formulations, and since there are large differences in the release patterns, for example, liposomes vs polymers versus micelles, it is of the utmost importance to visualize and analyze drug release, not only under semiartificial *in vitro* conditions but also under physiologically relevant *in vivo* conditions [20, 21]. *In vitro*, drug release can generally be analyzed relatively easily, for example, using HPLC, but *in vivo* this is much more complicated: after harvesting the target tissue, for instance, the material generally needs to be homogenized, and the cells need to be lysed, in order to release the agents from certain intracellular compartments [22]. During these processing steps, and especially during cell lysis (using detergents), many types of carrier materials are destabilized, and, for example, in the case of liposomes, it is then impossible to discriminate between the amount of drug that was still present within liposomes at the point of harvesting and the amount that was already released into the extra- and intracellular environment [23, 24]. The opsonization of intravenously administered nanoparticles decreases their circulation time, thus affecting the drug delivery efficacy of nanomedicine at the inflamed site [25]. The disturbed vasculature in the inflamed joints is also a limiting factor. The most remarkable quality of nanostructures is the engineered capability to carry substances of choice; they can be functionalized more biocompatible by appropriate designing procedures [26]. As a result of this freedom, researchers have been able to develop more targeted, biocompatible, and biodegradable nanomedicines, which is a step toward providing a sustainable solution to the long-standing ailments. The novel nanotheranostic and nanotherapeutic strategies being researched not only retain the potential to specifically target inflammation sites but could also reduce the dose and administration frequency of drugs to a minimum. Nanoparticles are composed of inorganic or organic material and are of diameter 1–100 nm; they exhibit novel and unique properties as compared with bulk materials but also exhibit considerable toxicity because of their high reactivity with chemicals, increased cell permeability, and their large surface area, and inner pore dimensions [27]. Liposomes are lipid vesicles that are composed of phospholipids, cholesterol, and other lipid conjugated polymers with an inner aqueous phase. The liposomes can load hydrophilic drugs in the inner aqueous core and lipophilic drugs in the lipid bilayers. Polymeric NPs can be engineered to load a high content of drugs and provide controlled drug release for prolonged periods of time. Dendrimers are globular, nanostructured polymers with a well-defined shape and narrow polydispersity (3–20 nm). Drugs could be either entrapped in the dendrimer core or conjugated to the dendrimer surface functional groups [28]. The drug-loading capacity and drug-release profile of dendrimers can be controlled by the dendrimer generation, surface chemistry and conjugation method. Micelles are self-assembled spherical vesicles consisting of hydrophilic corona and a hydrophobic core, which shows the potential to solubilize and stabilize hydrophobic drugs [29] (Fig. 5).

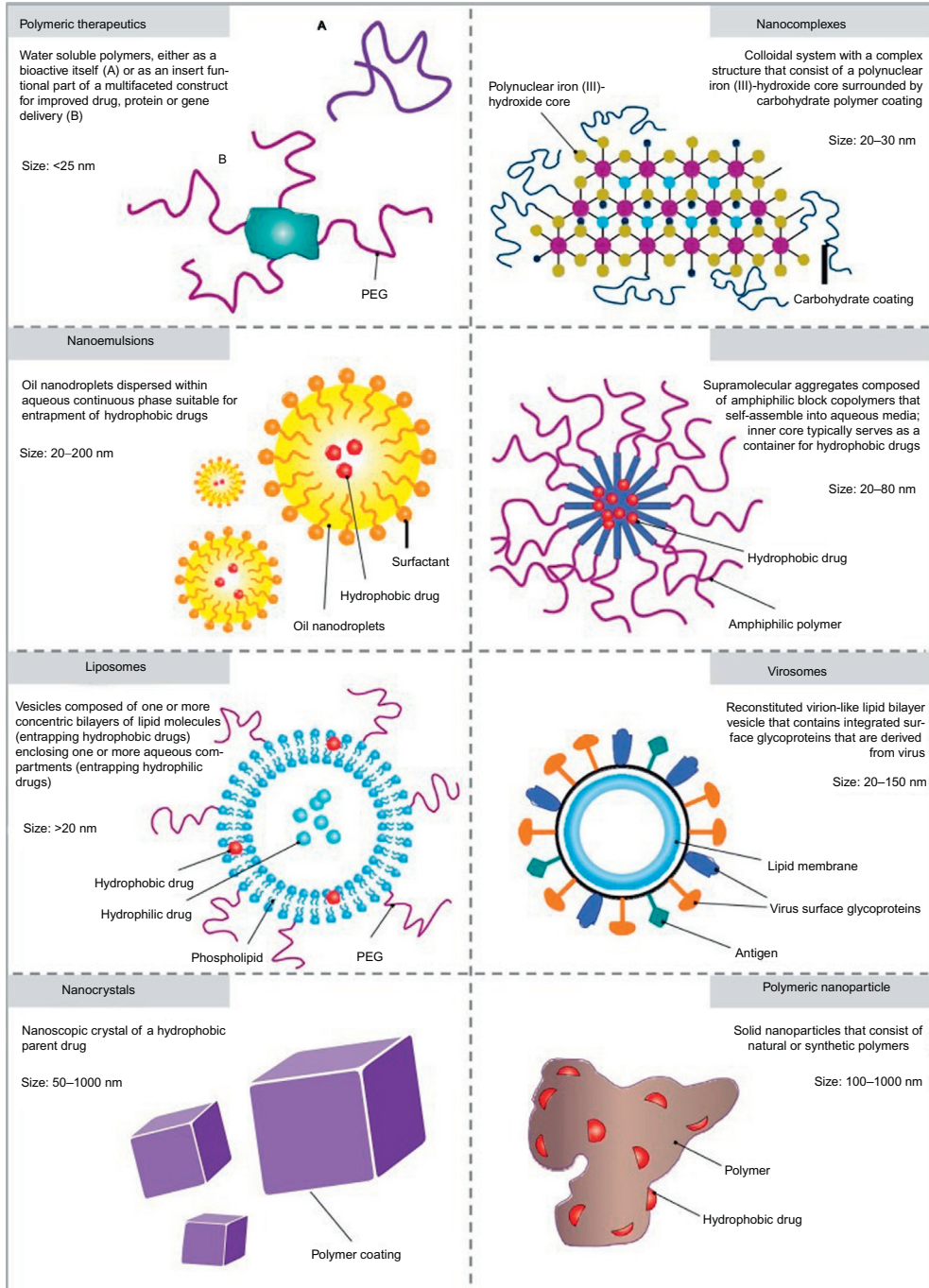


Fig. 5 A schematic overview of nanotherapeutic formulations with their respective size averages. (Adapted with permission from A. Hafner, et al., *Nanotherapeutics in the EU: an overview on current state and future directions*, *Int. J. Nanomedicine* 19 (2014) (9) 1005–23. Copyright © 2014 Dove Medical Press Limited).

2.1 Nanomedicines with natural polymers

Biopolymer nanoparticles can be used efficaciously to provide bioactive molecules for in vivo and in vitro applications. Nano-biopolymers also find applications in the field of enzyme replacement therapy (ERT). The emergence of stimuli-responsive polymeric systems and polymer-drug conjugates has greatly influenced the rational design of polymers tailored for specific cargo and engineered to exert distinct biological functions [30, 31]. Indeed, the possibility of using nanotherapeutic agents constituted by biocompatible and biodegradable polymers to deliver enzymes in those tissues where they are lacking or absent represents an enormous advantage by overcoming a series of ERT problems [32]. Natural polymeric nanomedicines are proved to be effective in stabilizing and protecting biologically active components, including vaccines, DNA, proteins, etc., from various environmental hazards and degradation. It was demonstrated that natural polymer-based nanomedicines have enhanced therapeutic efficacy as a result of the prolonged systemic circulation, targeted drug delivery, and cellular uptake [33, 34]. For example, alginate NPs proved to be good delivery vehicles for vaccine adjuvants, such that they stabilize and protect antigens from the immediate biological environment, slow down antigen clearance, and enhance delivery to antigen-presenting cells, especially dendritic cells [35] (Fig. 6).

During the recent years, preparation and processing of natural products-based nanomedicines are considered as the promising scientific arena because they have interesting characteristics, such as being biodegradable, biocompatible, being renewable with better drug availability, and also exhibiting very less toxicity compared to conventional pharmaceutical candidates [36]. The engineering of new polymeric derivatives that are

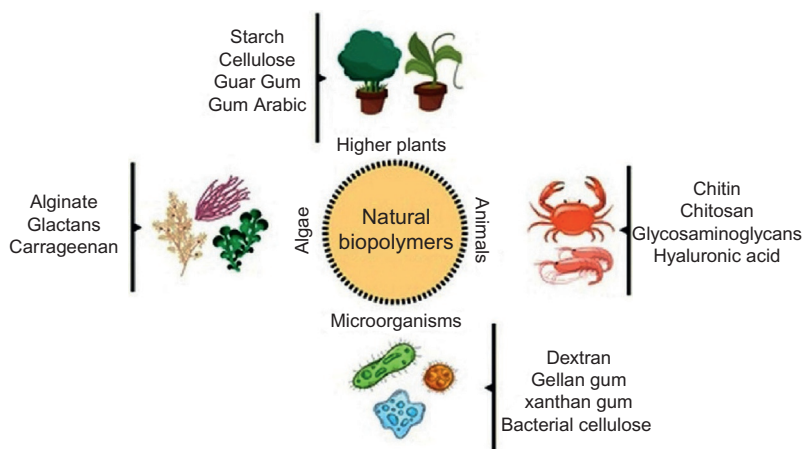


Fig. 6 Various biological sources from which natural biopolymers can be extracted and used in nanomedicine applications. (Adapted with permission from Patra, et al., *Nano-based Drug delivery systems*, *J. Nanobiotechnol.* 16 (2018) 71).

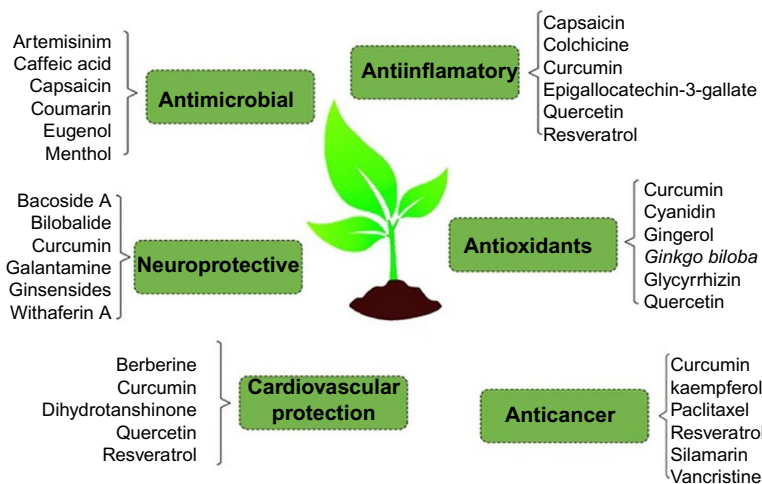


Fig. 7 Several ethno-pharmaceutical compounds were identified and extracted from herbs and higher plants which are used widely for nanotherapeutic applications. (Adapted with permission from Patra, et al., *J. Nanobiotechnol.* 16 (2018) 71).

capable of drug release by endogenous or exogenous stimuli has been introduced during the last 10 years and continue to be investigated [37]. These stimuli-responsive mechanisms are based on pH change, ionic strength change, enzyme-substrate interaction, magnetic stimuli, thermal change, electrical, and ultrasound stimuli. Stimuli induce changes in the surrounding environment that affect polymer physical and chemical properties [38] (Fig. 7).

Biodegradable polymers (synthetic, semisynthetic, and natural) used for the development of NPs possess unique characteristics including nanoscaled structures, high encapsulation capacity, biocompatibility, and controlled-/sustained-release profile for lipophilic/hydrophilic drugs. Despite the tremendous effort that was made to enhance natural polymeric nanocarrier properties, still some limitations can be observed, manifested by their poor drug-loading capacity, drug expulsion after polymeric transition during storage, and the tendency for particle-particle aggregation as a result of their large surface area [39].

2.2 Nanomedicines with synthetic polymers

There are various synthetic biodegradable polymers such as poly(hydroxybutyrate), poly anhydride copolymers, poly(orthoester)s, polyphosphazenes, poly(amidoester)s, poly(cyano acrylate)s, and PLGA. PLGA is a widely used polymer that has been approved by the US Food and Drug Administration (FDA) for various therapeutic/diagnostic applications [40]. Principally, drug release from polymeric nanomedicine involves the movement of a drug molecule from the initial position in the polymeric matrix to the

polymer's outer surface and finally into the surrounding environment. It should be noted that PLGA undergoes hydrolytic degradation in an aqueous environment where ester linkages along with the polymer backbone are randomly hydrolyzed. Drug release may occur via one or a combination of the following mechanisms: diffusion, dissolution, degradation, or swelling [41]. Generally, if drug diffusion across the polymeric matrix is faster than matrix degradation, then the mechanism of drug release is driven mainly by diffusion, otherwise, polymer degradation is the limiting step in drug release. Consequently, drug release normally follows first- (via matrix degradation) rather than zero-order (via diffusion) kinetics [42]. Particle size also strongly influences drug release through mediating both diffusion and matrix degradation. Drug release from a synthetic polymeric nano-formulations is also highly influenced by desorption of the surface-bound/adsorbed drug by diffusion and erosion [43]. Rapid initial or burst release can be attributed to the fraction of the drug that is adsorbed or weakly bound to the large surface of the polymeric nanocarriers (NCs), rather than drug molecules incorporated in the NCs itself (Fig. 8).

Hence, recent efforts to design and develop biodegradable polymeric nanomedicines have been focused on custom designing and synthesizing polymers with tailored properties for specific applications by (i) developing novel synthetic polymers with unique chemistries to increase the diversity of polymer structure, (ii) developing biosynthetic processes to form biomimetic polymer structures, and (iii) adopting combinatorial and computational approaches in biomaterial design to accelerate the discovery of novel resorbable polymers [44].

2.3 Nanomedicines with multifunctional adaptations

The morphological and chemical modifications of natural polymers produce semisynthetic polymers that are better suited for processing and production of materials with potential of mineralization and conversion to biomass. To improve the applicability of such semisynthetic polymeric forms and its various derivatives (e.g., carboxylated, thiolated, and acylated structures) for pharmaceutical/biomedical applications, they have so far been decorated with various functional groups such as polyelectrolyte/polyionic complexes [45]. Biodegradable polymeric micelles composed of PEG and polycarbonate functionalized with disulfide and carboxylic group can be synthesized as pH and redox dual responsive drug delivery systems [46]. Hydrophilic thermosensitive biodegradable polymeric nanocarriers, are another example of smart drug delivery systems that are collapsed at the hyperthermic condition of 42 °C which causes greater drug release and may lead to a synergistic effect of chemotherapy and hyperthermia for treatment of solid tumors [47]. Furthermore, the biodegradable polymeric carriers have been modified by tumor-targeting agents such as specific ligands (e.g., folic acid), antibodies and aptamers to enhance the nanomedicine translocation into tumor cells [48] (Fig. 9).

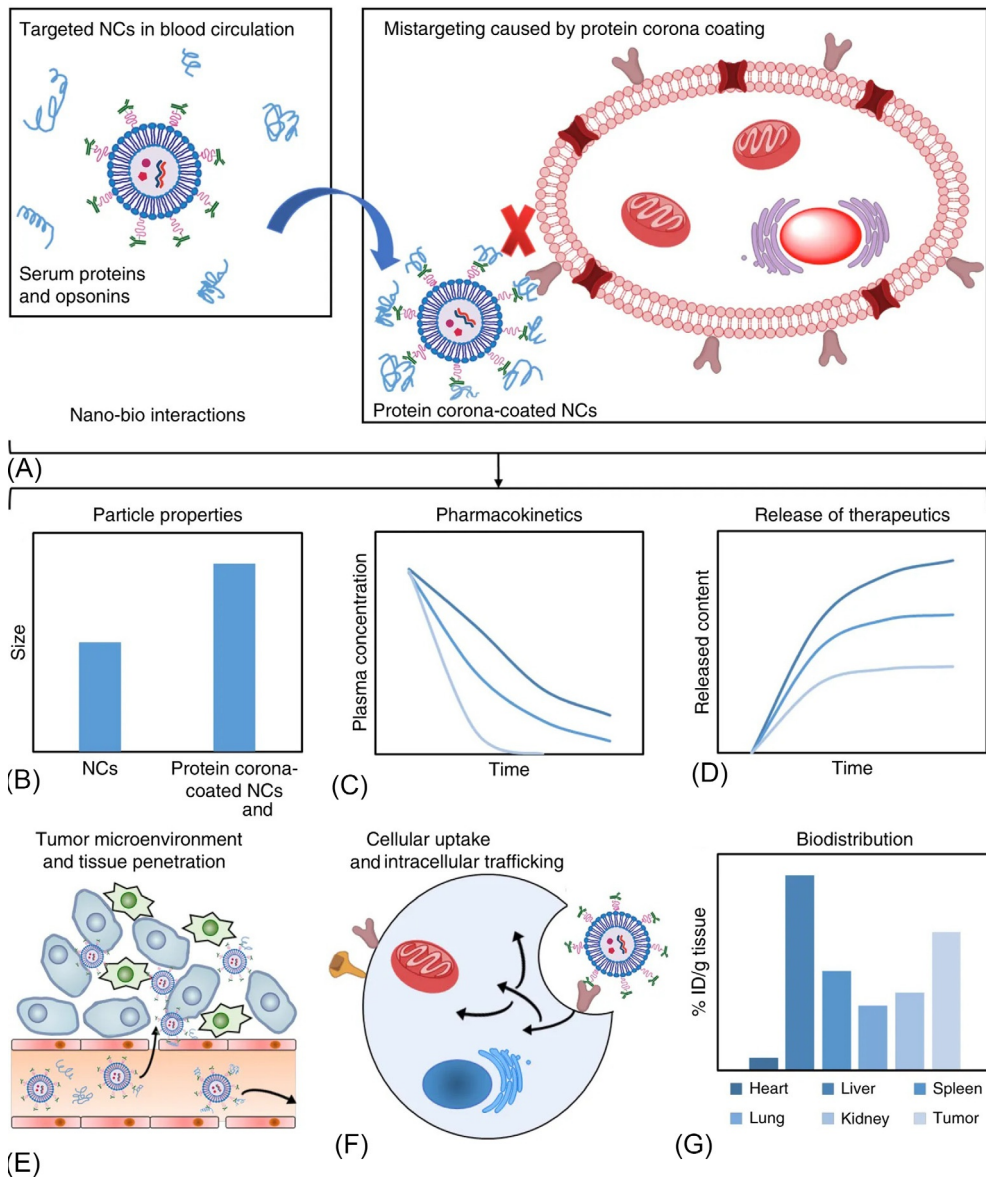


Fig. 8 The impact of nano-bio interactions on the systemically administered NCs. (A) During systemic circulation, targeted NCs get coated with serum proteins and opsonins, which impacts the targeting efficiency and many other properties of NCs, including (B) particle size, (C) pharmacokinetics, (D) release profiles, (E) tissue penetration, (F) cellular uptake and intercellular trafficking, and (G) biodistribution (ID injected dose). (Adapted with permission from D. Rosenblum, N. Joshi, W. Tao, et al., *Progress and challenges toward targeted delivery of cancer therapeutics*, *Nat. Commun.* 9 (2018) 1410).

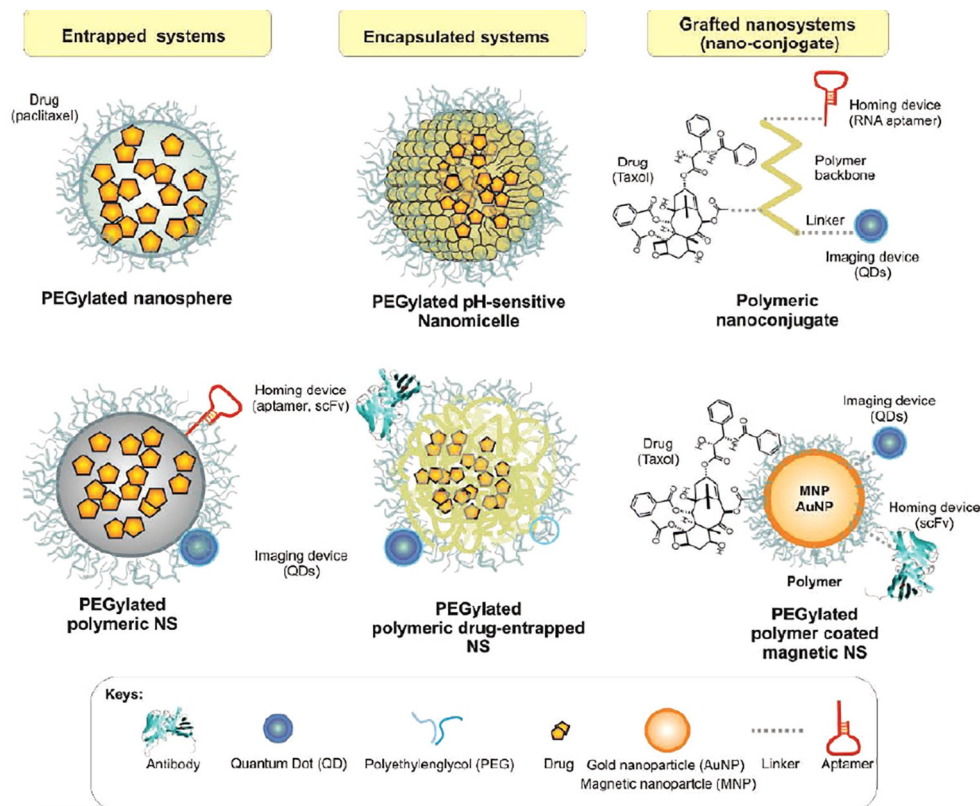


Fig. 9 Schematic illustration of nanomedicines with multifunctional adaptations. (Adapted with permission from M. Fathi, et al., *Int. J. Polym. Mater. Polym. Biomater.* 64 (2015) 541–549).

Taken all these understandings to the consideration, an ideal biodegradable polymeric drug delivery system for nonparental routes must be tailored in a way that it provides a number of imperative characteristics such as (a) suitable permeability and drug release profile based on physicochemical properties (e.g., lipophilicity and hydrophilicity) of cargo molecules, (b) biodegradability and biocompatibility, (c) tensile strength, and (d) possibility for surface modification and decoration.

3. Nonparental nanodrug delivery systems: Overview

Theranostic nanomedicines can be used for different purposes. By enabling a noninvasive assessment of the pharmacokinetics, the biodistribution and the target site localization of conjugated or entrapped pharmacologically active agents, nanotheranostics allow for the optimization of drug delivery systems. In addition, by combining information on overall target site localization with noninvasive imaging insights on the local distribution of the

drug and/or the carrier material at the target site, nanotheranostics can also be used for predicting treatment responses [49]. Furthermore, by noninvasively imaging drug release in vivo, some of the basic properties of drug delivery systems can be visualized and analyzed, and attempts can be made to correlate the in vitro characteristics of carrier materials with their in vivo capabilities. Related to this, by using contrast agents to monitor the release of pharmacologically active agents from stimuli-sensitive nanomedicines, the efficacy of triggerable drug delivery systems can be optimized, as exemplified by several studies on thermosensitive liposomes. And finally, by providing real-time feedback on the efficacy of targeted therapeutic interventions, theranostic nanomedicines can also be used to facilitate (pre) clinical efficacy analysis, to prescreen patients, and to realize the potential of personalized medicine [50]. During phase I and phase II clinical trials, nanomedicine formulations could be labeled with radioactive compounds, in order to obtain some initial noninvasive information with regard to target site accumulation. On the basis of this, rational predictions could then be made with regard to the potential effectiveness of nanomedicine-based therapeutic interventions.

3.1 Oral nanodrug delivery systems

The site-specific delivery of the drug to the oral cavity can be used to treat a number of diseases of the mouth, such as stomatitis, periodontal disease, fungal and viral infections, and oral cavity cancers, thereby avoiding the first pass metabolism effect [51] (Table 1).

3.2 Colorectal nanodrug delivery systems

Colorectal-specific drug delivery systems are gaining importance for use in the treatment of chronic diseases, such as irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, and also for the systemic delivery of protein and peptide drugs. In the frame of colorectal cancer therapy, the most employed approach is the use of intravenously administered nanovectors in order to improve the pharmacokinetic behavior of otherwise problematic drugs [52]. The same concepts of pharmacokinetic improvement can also be applied to diagnostic nanovectors, in order to deliver a higher amount of labeling molecules to the site of colorectal cancers (CRCs), avoiding their toxic effects and improving their sensitivity. Despite the remarkable progresses in the development of more complex and efficient nanovectors, in the large majority of studies, the biological testing of nanoparticles still relies on 2D cell cultures and ectopic murine models of CRC. These preclinical models are well known and validated, but they give only limited insight into the potential clinical efficacy of the formulations in the study [53]. The 2D cell cultures are characterized by a simple and unrealistic environment in which cancer cell lines are forced to grow only on a surface. This condition can alter the cells gene expression and polarization, inducing a phenotype different from the one found in the actual CRC tissue. Use of these tissue-like environments and drug-loaded nanovectors provides

Table 1 Examples of commercially available nano-therapeutic products for oral administration.

Nanotechnology approach	Drug	Major indication	Drug Form	Brand name (manufacturer info)
Nanocrystals	Sirolimus	Graft rejection	Tablet	Rapamune (Pfizer Ireland Dublin)
	Fenofibrate	Kidney transplantation Hypercholesterolemia	Tablet	Tricor/ Lipanthyl/Lipidil (Recipharm, Fontaine, FR)
	Aprepitant	Postoperative nausea and vomiting, Cancer	Capsule	Emend (Merck Sharp and Dohme Bv, Haarlem, NL)
Nanoemulsions	Cyclosporine	Prophylaxis of organ rejection following organ transplant	Capsules	Neoral (Novartis AG, Basel, CH)
	Ritonavir	HIV infections	Capsules	Norvir (Aesica Queenborough Ltd., UK)
Polymeric drugs	Sevelamer	Hyperphosphatemia Renal dialysis	Tablet	Renagel (Genzyme Ltd., Oxford UK)/Renvela (Genzyme Ireland)

Adapted with modifications from Hafner, et al., *Int. J. Nanomedicine* 9 (2014) 1005–1023.

unprecedented opportunities to study and exploit intercellular communication to achieve more specific targeting and even drug-free therapeutic actions (Fig. 10).

3.3 Nasal nanodrug delivery systems

Owing to nasal obstacles such as low membrane permeability, a short local residence time, and high turnover rate of secretion in nasal cavities, the bioavailability of nasally administered drugs is often comparatively poor [54]. The nasal drug delivery systems are promising adjuvant/delivery systems for nonparenteral delivery of antigens as well as for other immune-specific molecules. Moreover, the nasal administration of vaccines can induce specific IgA antibody responses at distant mucosal sites, including the upper and lower airway mucosa and the small and large intestines, as well as the nasopharynx, salivary glands, genital tract, and tonsils, because of the dissemination of antigen-specific

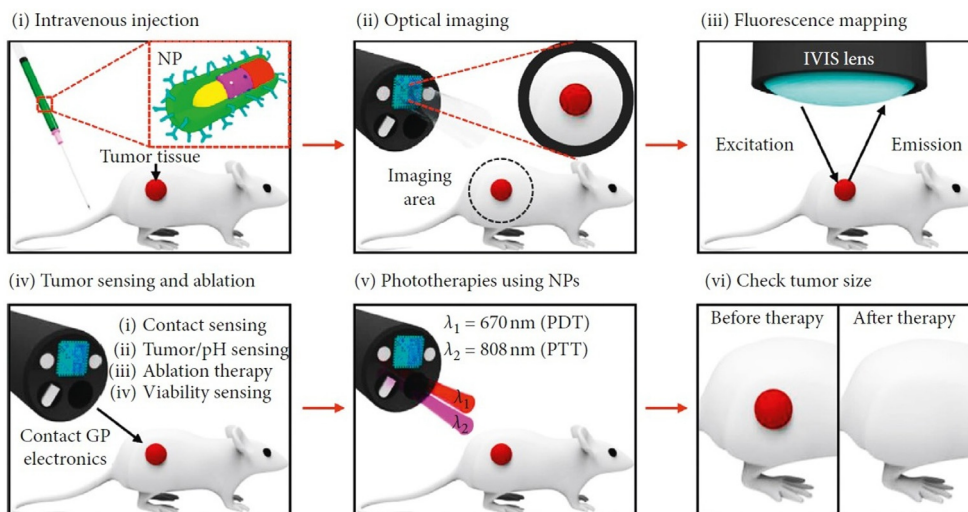


Fig. 10 Advanced nanotheranostic tools and strategies for endoscopic device development for the treatment of colorectal cancers. (Adapted with permissions from R. Rampado, et al., *J. Oncol.* (2019) 740923. Copyright © Hindawi).

lymphocytes in the common mucosal immune system [55]. The olfactory region is located at the top part of the nasal cavity under the cribriform plate in close proximity to the olfactory bulb, interlocking the nose with the brain. This region consists of three types of cells, namely the basal epithelial cells, sustentacular cells, and the olfactory neurons with their cilia extending toward the nasal cavity. After administration of the drug into the nasal cavity, the drug transport may occur through the olfactory epithelium, either (i) by axonal transport after internalization into the neurons, (ii) by paracellular transport across the spaces between cells and, notably across the channels next to the olfactory nerves, or (iii) by transcellular transport across the basal epithelial cells (Fig. 11).

3.4 Pulmonary nanodrug delivery system

Due to the complexity of respiratory disorders and lung morphology, it should be kept in mind that disease severity, age of the patient, breathing pattern, and device design, and structure decide the actual outcome of aerosol use and the final success of pulmonary therapy [56]. At the onset of the recent COVID-19 pandemic, enormous interest has been generated in the development of nonparenteral nanomedicine especially for treating Pulmonary Fibrosis. There was a recent development made in the formulation of a novel nanocarrier consisting of Lipoid S100 and chitosan or glycol-chitosan for the systemic delivery of low molecular weight heparin upon pulmonary administration. These nano-systems, formed by ionic gelation technique, provided both sufficient entrapment efficiency and mucoadhesive properties. Aerosolization of these formulations indicated

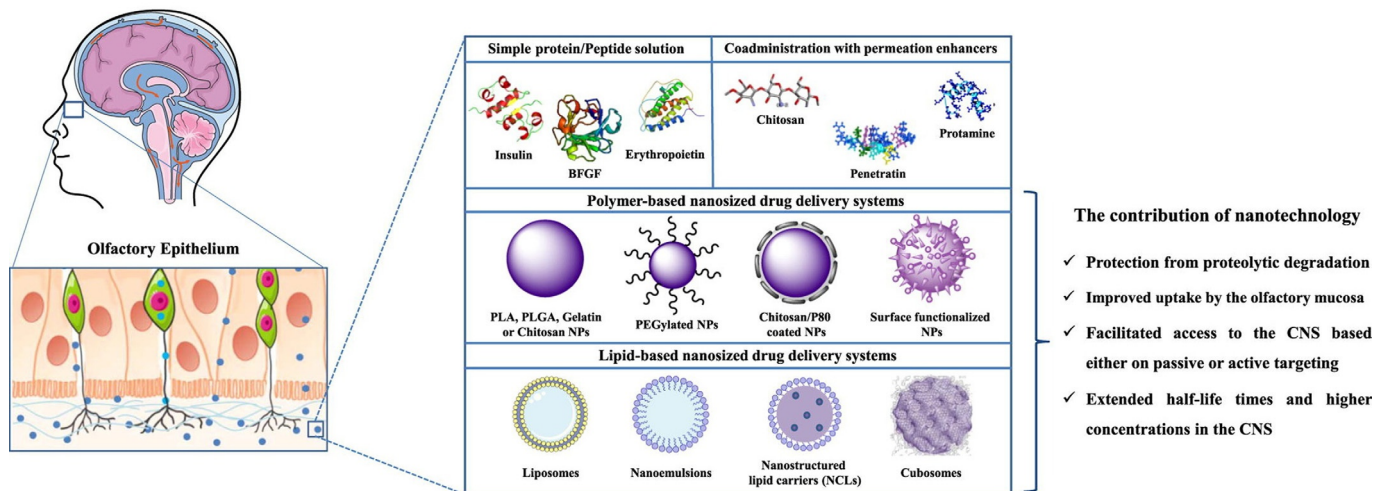


Fig. 11 Schematic representation of Nasal nanodrug delivery systems and possible uptake mechanisms involving olfactory pathway and transport of peptides from the nose directly to the brain. (Adapted with permissions from E. Samaridou, M.J. Alonso, *Bioorg. Med. Chem.* 26 (2018) 2888–2905. Copyright © 2018 Elsevier).

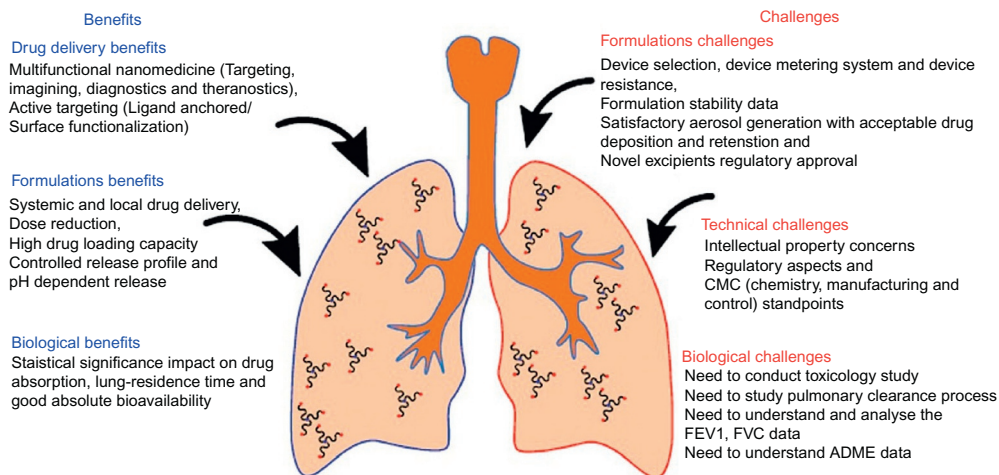


Fig. 12 Benefits and challenges of nanomedicines for pulmonary drug delivery. (Adapted with permission from Mehta, et al., *New J. Chem.* 43 (2019) 8396. Copyright © 2019 Royal Society of Chemistry).

that heparin could be delivered to the lung. Overall, these nanocarriers might have a use potential for systemic delivery of low molecular weight heparin as compared to the free drug with a therapeutic potential effect for the treatment of pulmonary embolism and other thrombo-embolic disorders [57]. Generally, the successful delivery of any active compound to the lungs by aerosol depends on four mutually dependent features: the formulation, the aerosol device design, the metering system and, finally, the patient's understanding/responsiveness (Fig. 12).

3.5 Transdermal nanodrug delivery systems

Transdermal drug delivery system refers to a route of drug delivery through the skin to achieve local or systemic therapeutic action. It is one of the focus areas of research for the third-generation pharmaceutical preparations, next only to oral medication and injection [58]. The reasons lie in the administration route of the drug, which is convenient, easy to use, noninvasive, and also improves patient compliance. It also reduces the fluctuation of the drug concentration in the blood, provides steady plasma levels and fewer chances of overdose and easy detection of the drug. At the same time, it evades the gastrointestinal environment, such as pH, enzymatic activity, and the interference of drug and food interaction on the drug efficacy and the "first pass effect" (where active drug molecules can be converted to inactive molecules or even to molecules responsible for side effects) by the liver [59]. Although TDDS has many advantages, the use of drugs in TDDS is currently limited. As mentioned above, the most resistance during the percutaneous permeation of the drugs comes from the SC of the skin. When many drugs are delivered through the

skin, adequate permeability rate is difficult to achieve as per therapeutic requirements. To overcome these difficulties, nanotechnology may be a good choice. Nanotechnology refers to the technology of using a single atom or molecule to produce or process macromolecular matter into a material with a particle size of 1–100 nm. One of the important areas of nanotechnology is nano-formulations [60]. Given their small particle size, nano-formulations have a better effect on drug retention, specificity and targeting, which makes an ideal TDDS. They have many advantages, such as being painless, minimal skin injury (does not change the general structure of SC of the skin and does not destroy the skin barrier function), and promotes permeation of macromolecular drugs, which has become a very popular field of research on TDDS. Nano-formulations can be divided into vesicles including liposomes, transfersomes, ethosomes, niosomes, invasomes, and nanoparticles including lipid nanoparticles, polymeric nanoparticles, and nano-emulsions [61]. As for active transdermal administration, microneedles are not involved, instead, ultrasonic, electroporation, hot perforation, and comprehensive application of other methods enhancing penetration are used.

3.6 Ocular nanodrug delivery systems

Anterior eye diseases are generally treated by eyedrops, but the rapid tear film turnover (15–30s) will quickly dilute the eyedrops and drain the drugs through the nasolacrimal duct, and the remained drugs will have to penetrate the cornea to reach the anterior chamber [62]. Various ophthalmic vehicles, such as inserts, ointment, suspensions, and aqueous gels, have been developed in order to lengthen the resident time of instilled dose and enhance the ophthalmic bioavailability and for improving the retention and biodistribution of drugs applied topically onto the eye. The poor corneal penetration and retention of drugs, resulting in limited ocular bioavailability, require repeated instillations to achieve therapeutic drug concentrations in the eye. Topical eye drops are still the preferred dosage form because of convenience and good patient acceptance. The drug clearance typically occurs within 15–30s owing to the tear film turnover, resulting in the intraocular bioavailability of topically applied [63]. One of the most investigated recent pharmaceutical forms is the in situ gels, which have been developed to prolong the precorneal resident time of the drug and to improve ocular bioavailability. The main challenge for retinal disease treatment is the ineffective drug delivery to the posterior segment. Owing to the nonspecific absorption and blood–retinal barrier, the systemic route delivers drugs to the eye at low rates with a high risk of systemic toxicity to other tissues. Many other factors also need to be addressed in detail, including the polymer purity; NP manufacturing technology, solvent residue, and potential local acidic environment during polymer degradation, material buildup in the eye after repeated dosing, foreign-body reactions, and the potential snow globe effects in the vitreous to disturb the visual axis. Success in the translation of nanomedicine would require a careful risk: benefit analysis, which is often skewed toward risk when it comes to novel therapeutics.

3.7 Regenerative nanomedicine systems

Nanotechnology applications to regenerative medicine have all the potential to revolutionize tissue regeneration and repair. However, the development of ideal nanomaterials capable of sending signals to the diseased or damaged cells and tissues to trigger the regeneration process still remains a challenge. In order to regenerate some loss or damaged tissue and organ, *in vitro* seeding and attachment of human cells onto a scaffold, followed by the culturing of the cells to form the new organ or tissue must be performed to avoid some transplantation of them. Scaffold design is a niche in regenerative medicine that involves creating a foundation for cell adherence that directs proliferation in an appropriate configuration and differentiation scheme. Nanoscale fibers have shown considerable success in the reparation and regeneration of soft tissues through tissue scaffolding in the skin, blood vessels, nerves, tendons, and cartilage applications [64]. Common design criteria include biocompatibility, porosity for cell growth and nutrient and waste flow, natural extracellular matrix (ECM) architecture, biodegradability at a rate consistent with new tissue growth, and mechanical support. One of the major applications in this field is the use of nanostructures having native tissue-mimicking ability, which has resulted in the development of long-lasting and better-performing scaffolds. Extensive research is being conducted on the use of scaffolds seeded with stem cells to generate bone and cartilage. However, the success of this technique is limited by the availability of stem cells and their efficiency in regeneration. The enhancement of axonal growth using nanofiber conduits for the treatment of neuronal injuries is also being explored [65]. Efforts are presently directed toward the development of nanofibers, which help provide properties similar to those of natural cardiac tissue. The clinical use of growth factors in wound healing has generated considerable research interest in recent years. Biodegradable scaffolds integrated with multiple growth factors appear to be the most promising therapeutic option for skin tissue regeneration. Progress made in molecular and stem cell biology, material sciences, and tissue engineering has enabled researchers to develop cutting-edge technology, which has led to the creation of nonmodular tissue constructs such as skin, bladders, vessels, and upper airways. In all cases, autologous cells were seeded on either artificial or natural supporting scaffolds. However, such constructs were implanted without reconstruction of the vascular supply, and the nutrients and oxygen were supplied by diffusion from adjacent tissues (Fig. 13).

4. Toxicity and safety concerns of nanomedicines

Regardless of various advantages, there are also some limitations associated with the usage of nanomedicine, particularly the possibility of generating toxicity at the cellular level. In this context, it is important to identify the properties to understand the mechanisms by which nanomedicines interact with living systems and to understand exposure, hazards, and their possible risks [66]. The toxicity of nanoparticles is currently a major issue in

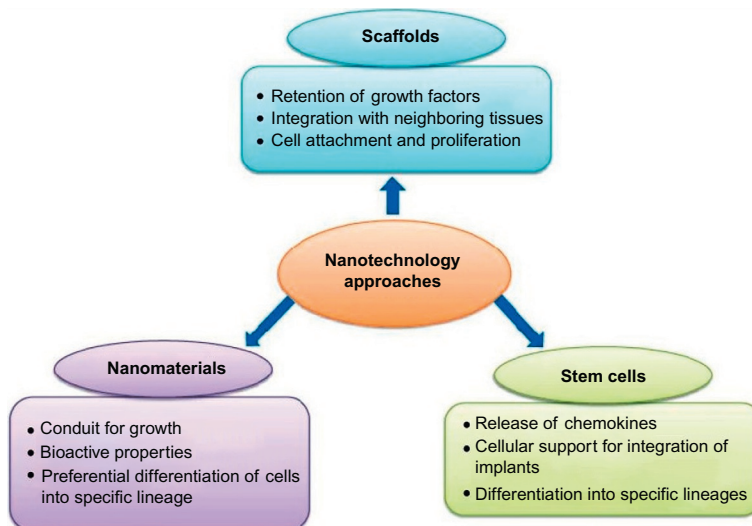


Fig. 13 Commonly used Nanotechnology approaches for regenerative medicine. (Adapted from Chaudhury, et al., *Int. J. Nanomedicine* 9 (2014) 4153–4167).

biomedical applicability since it is a multiparameter problem comprising of materials and morphological parameters such as composition, degradation, oxidation, size, shape, surface area, and structure. Nanomaterials are capable of disrupting the balance of the redox systems and, consequently, lead to the production of reactive species of oxygen (ROS). ROS comprise hydroxyl radicals, superoxide anion, and hydrogen peroxide. Under normal conditions, the cells produce these reactive species as a result of the metabolism [67]. When compared to micron-sized particles, nano-sized particles can be generally more toxic because they have a larger surface area (hence, more reactive), for a given mass, to interact with cell membranes and deliver toxicity. They are also retained for longer periods in the body (more circulation or larger clearance time) and, in principle, can be delivered deeper into the tissue due to their size. Hence, for understanding their pharmacokinetics it is important to define the critical parameters such as physicochemical properties, including size, size distribution, composition, surface characteristics, purity, and stability because they can directly affect in vivo activity of the nanomedicine. Nanomaterials must be evaluated for their toxic effects to assess their safety, along with the therapeutic agent itself. Examining how the nanomedicine and its components interact with blood and immune cells in vitro can help prevent serious and potentially lethal reactions during clinical evaluation. The immune response can directly rely on the adsorption pattern of body proteins. For example, during inflammation, certain matrix-degrading enzymes released by endothelial cells are adsorbed and migrate through the basal membrane and lead to angiogenesis; circulating nanomedicine targets this disturbed vasculature to eradicate the angiogenesis or stop its further spread across the endothelium to

access the joint cavity and other sites of inflammation. An assessment of the *in vivo* protein profile is therefore crucial to address these interactions and to establish biocompatibility. The clearance of nanoparticles is also size and surface-dependent. Small nanoparticles, below 20–30 nm, are rapidly cleared by renal excretion, while 200 nm or larger particles are more efficiently taken up by mononuclear phagocytic system (reticulo-endothelial system) located in the liver, spleen, and bone marrow.

4.1 Immunotoxicity of nanomedicines

Activation of the immune system is the most observed immune response in animal models, following the administration of nanomedicines. It is completely independent of the category of the nanomedicines. Moreover, excessive immune stimulation can result in autoimmune disorders and alternatively cause inflammation in tissues, resulting in long-term damage [68]. Nanoparticles can be taken up by immune cells, including monocytes, macrophages, platelets, dendritic cells in the bloodstream as well as within tissues such as Kupffer cells of the liver, dendritic cells in the lymph nodes and macrophages, and B cells in the spleen. Since the introduction of nanomedicines to the clinic, there have been several cases of acute immune responses to the NMP product in the form of hypersensitivity reactions, this is often due to the structural similarity of NM to viral antigens, which can trigger nonspecific humoral immunity and cause the complement system to produce an immediate eliminatory response. Endotoxin is a major contaminant in early nanomedicine formulations. If endotoxin levels are above certain thresholds, many immunotoxicity assays could give false-positive readings. Taking precautions early in the development process to reduce endotoxin contamination will allow for a more accurate assessment of the toxicity profile of the nanomedicine and its components. However, some nanomaterials can interfere with commonly used assays that assess contaminants and they may exaggerate the inflammatory properties of endotoxin. Controlling bacterial and endotoxin contamination is highly recommended before conducting toxicity or immunology assays (Table 2).

4.2 Challenges in the safety assessment

In spite of efforts to harmonize the procedures for safety evaluation, nanoscale materials are still mostly treated as conventional chemicals, thus lacking clear specific guidelines for establishing regulations and appropriate standard protocols. All nanoparticles rely on control at the nanoscale, meaning small variations may cause significant changes to the nanoformulation. However, not all techniques are sensitive enough to detect small changes in physicochemical properties, so orthogonal techniques are recommended for a more thorough evaluation. Despite the importance of surface evaluation, it remains one of the most challenging physicochemical tests. There are only a few widely applicable assays for surface characterization. Most assays must be individually tailored for the specific surface

Table 2 Major toxicity mechanisms identified during nanomedicine administration.

Types of toxicity	Trigger for toxicity	Consequences
Oxidative stress	Nanoparticle (reactive surface, dissolution of toxic ions); LMP; mitochondria dysfunctions; activation of immune cells	ROS toxicity; damage of other organelles; induce inflammation and genotoxicity; apoptosis
Inflammation	Activation of TLRs and NLRs; uptake by immune cells; release of alarmins	NLRP3 inflammasome activation; release of cytokines
Genotoxicity	Nanoparticle interruption; ROS accumulation; Dissolution of toxic ions; inflammation	Chromosomal fragmentation, DNA strand breakages, point mutations, oxidative DNA adducts and alterations in gene expression profiles
Lysosome dysfunction (LMP)	Proton sponges hypothesis; ROS toxicity; Increase of lysosomal pH; Disruption of lysosomal trafficking	NLRP3 inflammasome activation; release of ROS, ions and hydrolytic enzymes; induce other organelles dysfunction; apoptosis
Mitochondria dysfunction ER stress	Mitochondria outer membrane depolarization; release of ROS Unfolded protein accumulation of ER	NLRP3 inflammasome activation; autophagy induction; apoptosis Activation of ER stress signaling pathway and autophagy to balance homeostasis; apoptosis
Autophagy dysfunction	Blockage of autophagy reflex caused by particle overloading; excessive autophagy induction	Apoptotic and autophagic cell death

Adapted with modifications from Wang, et al., *J. Mater. Chem. B* 3 (2015) 7153–7172.

ligand–nanoparticle combination being evaluated. Among the most important limitations that can negatively impact the use of natural polymers as nanocarriers are their antigenicity and nonuniformity of properties from batch to batch. Variability in the composition is also accompanied by variability in trace impurities, cross-linking density, enzymatic degradation rate as compared with hydrolytic degradation [69]. The risk of viral infection in collagen and gelatin-based materials due to contamination with bovine spongiform encephalopathy is another drawback. Some of the advanced characterization techniques like reverse-phase high-performance liquid chromatography (RP-HPLC) and thermogravimetric analysis (TGA) can be used to quantitatively measure various surface coatings on a variety of nanoplatforms. Imaging by immunoelectron microscopy can also serve as a qualitative method to illustrate nanoparticle surfaces with the help of appropriate antibodies. Certain biological surface moieties have additional complexities that need to be elucidated through specific structural evaluations. For example, the specificity of targeting ligands can be assessed using immune-specific precipitation or titration assays

like ELISA, EIA, etc. Therefore, a combination of different surface characterization techniques along with biological assays may be required for molecularly targeted nanomedicines.

4.3 Strategies for engineering nontoxic nanomedicines

Combinatorial delivery of multiple therapeutic agents, not limited to chemotherapeutic agents, could potentially provide a strategy to combat drug resistance exhibited in many aggressive pathological cases. In addition to passively and actively targeted nanoparticles, targeting the intended disease site can also be achieved with stimuli-responsive drug delivery nanoparticles. New approaches have arisen from the pharmaceutical innovation and the concern about the quality and safety of new medicines by regulatory agencies. Quality-by-design (QbD), supported by process analytical technologies (PAT) is one of the pharmaceutical development approaches that were recognized for the systematic evaluation and control of nanomedicines. Responsive nanoparticles can be designed to deliver their cargo in reaction to some intrinsic or external stimulus. The payload can thus be released to the site of action upon the specific detection of stimulus and nanoparticles can thus undergo transition trafficking to the therapeutic site. Intrinsic stimuli can either be one or combinations of parameters like the pH, enzyme concentration, or temperature of the disease microenvironment [69]. Extrinsic stimuli consist of certain magnetic or electrical fields, ultrasound, or radiation. The goal of this dynamic design of particles is for improving drug accumulation at the site of action; however, assessing drug kinetics in this type of system requires additional understanding of the particle's mechanism of physical transition, the level of stimulation required, and drug release profiles before and after stimulation. Also, externally stimulated nanoparticles have the added complexity of potentially being a drug-device combination, which requires additional know-how and may complicate translation and adoption by physicians. In general, nanomedicines are designed to increase the half-life of the drug, enabling delivery of the active pharmaceutical ingredient (API) to its intended site of action. If the drug releases too quickly, it can produce off-target toxicities. On the other hand, if the formulation is too stable, the API will not be delivered in appropriate concentrations making it therapeutically ineffective. Drug release is, therefore, an important measure of nanoparticle stability. However, determining drug release in vivo is challenging because drug binding can equilibrate between the nanoparticle and abundant proteins in the blood.

5. Conclusion and future perspectives

Incorporation of nanomaterials for nonparenteral drug delivery application is an interdisciplinary research subject involving aspects of biology, medical science, material science, and nano-biotechnology innovations. The key focus of the subject is to achieve and reproduce multicomponent fabrication and designing that control and

measure property response at the nano-size scale efficacy. Biologists and Physicians should focus on ways to introduce multifunctionality without sparing enhanced performance and to increase biocompatibility and sustain enhanced multifunctionality in vivo. The first challenge stems from nanoparticle design and targeting with special emphasis to fine-tune the surface morphology, particle size, and surface charge determine pharmacokinetics, toxicity, and biodistribution. The efficiency of site-specific delivery depends on the profile of cargo-loaded MNPs, field strength, depth of target tissue, rate of blood flow, and vascular supply. Application-driven functionalization is a key ingredient for their successful multifunctional implementation in modern theranostics. Importantly, physicochemical properties of the nanoformulation need to be linked to their performance characteristics such as pharmacokinetics, biodistribution, efficacy, and toxicity profiles. Because of the demanding characterization needs, a clear advantage of the nanomedicine over existing formulations should be established early on in the development stage, along with a feasible manufacturing strategy to prevent expensive failures later on. Successful translation of research from academia to production lines has been identified as one of the major challenges in nanotherapeutic development. Strategies to foster and initiate this translation have yet to be developed to help European research institutions and industries remain competitive in global markets. A quick and successful translation of emerging nanotherapeutics is expected to adapt the established quality-by-design approach. The quality-by-design approach, in the field of nanotherapeutic development, promotes the idea that control over the quality, efficacy, and safety should be incorporated into the formulation development. This approach includes clear definitions of the desired performance (i.e., the expected specifications of the target formulation), nanoparticle design (i.e., the nanoparticle attributes providing efficacy and safety), manufacturing design (i.e., establishing the process parameters ensuring reproducibility of nanoparticle properties), and therapy design (i.e., the treatment modalities providing efficacy and safety of the therapeutic application). A process of developing an optimal formulation is influenced by a complicated matrix of interlinked or independent input and output parameters, which include critical process parameters, critical product quality attributes, and clinical properties such as safety and efficacy. For instance, in order to induce hyperthermia, a major objective is to control the heat distribution using multiple trajectories and also to enhance the formation of aggregates selectively on malignant cells. For magnetic resonance imaging, steps should be made for enhanced cellular internalization, slower clearance from tumor site and size-dependent tissue distribution. In the case of cell imaging and tracking, triggering should be improved to promote cell membrane receptor recognition, long-term in vivo monitoring, uptake initiation, and/or enhancement. Hence, multidisciplinary expertise and testing are essential to grasp a complete understanding of the design features that contribute to a safer and more effective therapy.

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