Review

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Therapeutic Delivery

Nanocarrier fabrication and macromolecule drug delivery: challenges and opportunities

Macromolecules (proteins/peptides) have the potential for the development of new therapeutics. Due to their specific mechanism of action, macromolecules can be administered at relatively low doses compared with small-molecule drugs. Unfortunately, the therapeutic potential and clinical application of macromolecules is hampered by various obstacles including their large size, short *in vivo* half-life, phagocytic clearance, poor membrane permeability and structural instability. These challenges have encouraged researchers to develop novel strategies for effective delivery of macromolecules. In this review, various routes of macromolecule administration (invasive/noninvasive) are discussed. The advantages/limitations of novel delivery systems and the potential role of nanotechnology for the delivery of macromolecules are elaborated. In addition, fabrication approaches to make nanoformulations in different shapes and sizes are also summarized.

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Macromolecule drug delivery

Macromolecular drugs (protein and peptides) are highly specific and potent agents. They have shown great promise as a novel therapeutics in the treatment of many diseases. These large molecule drugs offer many advantages compared with small molecule drugs with respect to high potency, activity, low unspecific binding, less toxicity, minimization of drug–drug interaction, biological and chemical diversity [1]. The chemical structure of macromolecules enables them to perform several specific functions in the body. However, these drugs are subjected to the physical and chemical degradation, short *in vivo* circulation half-life and biodistribution, lack of an efficient, safe and specific delivery. In addition, clearance by the mononuclear phagocytes of the reticuloendothelial system, risk of immunogenic effect, solubility challenges, high molecular weight (MW), structural complexity and failure to permeate cell membranes further reduce their therapeutic efficacy [1–3]. Thus, to achieve a high therapeutic efficacy of macromolecules, appropriate delivery platforms are needed to be designed.

Macromolecules delivery via oral route of administration is very challenging. The large molecular size, complex 3D structure and low permeation of these drugs across biological barriers such as the gastrointestinal (GI) mucosa lead to poor absorption of macromolecules following their oral administration [4]. In addition, low gastric pH and digestive enzymes degrade a significant fraction of the macromolecules prior to their oral absorption. Hence, a large portion of approved and investigational macromolecules is administered via parenteral (invasive) routes mainly through intravenous, intramuscular and subcutaneous injections [1–3,5]. However,

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vulnerability toward enzymatic degradation under *in vivo* condition results into short half-lives of macromolecules even with parenteral administration. Moreover, the short half-lives of protein and peptide drugs require frequent parenteral administrations to maintain their therapeutic levels and are not patient compliant. These drugs also suffer from a number of physicochemical and biological instability due to their complex secondary, tertiary and quaternary structures. Any alteration in active conformation may result in loss of activity as well as irreversible aggregation of macromolecules.

In general, systemically delivered formulations either for small or macromolecule drugs face several barriers before reaching the target cell/organs. Hence, there is a requirement to develop novel formulation strategies to deliver these highly potent molecules. However, due to several physiochemical instability and enzymatic barriers of macromolecules delivery, it is very difficult to develop a suitable formulation for these drugs (Figure 1).

Considering the above facts, various routes of administration (noninvasive and invasive) and respective barriers for the macromolecular drugs (protein and peptides) are discussed in this review. The advantages and limitations of various novel delivery systems including nanotechnology approaches for macromolecule therapeutics are summarized. The challenges of nanotechnology surface modification approaches, design consideration and various novel fabrication methods to make nanocarriers (NCs) in different shape, size and surface engineering that could enhance their *in vivo* circulation time are also summarized.

Route of administration for macromolecule

Currently, a large number of protein therapeutics is under clinical trials. The next generation biologics attained a market value of more than US$1.5 billion in 2013 according to vision gain analyst. This study predicted that the market for biologics spread dramatically and will increase to $30 billion by the end of 2024 (www.pharmamanufacturing.com/articles/2014/next-gen-biologics-market-worth-30b-by-2024). A majority of currently available macromolecule drugs are administered via parenteral routes to achieve the desired therapeutic effects [2]. The route of administration has a significant impact on the therapeutic outcome of a macromolecule drug. Macromolecules can be administered via various delivery routes categorized into two major classes:

Figure 1. Challenges of delivering macromolecule drugs.

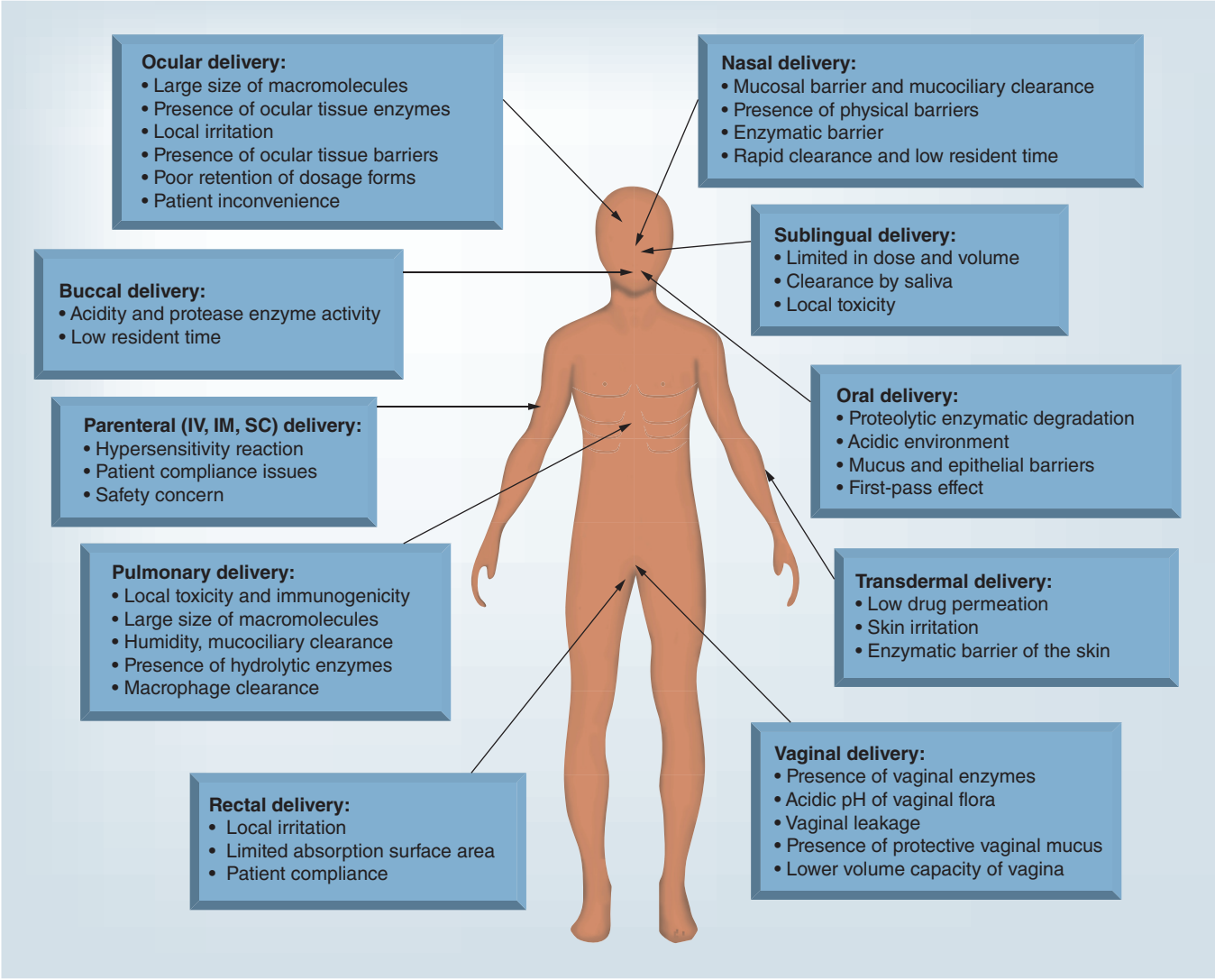

Figure 2. Barriers in invasive and noninvasive delivery of macromolecules.

parenteral, in other words, invasive (mainly intravenous, subcutaneous and intramuscular injections), and noninvasive routes [6] as indicated below. However, noninvasive and invasive routes of macromolecule delivery are limited by the presence of several barriers as illustrated in Figure 2.

Parenteral (invasive) routes of administration

Most of the currently available macromolecule products are designed for the parenteral route of administration. Parenteral delivery of macromolecules can overcome the issue of low absorption and bioavailability as observed in noninvasive route of administration (summarized later in this manuscript). However, in addition to being invasive, several other factors limit the bioavailability of macromolecule therapeutics [5]. Some of these limitations related to parenteral delivery of macromolecules are enlisted below:

- Lack of patient compliance and difficulty associated with parenteral routes of administration;
- Instability of macromolecules affected by pH, humidity, ionic strength, temperature and various other environmental factors;
- Higher viscosity of macromolecule solutions is affecting their syringeability. This makes necessary to deliver the solution using acceptable needles and has a strong impact on patient acceptance;
- Opsonization and rapid clearance of macromolecule and associated formulations from the blood making it necessary for patients to take repeated and high doses of macromolecules which may lead to dose-dependent toxicity and side-effects. In general, opsonization is a process in which external components in the body are coated with opsonin proteins, marking them recognized by the immune system for phagocytosis [7];

Table 1. Clinically approved macromolecule formulations delivered via invasive route.

Drug name	Trade name	Indication	MW (kDa)	Route of admin.	US FDA approval year
Secukinumab	Cosentyx™	Plaque psoriasis	151	sc.	2015
Dinutuximab	Unituxin™	Neuroblastoma (pediatric)	NA	iv.	2015
Ramucirumab	Cyramza®	Advanced gastric or gastroesophageal junction adenocarcinoma and metastatic non-small-cell lung cancer	147	iv.	2014
Siltuximab	Sylvant™	Multicentric castleman's disease	145	iv.	2014
Vedolizumab	Entyvio®	Ulcerative colitis and adult patients Crohn's disease	146.8	iv.	2014
Peginterferon	Plegridy™	Multiple sclerosis	44	sc.	2014
Pembrolizumab	Keytruda®	Unresectable melanoma	49	iv.	2014
Blinatumomab	Blincyto™	B-cell precursor ALL	554.1	iv.	2014
Nivolumab	Opdivo®	Unresectable melanoma	146	iv.	2014
Adotrastuzumab Emtansine	Kadcyla®	Her2-positive, late-stage (metastatic) breast cancer.	148.5	iv.	2013
Obinutuzumab	Gazyva®	Combination with chlorambucil to treat patients with previously untreated CLL	150	iv.	2013
Ziv-aflibercept	Zaltrap®	Metastatic colorectal cancer	115	iv.	2012
Ocriplasmin	Jetrea®	Symptomatic vitreomacular adhesion	27.2	iv.	2012
Raxibacumab	Abthrax®	Inhalational anthrax	146	iv.	2012
Belimumab	Benlysta®	Systemic lupus erythematosus	147	iv.	2011
Ipilimumab	Yervoy®	Unresectable or metastatic melanoma	148	iv.	2011
Belatacept	Nulojix®	Prophylaxis of organ rejection	90	iv.	2011
Brentuximab Veotin	Adcetris®	Hodgkin lymphoma and systemic anaplastic large cell lymphoma	153	iv.	2011

ALL: Acute lymphoblastic leukemia; CLL: Chronic lymphocytic leukemia; iv.: Intravenous; MW: Molecular weight; sc.: Subcutaneous.

- Conformational structures of the macromolecule must be preserved;
- Complex formation with blood proteins and degradation of labile side groups;
- Pain at the site of injection, and potential hypersensitivity reactions;
- Low therapeutic value of the drugs especially for long-term management of certain diseases;
- Parenteral administration is dependent on several other factors such as macromolecules MW, injection site and pathological conditions.

In spite of several challenges associated with invasive route, formulations and delivery strategies have enabled the launch of numerous successful macromolecule-based products as given in Table 1.

Noninvasive routes of administration

Due to several challenges of parenteral routes of administration, scientists have focused on more effective, easier and safer alternative routes of administration of macromolecule drugs. Noninvasive routes such as transdermal [1,3,8,9], pulmonary [1,3,9,10], oral [1,3,9,11], nasal [1,3,6,9,12], vaginal [1,3,9,13], buccal [1,9,14], sublingual [15], rectal [3,9,16] and ocular [1,3,9,17,18] are considered as painless and effective methods of macromolecular delivery. The drug delivery via the nonparenteral route is highly appealing owing to their noninvasive nature. However, presence of several barriers associated with nonparenteral routes led to poor absorption of macromolecules (Figure 2). The advantages and limitations of various noninvasive routes of macromolecule administration are elaborated in Table 2.

Formulation development of macromolecule

Therapeutic potential and clinical application of mac-

Table 2. Noninvasive administration routes of macromolecular administration: advantages and limitations.

Delivery system routes	Advantages	Limitations
Transdermal delivery [1,3,8,9]	• Painless and sustained delivery. • Allows active control & discontinuation of delivery. • Large surface area (1–2 m^2) for drug absorption. • Reduced systemic side effects. • Avoidance of first-pass effect. • Potential for improved patient compliance due to flexibility of altering the typical dosing schedule.	• Low bioavailability. • Limited to low MW hydrophobic drugs. • Relatively impermeable to large hydrophilic molecules. • Variability in dosing. • Delivery dependent on the MW, physicochemical properties and susceptibility to metabolism by skin enzymes.
Pulmonary delivery [1,3,9,10]	• Ease of use. • Rapid systemic uptake. • Large surface area (100–140 m^2) for drug absorption. • Avoidance of harsh conditions in the GI tract as well as first-pass metabolism. • High bioavailability and permeability.	• Potential for local toxicity and immunogenicity. • Limited delivery efficiency and short duration of action. • Some devices are bulky and expensive. • Variation in drug absorption due to age, and respiratory tract infection. • Physiological factors (e.g., breathing pattern) and properties of macromolecules (e.g., MW, lipophilicity) affect the delivery. • Protective mucus layer covering the airway epithelium acting as a barrier to macromolecular absorption. • Therapeutic molecules are subject to *in vivo* mucocilliary and macrophage clearance as well as degradation enzymes.
Oral delivery [1,3,9,11]	• Easy and convenient. • High patient compliance. • Easily accessible route. • Absorption enhancers can improve the oral delivery of macromolecules.	• Macromolecules are susceptible to harsh conditions in the GI tract (acid and proteolytic enzyme dependent degradation). • Limited permeation across intestinal epithelia. • Variable rate of absorption. • Electrostatic repulsion between the negatively charged protein and mucus layer creating a diffusion barrier, thus, poor absorption and low bioavailability (approximately <2%). • Presence of food may affect the absorption. • Presystemic elimination in the liver and gut.
Nasal delivery [1,3,6,9,12]	• Large absorptive surface area (approximately 150cm^2) for drug absorption. • Noninvasiveness and ease of administration. • Highly vascularized and permeable mucosal surface. • No first-pass metabolism.	• Mucosal and enzymatic barriers. • Physical barrier of the nasal epithelium hinders absorption of large hydrophilic proteins and peptides. • Rapid clearance, and low residence time. • Low and variable bioavailability. • Drug degradation by proteolytic enzymes. • Nasal irritation. • Mucociliary clearance. • Variable and inconsistent absorption. • Relatively small amount and volume can be administered.

MW: Molecular weight.

Table 2. Noninvasive administration routes of macromolecular administration: advantages and limitations (cont.).

Delivery system routes	Advantages	Limitations
Vaginal delivery [1,3,9,13]	• Noninvasive and ease of administration.	• Enzymatic/pH dependent degradation in vagina.
	• Higher bioavailability due to rich blood supply and large surface area of the vagina.	• Variable absorption.
	• Bypasses the first-pass metabolism.	• Personal hygiene, gender specificity, local irritation and influence of sexual intercourse alter the vaginal formulation.
	• High permeability for low MW drugs.	• Vaginal leakage is an issue.
		• Protective vaginal mucus layer limits the absorption of drug.
Buccal delivery [1,9,14]	• Formulation can be retained for a longer time.	• Biocompatibility of the drug/device and device/environment interfaces.
	• Convenient dosing, easy removal.	• Low bioavailability.
	• Avoidance of first-pass metabolism.	• Acidity and protease activity in the GI tract causing degradation.
	• Higher tolerance in comparison with the nasal mucosa and skin.	• Formulations need to exhibit suitable rheological properties, high spreadability and prolonged residence.
		• Taste liability.
Sublingual delivery [15]	• Convenient dosing.	• Limited in dose and volume.
	• Bypasses the first-pass metabolism.	• Clearance by saliva.
	• Drug stability can be retained due to the neutral pH of saliva.	• Local toxicity.
	• More robust mucosa.	• Taste liability.
	• Several dosage form options (film, spray, tablet, patch, etc.).	• May lose some part of the drug dose if swallowed.
Rectal delivery [3,9,16]	• Avoids local enzymatic degradation.	• Local adverse reactions.
	• Higher systemic bioavailability with absorption enhancers.	• Low and variable levels of absorption.
	• Controlled absorption.	• Local irritation.
	• Absorption enhancement in the rectal environment.	• Low bioavailability (approximately 10–20%).
	• Large dose can be administered.	• Limited absorption due to limited surface area.
		• Drug metabolism in micro-organisms and rectal mucosa.
		• Patient non-compliance.
Ocular delivery [1,3,9,17]	• Rapid rate of systemic absorption.	• Low bioavailability, local irritation.
	• Bypasses the first-pass effect.	• Patient noncompliance.
	• Convenient dosing, easy access.	• Limited dose, dose-volume capacity.
	• Various routes of ocular administration of drugs.	• Large size of macromolecule limits their diffusion through ocular tissue barriers.
		• Ocular tissue enzymes may degrade the macromolecules.

MW: Molecular weight.

Table 3. Macromolecule (protein/peptide) delivery systems: advantages and limitations.

Delivery systems	Advantages	Limitations
MPs and NPs [1,3]	• Controlled and long-term drug releases are possible with various routes of administration.	• Burst release may lead to potential toxicity.
	• Small size allows enhanced permeation into various organs.	• Nonspecific uptake in RES system and phagocytic clearance.
	• Greater flexibility of surface modification by ligand molecules.	• Biocompatibility, safety, stability and immunogenicity issues.
	• Encapsulation and delivery of multiple drugs in a single NC.	• Polymer can alter drug release and stability.
	• Adjustable physicochemical properties (size, shape, surface functionality).	• Size, shape, surface properties of carriers can determine release behavior, stability and targeting efficiency.
	• Higher possibility of stimuli sensitive delivery.	• Scale-up of nanoformulations.
	• Targeted delivery system.	• Small size and large surface area may lead to particle aggregation.
		• Nonuniform size distribution.
		• Polymers hydrophobicity and acidic environment by polymer degradation lead to protein denaturation/ aggregation.
		• Chemical reactions between macromolecules and polymers.
NFs [19]	• Variety of possible geometries and mechanical properties.	• Organic solvents used in the electrospinning process may be toxic.
	• Allows sustained and long-term bioactivity.	• The physical and chemical stability of these systems has not yet been thoroughly investigated. Thus, poses an additional challenge in long-term biologic development.
	• Macromolecules can be incorporated in the polymeric matrix or immobilized on the surface of the NFs.	
	• Polymeric nature of macromolecules makes it spinnable, thus enabling the formation of NFs.	
LPSs [3,20]	• Versatility of surface chemical modification and specific targeting.	• Instability in biological media.
	• Delivery to CNS through blood–brain barrier due to lipophilic nature of liposomes.	• Phagocytic uptake.
	• Entrapment of hydrophilic and hydrophobic drugs to aqueous and lipid phases, respectively.	• Liposomeal formation development can cause instability of macromolecules.
	• Can provide a sustained and controlled release.	• Manufacturing cost, scale up, batch-to-batch reproducibility.
	• Drug release can be controlled, depending on the bilayers number and composition.	• Productions of sterile liposomes are expensive.
	• Possibility of stimuli sensitive delivery system.	• Interactions of phospholipids with protein drugs.
	• Higher biocompatibility and nonimmunogenicity.	• Heterogeneous particle size distribution.
SLNs [3,20,21]	• Large-scale production.	• Complexity of the physical state of the lipid.
	• Small size, large surface area, high drug loading.	• Phagocytic uptake and clearance.
	• Improved drug stability.	• Lipid particle growth and tendency to gelation.

ARCS: Archaeosome; LPS: Liposomes; MP: Microparticle; NC: Nanocarrier; NF: Nanofiber; NMC: Nanomicelle; NP: Nanoparticle; SLN: Solid lipid NP.

Table 3. Macromolecule (protein/peptide) delivery systems: advantages and limitations (cont.).

Delivery systems	Advantages	Limitations
	• Avoidance of organic solvents in the production may reduce the stability problems.	• Low drug loading capacity due to the lipid crystal matrix formation.
	• Potential of carrying both lipophilic and hydrophilic drugs.	
	• Excellent biocompatibility.	
Dendrimers [22]	• Can be tailored by manipulating the structure/composition or surface functional groups.	• Complexity of formulation development.
	• Thermodynamically stable system.	• Toxicological issues limiting clinical application.
	• Uniform size distribution.	• Dendrimers structure core is difficult to access as the complexity of the system increases with multiple generation structures.
	• Drug molecules can be loaded both in the interior as well as attached to the surface groups.	
	• High transfection efficiency not only due to well-defined shape, but may also be caused by the amine functionality.	
Hydrogels [23–26]	• Porous nature of hydrogels can be finely tuned to allow for drug loading.	• High water content and soft nature of hydrogels typically results in relatively rapid release of proteins from the gel matrix.
	• Pharmacokinetic properties for release of the loaded therapeutic molecule can be easily adjusted to the requirements.	• Low mechanical strength and short durability.
	• Higher biocompatibility due to the high water content and soft nature.	• Stability of hydrogels is low in most cases which represent a major limitation.
	• Unlike other delivery systems, organic solvents are not required in preparation. This is beneficial in preserving protein stability, as very mild conditions (aqueous environment, room temperature) are normally required.	• Low tensile strength of many hydrogels limits their use in load-bearing applications and can result in the premature dissolution or removal of the hydrogel from a targeted local site.
	• Proteins have a limited mobility in the hydrogel network, which is favorable for preservation of their fragile 3D structure.	• Quantity and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs.
	• Soft and hydrophilic nature and mild preparation conditions are well-suited to enhance the efficacy, reduce dosing interval, which provide a more convenient dosage administration of large and labile protein.	•Sometimes, hydrogels are not sufficiently deformable for injection, necessitating surgical implantation.
	• Hydrogels can conform to the shape on the applied surface.	• Each of the above issues significantly restricts the use of hydrogel-based drug delivery therapies in the clinic.
	• Stimuli sensitive hydrogel delivery is feasible.	
NMCs [27,28]	• Suitable for intravenous administration.	• Toxicity and immunogenicity.
	• Easy and reproducible formulation process.	• Lack of suitable formulation methods for scale-up.
	• Easy sterilization by simple filtration process.	• Formulation instability.
	• High biocompatibility, biodegradability and the multiplicity of functional groups.	• Low cellular uptake and tissue accumulation.
	• Possibilities of different polymer block arrangements based on the requirements.	• Self-assembled polymeric micelles are not stable and may dissociate upon dilution. However lipid-core micelles demonstrate high stability, biocompatibility and prolonged blood circulation time.

ARCS: Archaeosome; LPS: Liposomes; MP: Microparticle; NC: Nanocarrier; NF: Nanofiber; NMC: Nanomicelle; NP: Nanoparticle; SLN: Solid lipid NP.

Table 3. Macromolecule (protein/peptide) delivery systems: advantages and limitations (cont.).

Delivery systems	Advantages	Limitations
	• Hydrophobic core serves as a solubilization depot for drugs with poor aqueous solubility.	• Instability in the physiological environment.
	• Hydrophilic shell limits the opsonin adsorption, which contributes toward a longer blood circulation time.	• Nanomicelles are liable to dissociate, especially upon administration when they are diluted to a concentration below the critical micelle concentration.
	•Small size of polymeric micelles provides longer blood circulation time by evading scavenging by the MPS system in the liver and bypasses the filtration of interendothelial cells in the spleen.	• Limitations in entrapping hydrophilic small as well as macromolecule drugs.
	•Longer circulation time leads to improved accumulation at tissue sites with vascular abnormalities.	
ARCSs [29,30]	• Suitable for oral delivery of macromoecuels.	• Uptake of archaeosomes by phagocytic cells can be up to 50-fold greater than that of conventional liposome.
	• Stability at high temperature, pH, pressure and oxidative degradation.	
	• The archaeal lipids are more stable than phospholipids used in liposomes preparation.	
	• Due to high thermostability archaeosomes formulations can be sterilized by autoclaving.	
	• Specific organ targeting.	
Composite nanoformulations (NPs-in-gel) (cs-NFs) [31–33]	• Minimizes the burst effect (dose dumping) of nanoformulations which may result in severe dose related toxicity.	• NPs can be suspended in the gel at the time of delivery, otherwise drug will be released from the NPs and accumulate in the gel which could give burst effect. Therefore, this novel approach requires dual chamber mixing device.
	• Exhibit nearly zero order release for longer time period with no or minimal burst effect.	• Storage at cool temperature.
	• Provides stable environment for macromolecules against enzyme.	
Cellular carriers (erythrocytes) [34–37]	• Biodegradable and nonimmunogenic.	• Long-term storage is difficult.
	• Longer circulation half-life in comparison to the synthetic carriers.	• Liable to biological contamination due to the origin of the blood and the equipment used.
	• Considerable protection against the toxic effects of the encapsulated drug.	• Rigorous controls are required for the collection and handling.
	• Possibility of targeted drug delivery to the RES system organs.	• Risk of rejection if immunogenic species are not removed during fabrication steps.
	• Possibility of ideal zero-order kinetics of drug release.	• Restricted space of activity within blood.
		• Leakage of encapsulated drug.
		• Therapeutic molecules may alter the physiology of the erythrocyte.

ARCS: Archaeosome; LPS: Liposomes; MP: Microparticle; NC: Nanocarrier; NF: Nanofiber; NMC: Nanomicelle; NP: Nanoparticle; SLN: Solid lipid NP.

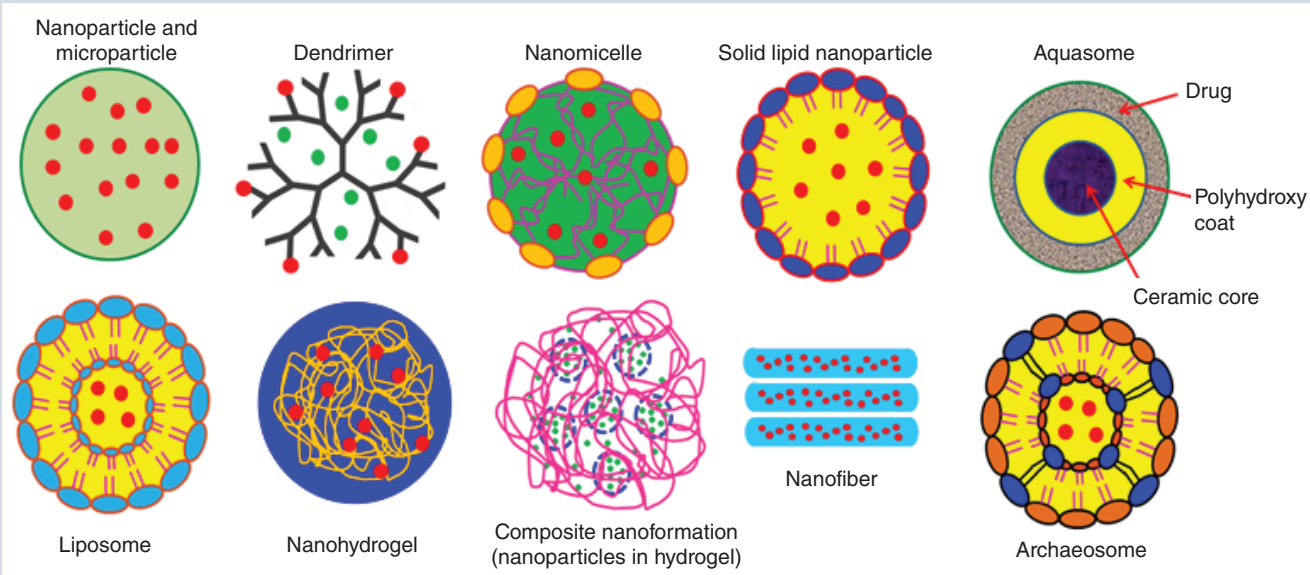

Figure 3. Different types of macromolecule formulation systems.

romolecule drugs is frequently hampered by various obstacles in their successful delivery. Nanotechnology-based drug delivery systems have demonstrated great promise in pharmaceutical applications and can enhance the macromolecule therapeutic efficacy by:

- Enhancing the stability by preserving the macromolecules from denaturation or degradation in biological fluids;
- Controlled/sustained or tunable release profile by optimizing the MW and polymer used, thus, minimizing the burst release effect of macromolecule drugs;
- Improving the biodistribution by enhancing systemic circulation half-life of macromolecules;
- Tissue targeting *in vivo* by receptor-mediated targeting or due to small size of NCs, thus improving the safety and efficacy of macromolecules;
- Enhancing the bioavailability by encapsulating and protecting the macromolecules from harsh GI environment (enzymatic and pH degradation) and by enhancing tissue uptake.

There are various delivery systems designed for the delivery of macromolecule therapeutics. The advantages and limitations of different types of delivery systems are shown in Table 3.

The role of nanotechnology in macromolecule formulation development

Nanotechnology-based delivery systems are one of the most studied colloidal systems and offered exciting therapeutic options for macromolecule delivery [21,38–42]. Because of the small size and use of biodegradable materials in formulation development, NCs offer various advantages such as targeted delivery and improve bioavailability, biocompatible in nature, sustain/controlled drug release profile, protection of therapeutic agents against enzymatic degradation and under harsh pH conditions, potential to combine diagnosis and therapy in one system. These all advantages associated with NC systems are useful to overcome the challenges associated with other dosage forms and delivery vehicles of macromolecules. A summary of different types of NC formulation systems is illustrated in Figure 3. The brief description of these carrier systems is also provided below.

Polymeric nanoparticles & microparticles

Nanoparticles (NPs) are classified as particle dispersions or solid particles with a nanoscale size range of about 10–1000 nm. Therapeutic entity is dissolved, encapsulated or chemically conjugated to the system. Depending on the specific method of preparation, either nanoconstructs can be formulated as NPs, nanospheres or nanocapsules. On a similar note, microparticle systems are drug delivery systems having the micrometer size range of about 1 to 1000 microns. The ability of NPs and MPs to improve oral bioavailability of macromolecule drugs by encapsulating and protecting them from harsh GI environment (enzymatic and pH degradation) makes them a promising carrier system for oral delivery. Encapsulations of macromolecules in these carriers

also control their release and enhance their absorption. In addition, their physicochemical properties can be optimized by changing the MW and composition of the polymers used. Both, NPs and MPs have also been extensively studied in stimuli-sensitive drug delivery applications of macromolecule [43] and small molecule drugs [44,45], however; further explanation of these applications is beyond the scope of this review.

Dendrimers

Dendrimers are characterized by highly branched and star shaped polymeric systems in the nanosize range [22]. These constructs are available with terminal end groups of amine, hydroxyl or carboxyl functionality. These functional groups may be utilized to conjugate targeting moieties or therapeutic molecules. The highly branched structure of dendrimers allows them to incorporate a wide variety of therapeutic (hydrophilic or hydrophobic) molecules. Due to their unique structure, as compared with other polymeric delivery systems, dendrimers exhibit improved physicochemical properties including monodispersity in size distribution, and higher biocompatibility [22].

Polymeric nanomicelles

Polymeric nanomicelles are self-assembled structure from biodegradable and biocompatible amphiphilic block polymers in the nanoscale size range of around 10–100 nm [27,28]. Owing to their small size, micelles can selectively leave the circulation at the tumor site via the enhanced permeability and retention effect. Their amphiphilic structure allows them to carry hydrophobic drugs, prolongs circulatory half-life and thus, an enhanced therapeutic efficacy.

Liposomes

Liposomes are lipid vesicles with phospholipid bilayers enclosing an aqueous core in the size range from 0.1 to 10 μm. Based on the size and lipid bilayers, liposomes can be classified as small unilamellar, large unilamellar and multilamellar vesicles. Because of their high versatility of surface chemical modification, specific targeting and potential of encapsulating both the hydrophilic and hydrophobic drugs, liposomes have been extensively studied in various drug delivery applications [3,20].

Solid lipid NPs

Solid lipid NPs are colloidal systems (average size of 40–1000 nm) like nanoemulsions. However, a liquid-lipid incorporated in emulsions is replaced by a lipid-solid in solid lipid NPs [3,20,21]. These systems provide several advantages and avoid the limitations of other colloidal carriers such as NPs and MPs as given in Table 3 [3,20,21].

Niosomes

Niosomes are novel hydrated vesicular systems composed of nonionic surfactants with cholesterol or other lipids and the enclosed interior usually contains a buffer solution at appropriate pH [46,47]. Niosomes can deliver the hydrophilic and lipophilic drugs to their targeted site and they are nontoxic, require less production cost and have higher chemical stability over a longer period of time in different conditions. Like liposomes, niosomes can be unilamellar or multilamellar. However, further investigation of the toxicity of niosomes after *in vivo* administration has not been performed and needed for their extensive drug delivery applications.

Aquasomes

Aquasomes have shown immense potential as carriers capable of preserving the structural integrity of protein pharmaceuticals [40,48]. These are three-layered (core, coating and drug) self-assembled delivery systems where the ceramic core surface is noncovalently modified with a carbohydrate (cellulose, sucrose, trehalose, etc.). The system is then exposed for adsorption of therapeutic molecules. The solid core provides the structural stability, while the carbohydrate coating protects the therapeutic molecules. However, an in-depth pharmacokinetics, toxicology and animal studies of aquasomes is required to validate their safety, efficacy and other parameters to confirm their efficiency for clinical applications.

Archaeosomes

Archaeosomes are based on a lipid-based delivery system in the size range of 20–1000 nm and are made of polar lipid fraction E extracted from *Sulfolobus acidocaldarius* [29,30]. They are made of archaeobacterial membrane lipids containing diether and/or tetraether lipids. Archaeosomes are biodegradable, not toxic *in vivo* and have been used for the oral delivery of macromolecules [29].

Electrospun nanofibers

Electrospinning is one of the most efficient techniques for the production of polymeric nanofibers (NFs) in nanoscale size range [19]. NFs exhibit special properties due to their high drug loading efficiency, surface area to volume ratio and porosity compared with conventional fibers and other delivery systems such as liposomes, NPs, micelles, etc [19]. Such unique properties of NFs make them suitable for a wide range of applications. Based on the polymer and electrospinning apparatus (monoaxial, coaxial or triaxial) used, several modifications in the NF geometry and mechanical properties can be achieved to develop a controlled, fast or stimuli-sensitive NF drug release system.

Table 4. Application of nanocarriers system in macromolecule (protein and peptide) delivery.

Carrier system	Active molecule	Carrier material	Comments	Ref.
NPs	Insulin	CS	The insulin-loaded CS-NPs induced a prolonged hypoglycemic effect compared with the control samples. The developed system could be a promising alternative to the systemic delivery of macromolecules to the lungs, in addition to provide a local effect.	[52]
	Immunogenic W-1 L19 peptide	PLGA	W-1 L19 peptide-loaded PLGA NPs were successfully phagocytized by macrophages and increased NO production (twofolds) in contrast to free W-1 L19 peptide, and threefolds higher compared with control. Based on the results, these NPs can be a potential vaccine candidate against Canine Parvovirus.	[53]
	Insulin	PLGA	The *in vitro* results showed that NPs were capable to overcome the gastrointestinal barrier and appropriate for oral delivery of insulin.	[54]
	Renin substrate I (RSI) peptide	CS	The CS-NPs formulations displayed about 100% of encapsulation efficacy, low burst release effects, and a linear release of the model RSI peptide.	[55]
	PSC-RANTES	PLGA	Results showed that that the PSC-RANTES was readily encapsulated into PLGA-NPs, retain its anti-HIV-1 activity and delivered to the target site.	[56]
MPs	Salmon calcitonin, Urokinase and Rituximab	Poly(itaconic acid-co-N-vinyl-2-pyrrolidone) (P(IA-co-NVP))	The hydrogel-MPs offer ideal loading and release behavior, showed no degradative release of encapsulated salmon calcitonin in gastric conditions. However, a rapid and almost 100% release of encapsulated protein within 1 h was observed in intestinal conditions.	[57]
	Octreotide	Triblock (TB: PCL_{10k}-PEG_{2k}-PCL_{10k}) and pentablock (PB: PLA_{3k}-PCL_{7k}-PEG_{2k}-PCL_{7k}-PLA_{3k} and PBB: PGA_{3k}-PCL_{7k}-PEG_{2k}-PCL_{7k}-PGA_{3k}) polymers	A significant fraction of released octreotide was acylated from PBA (53%) and PBB (92%) polymers. Complete release of octreotide was achieved from TB polymer over a period of 3 months with minimal acylation of peptide. Polymers having PLA and PGA blocks may not be appropriate for peptide delivery due to the observance of acylation and incomplete release.	[58]
	IGF-1	CS	The MPs showed a significant decrease in IGF-1 encapsulation efficiency, and cumulative release during the 2-week period.	[59]
	Peptide salmon calcitonin (sCT) coprocessed with alpha-1-antitrypsin (AAT), a model protein	ACC	The bioactivity of the sCT, coprocessed with AAT was maintained during MPs formulation and had excellent aerodynamic properties. The bioavailability of sCT after aerosol delivery as sCT and AAT-loaded composite MPs to rats was four-times higher than that of sCT solution. These all making the MPs a suitable system for pulmonary aerosol delivery.	[60]
LPSs	Vasoactive intestinal peptide	PEG grafted to distearoylphosphatidyl-ethanolamine	It was reported that a single intratracheal or subcutaneous administration of vasoactive intestinal peptide self-associated with sterically stabilized liposomes (VIP-SSL) normalized the mean arterial pressure in spontaneously hypertensive hamsters.	[61]
	FITC-labeled ovalbumin	Hydrogenated phosphatidylcholine from soya bean and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine	This work reported a stable double emulsion carrier, demonstrated an enhanced protection and release of encapsulated antigen containing PEGylated liposomes for oral vaccine delivery	[62]
SLNs	Salmon calcitonin (sCT) (peptide)	PEG2000-Stearate	SLN modified with peptide ligand CSKSSDYQC or a cell penetrating peptide IRQRRRR, reported to be the potential carriers for the transport of protein drugs across intestinal barriers to improve its oral bioavailability.	[63]

ACC: Amorphous calcium carbonate; ARCS: Archaeosome; AQS: Aquasome; BSA: Bovine serum albumin; CS: Chitosan; CS-NF: Composite nanoformulation hADSC: Human adipose-derived stromal cell; HSA: Human serum albumin; LPS: Liposomes; MP: Microparticle; NF: Nanofiber; NIS: Niosome; NMC: Nanomicelle; NP: Nanoparticle; OVA: Ovalbumin; PB: Pentablock; PEO: poly(ethylene-oxide); PLCL: poly(L-lactide-co-caprolactone); PLFE: Polar lipid fraction E; PLGA: Poly(lactic-co-glycolic acid); SLN: Solid lipid NP; TPL: Total polar lipid.

Table 4. Application of nanocarriers system in macromolecule (protein and peptide) delivery (cont.).

Carrier system	Active molecule	Carrier material	Comments	Ref.
	Octapeptide LSCQLYQR (LRp)		LRp loaded SLN, has been identified to reduce the growth of cis-platinum (cDDP) resistant ovarian carcinoma cells by inhibiting the monomer–monomer interface of the human enzyme thymidylate synthase. The cell cycle analysis by the propidium iodide test showed that SLNs peptide treated cancer cells elevated the apoptosis percentage indicating that SLNs were able to efficiently carry the peptide until its enzymatic target.	[64]
	rhEGF		The topical administration of rhEGF for wound healing with SLN-rhEGF and NLC (Nano structured lipid-carrier)-rhEGF significantly improved healing in terms of wound closure, restoration of the inflammatory process, and re-epithelization grade. The effectiveness of rhEGF-loaded lipid nanoparticles has been studied in a full thickness wound model in db/db mice.	[65]
	Thymopentin and insulin	Glyceryl palmitostearate, glyceryl tripalmitate and cetyl palmitate	This strategy demonstrated a promising approach with the outstanding encapsulation efficiency, low burst release and relatively high pharmacological availability.	[66]
NF	Antimicrobial peptide, LL-37	PEO	Results demonstrated the electrospinning conditions did not significantly affect the antimicrobial activity of LL-37. NFs effectively delivered antimicrobial peptides locally.	[67]
	Heparin and VEGF	PLCL	The release of both heparin and VEGF from the NF was observed for more than 1 month with no significant burst release. The degradation rate of NFs containing heparin and VEGF was faster than that of pure PLCL-based NFs.	[68]
	FITC-conjugated bovine serum albumin (FITC-BSA)	PEG as core material and poly(epsilon-caprolactone) (PCL) as shell material.	The core-shell PCL-r-FITC-BSA/PEG NFs pronouncedly alleviated the initial burst release and showed better sustainability compared with that of PCL/FITC-BSA/PEG NFs for tissue engineering applications.	[69]
AQS	Insulin	Calcium phosphate dihydrate core coated with various disaccharides (cellobiose, trehalose and pyridoxal-5-phosphate).	Pyridoxal-5-phosphate-coated AQSs were found to be more effective in reducing blood glucose levels than AQSs coated with trehalose or cellobiose. The prolonged activity of AQSs was attributed to slow release and preserved structural integrity of the peptide.	[70]
	OVA Ag	Calcium phosphate dihydrate core coated with trehalose.	Results showed that OVA adsorbed AQSs were able to induce a strong T-cell specific proliferative response, prevention of anaphylactic reactions and maintenance of low titers of IgE. Overall, AQSs shown to have possible application in peptide-based vaccines against allergic disorders.	[71]
ARCS	Insulin	PLFE extracted from sulfolobus acidocaldarius	It was observed that the ARCSs had little effect on the transport of insulin across the Caco-2 cell monolayers. However, ARCSs were superior in reducing the blood glucose levels in diabetic mice, compared with conventional liposomes. The modest hypoglycemic effect was due to the poor permeability of intestinal epithelium after oral administration.	[29]
	OVA Ag	TPL obtained from the archaea	Results indicated that ARCSs are versatile, potentially universal, vaccine delivery adjuvants.	[72]
NIS	BSA	Cholesterol (CH) to sorbitan monostearate (Span 60) in different molar ratio.	The results showed that the release profile of model protein can be controlled by the CH percentage. High EE% and sustained release of BSA in NISs was observed which showed their potential in drug delivery and tissue engineering applications.	[73]
	Insulin	Polyoxyethylene alkyl ethers (Brij)	Results indicate that niosomes have potential for sustained release oral dosage forms for delivery of macromolecules. The surfactant type, CH content and charge inclusion altered the %EE, size distribution and drug release rate from NISs. NISs were able to stabilize insulin against enzymatic degradation.	[74]

ACC: Amorphous calcium carbonate; ARCS: Archaeosome; AQS: Aquasome; BSA: Bovine serum albumin; CS: Chitosan; CS-NF: Composite nanoformulation hADSC: Human adipose-derived stromal cell; HSA: Human serum albumin; LPS: Liposomes; MP: Microparticle; NF: Nanofiber; NIS: Niosome; NMC: Nanomicelle; NP: Nanoparticle; OVA: Ovalbumin; PB: Pentablock; PEO: poly(ethylene-oxide); PLCL: poly(L-lactide-co-caprolactone); PLFE: Polar lipid fraction E; PLGA: Poly(lactic-co-glycolic acid); SLN: Solid lipid NP; TPL: Total polar lipid.

Table 4. Application of nanocarriers system in macromolecule (protein and peptide) delivery (cont.).

Carrier system	Active molecule	Carrier material	Comments	Ref.
NMC	Cyclosporine A (peptide)	Hydrogenated castor oil-40 and octoxynol-40	*In vivo* ocular tissue distribution has been studied on Cyclosporine A loaded NMCs formulation.	[75]
	Insulin	PLGA-PEG block copolymer	This study suggested that the PLGA-PEG block copolymers NMCs have been prepared by a new synthetic route are potent nanocarriers for poorly water-soluble drugs as insulin.	[76]
	HSA (protein)	Methoxy poly(ethylene glycol)-poly(β-amino ester) (PEG-PAE)	The rat was intravenously injected with the Cy5.5-labled albumin-encapsulated polymeric micelle. A gradual increase in fluorescence signals of the brain ischemic area was observed, indicating that the pH-tuning positively charged protein-encapsulated polymeric micelle could be effective for targeting the acidic environment and diagnostic imaging.	[77]
Hydro-gel	BSA	Poly(chloromethylstyrene)_PEG_ poly(chloromethyl styrene) (PCMS_ PEG_PCMS) triblock copolymer	Protein-loaded, redox-active, injectable, gel (RIG) has been studied for local protein therapy. Further, *in vivo*, study has been performed by using IL-12 as a model drug in tumor-bearing mice to observe the therapeutic effect. It was reported that the RIG is a promising approach for an injectable sustained-release carrier for proteins to provide a high therapeutic effect while suppressing side effects.	[78]
	Salmon calcitonin, urokinase and rituximab	poly(itaconic acid-co-N-vinyl-2-pyrrolidone) (P(IA-co-NVP))	In this study, high pI proteins of various size, varying hydrogel crosslinking density and different hydrogel particle size, have been utilized to demonstrate tunable material properties for the oral delivery of therapeutic proteins. This work also reported the *in vitro* use of an enzymatically responsive P(MAA-co-NVP) hydrogel with peptide crosslinker enabling rapid and complete release of protein within small intestinal conditions.	[57]
	BSA	PEG and hADSCs	The release of BSA as a model protein was studied to evaluate the effects of varying PEG mesh size and degradation on protein release over 2 months. The release of basic fibroblastic growth factor from an optimized hydrogel formulation over 35 days was also reported and its ability to stimulate cell proliferation has been assessed using hADSCs.	[79]
CS-NF	IgG-Fab	Pentablock polymers	The CS-NFs delivery system was designed to be utilized for the treatment of posterior segment ocular diseases such as age-related (wet) macular degeneration, diabetic retinopathy and diabetic macular edema. The CS-NFs provided a sustained delivery of macromolecules over a longer duration with negligible burst release effect. It may provide minimal side effects associated with frequent intravitreal injections in clinical applications.	[80]
	FITC-BSA, IgG and bevacizumab	PB polymers	The novel PB polymers are evaluated as a platform for sustained delivery of therapeutic proteins. CS-NFs demonstrated no or negligible burst release with continuous near zero-order release in contrast to NPs alone. It was reported that hydrodynamic diameter of protein therapeutics and hydrophobicity of PB copolymer exhibited significant effect on entrapment efficiency and *in vitro* release profile.	[33]
Cell carrier (erythrocytes)-based systems	L-asparaginase	Intact erythrocytes	The proposed method could be applied to the LPSs, NPs or CS-NMCs encapsulation into intact RBCs without any structural or functional damages.	[81]
	OVA	Mouse erythrocytes	The results showed the potential role of RBCs in an Ag-delivery system in future immunotherapy.	[82]

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Hydrogels

Hydrogels are crosslinked networks of hydrophilic polymers and biocompatible materials [26]. These polymers are capable of retaining large amounts of water yet remaining insoluble and maintaining 3D structures. The hydrogels have been studied for a wide range of drug delivery applications [23–26]. The biodegradable nature of hydrogels can be generated by a proper selection of polymers as well as the crosslinking agents. Porous and soft nature and high water content of hydrogels are extremely suitable for higher encapsulation of water soluble drugs including proteins and peptides.

Composite nanoformulations (NPs dispersed in a hydrogel)

Composite nanoformulation term is used for the type of delivery system in which NPs are dispersed in a thermosensitive gel or hydrogel. Such suspended NPs in the gel matrix encounter an additional diffusion barrier which in turn provides the long-term release of therapeutics especially for the macromolecules. Similarly, it minimizes the burst effect, reduces the dose dumping and follows zero order kinetics as reported by several *in vitro* release studies [31–33]. In addition, composite nanoformulations provide stability to macromolecules from enzymatic degradation and helps in improving the biological half-life.

Cellular carriers-based delivery systems

Recent advances in molecular and cellular biology have inspired scientists to model NCs modified with red blood cells (RBCs), platelets and leukocytes mimicking membrane and membrane components [49–51]. Out of these cells, RBCs camouflaged NCs have been studied a lot since RBCs are the most abundant cells in the human body (~5 million RBC/μl of blood), have a unique biconcave discoidal shape and can circulate in the bloodstream for up to 120 days. The unique shape of RBCs provides them a favorable surface area to volume ratio which allows these cells to undergo pronounced deformations while maintaining a constant surface area [34–37]. Meanwhile, RBC-membrane-camouflaged NCs have been synthesized using the ghost RBC membrane vesicle on NCs [50,51]. NCs, mimicking the RBC shape have also been formulated using various methods to prolong the NCs drug circulation time. However, RBC-mediated carrier systems suffer from several drawbacks such as the risk of rejection, immunogenic species need to be removed during fabrication as well as restricted space of activity of RBCs within blood (Table 3). Some of the recent applications of these carrier systems in macromolecule (protein and peptide) delivery are shown in Table 4.

Challenges of nanotechnology approaches in macromolecule drug delivery

Encapsulating drug in NCs prolongs their half-life, protects them from physicochemical degradation, improves site-specific targeting, reduces side effects and enhances therapeutic efficacy [83–85]. However, NCs have several major limitations that impact their targeted delivery. Upon entering the blood circulation, systemically injected NCs are tagged with opsonin proteins through a process called opsonization and subsequently removed by the mononuclear phagocytes system organs (liver and spleen) prior to reaching target organs [7,84,86]. Therefore, engineering a delivery system that is biocompatible and has long drug circulation time is highly desired to enhance the therapeutic efficacy for both, the macromolecule and small molecule drugs.

Several approaches have been discussed regarding avoidance of NC phagocytic clearance [87]. The most widely applied technique is the introduction of PEG molecule on the surface of NCs to reduce the serum protein binding through a process called steric hindrance [88–90]. However, it has been recently found that the use of PEG cannot completely prevent clearance and opsonization and nonspecific clearance remains a great challenging task [91,92]. PEG immunological response and hypersensitivity reactions have also triggered further investigation on the biological relevance and approaches to prevent the phagocytosis of NCs. In addition, desorption/degradation of PEG coating and excess PEG on NCs surface may lower their mobility and flexibility leading to shorter circulation time [91,92]. Beside the surface markers, NC size, shape, surface composition and aspect ratio are the critical parameters determining the opsonization and the reticuloendothelial system interaction with NCs [93–95] (Table 5).

Altering shape away from the spherical has been shown to influence the blood circulation/transport and biodistribution of NCs including enhanced binding and cellular internalization compared with spherical NCs [95,96]. Different approaches including modifications of NCs size, surface, shape and flexibility have been explored to extend their residence time *in vivo* [93,97]. Although, the unique shape of RBC is key to their exceptional morphological properties but, replicating RBCs shape has been extremely challenging. However, recent advances in approaches to particle fabrication have finally circumvented this barrier and produced exquisite replicas of RBC shape [93,97–100]. Few of these methods have been compared in Table 6 in terms of their applicability on producing unique size/shape NCs, process control, scale up and cost–effectiveness [93,97–100].

Table 5. Nanocarriers physicochemical parameters and *in vivo* effect [93–95].

Parameters	Recommendations for longer *in vivo* circulation of nanoformulations
Size	• 10 nm–200 nm particle size range is good for longer *in vivo* circulation. • Kidney allows particles <10 nm to pass through and particles in that range can be cleared rapidly. • Particles (>200 nm), get quickly cleared by the MPS organs (lung, liver and spleen) and often get filtered out by the lungs. Particles <1 μm and >200 nm are filtered out in the spleen, >2–3 μm can clog blood vessels.
Shape	• Nonspherical particles are recommended for longer *in vivo* circulation. • Erythrocyte shape mimicking NC are preferred. • Nonspherical particles with diameter >1 μm, resulting in increased clearance by MPS organs. • Spherical particles are mainly up taken by liver. • Cylindrical particles go mainly to liver and spleen. • Discoidal particles are mainly taken up by lung, liver and spleen.
Mechanical properties	• Soft and elastic particles are good for longer *in vivo* circulation. • Increasing elasticity enhances the properties of NC to avoid clearance by immune system (e.g., erythrocytes are elastic and soft). • MPS organs liver and spleen, have fenestrated endothelia to filter the particles from circulation. Rigid particles with diameters that exceed the cut-off limit of these fenestrations or discontinuation are easily cleared by these organs.
Surface properties	• Hydrophilic surface particles are good for longer *in vivo* circulation. • Opsonin proteins bind to particles mainly via hydrophobic interactions. Therefore, hydrophilic surface of NC is preferred to avoid opsonin binding and opsonization.
Surface charge	• Neutral or anionic surface of particles are recommended for longer *in vivo* circulation. • Positively charged particles more prone to sequestration by macrophages in the lungs, liver and spleen. • Neutral and slightly negatively charged nanoparticles have longer circulation lifetimes and less accumulation in the aforementioned organs of the MPS. • Serum proteins (negatively charged at physiological pH) interact easily with positively charged NC and may be cleared by immune cells.

MPS: Mononuclear phagocytic system; NC: Nanocarrier.

Future prospects of nanotechnology-based delivery system of macromolecule therapeutics

Macromolecule drugs are already proven in various therapeutic areas and have greater impact in the future. However, efforts should be concentrated on noninvasive and intracellular delivery to overcome the problems associated with invasive routes of macromolecule delivery such as opsonization and phagocytic uptake. A long drug circulation time of NCs is highly desired to enhance the therapeutic efficacy of active molecules. Recently, CD47 'marker of self' recognition system has been explored as a key factor toward the long RBC circulation time [101,102]. This self-recognition marker interacts with the inhibitory receptor signal regulatory protein alpha on macrophages and inhibits the phagocytosis of RBCs (Figure 4). Thus, the incorporation of such a 'marker of self' peptide into NCs may improve immune-compatibility *in vivo*. Recently, a smallest sequence of amino acids CD47, a minimal 'self' peptide can resemble and mimic the functions of human CD47 [101,102]. This approach can be applied along with the delivery systems to enhance the circulation time of macromolecule loaded NC in blood. Moreover, the fabrication of such systems can be advantageous to enhancing the protein and peptide-based therapy by:

- Enhancing macromolecule solubility;
- Controlled prolonged release with no or minimal burst effect thus, avoiding undesirable side-effects;
- Inhibit the phagocytic clearance, thus low dose is required with enhanced treatment duration and lower dose frequency;
- Protect the macromolecules from various environmental factors such as pH, temperature, electrolytes;

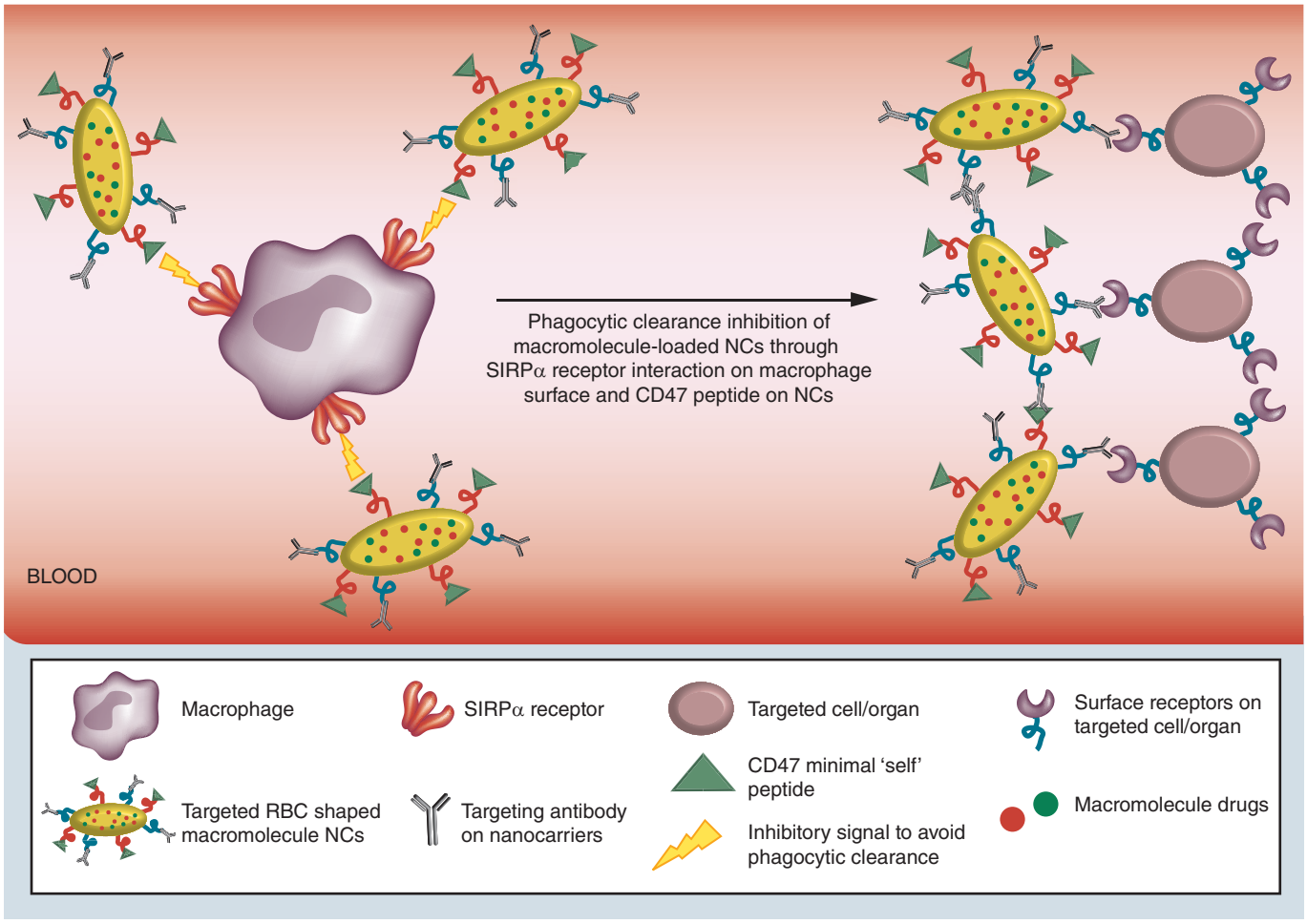

Figure 4. Interaction of self-recognition marker with the inhibitory receptor SIRPα on macrophages inhibiting the phagocytosis of nanocarriers.
NC: Nanocarrier; SIRPα: Signal regulatory protein alpha.

- Improved biodistribution and targeting efficiency;
- Avoiding off-target effect

Design of experiment approach is also a valuable tool for preformulation development and can be employed to optimize the formulation parameters for a small molecule and macromolecule drugs as several factors can be screened to analyze their individual/interactive effects in formulation development as used by several researchers [45,103–105]. Traditional approaches for formulation development involve the time consuming process of varying one factor at a time and examining its effect, which requires a large number of experimental runs. In macromolecule formulation development, characterization techniques may be used to screen a wide range of parameters including buffer ionic strength and types, pH, temperature and presence of other excipients for their potential impact on the thermal, structural, conformational and physicochemical stability of the macromolecules during preformulation steps. These initial screening parameters are important to identify a range of formulation and process parameters in macromolecule formulation development. Thus, formulation development of macromolecule through design of experiment approach may provide a potential way for their efficient delivery.

Concluding remarks

Macromolecules display an increasingly important role as therapeutic agents for the treatment of various diseases. The advantages of nanotechnology approaches in macromolecule formulation development may provide solutions to several problems encountered in their delivery. However, there are several challenges those need to be resolved in their clinical applications. First, macromolecule drug loading in NCs needs to be well controlled to avoid batch-to-batch inconsistency. The drug needs to be released in a controlled manner to maintain their concentrations in therapeutics range and to reduce the frequency of

Table 6. Fabrication techniques on nanocarriers [93,97–100].

Techniques	Shape	Approx. size	Process control, scale-up and cost–effectiveness
Particle film stretching	Multiple shapes	60–100 μm	• Lab-scale adaptability, monodispersed particles can be applied to various polymers. • Cost effective.
Particle replication in nonwetting templates (PRINT®)	Cube, rod, circular, disc, worm, cylinder, multiple other shapes	10–200 μm	• Greater flexibility, residue-free method, no wetting of the surrounding area. • Expensive
Self-assembly process	Cube, rod, circular, disc, cone	Length in few μm, diameter in nm	• High yield process, spherical particles can be produced in great control. • Limited in shape production of nonspherical particles. • Cost effective.
Step-flash imprint lithography	Square, triangle, pentagon	50 nm	• Great control over size and shape, removal of the residual layer exposes the polymer/drugs to a harsh environment. • Costly and time consuming.
Emulsification method methods	Spherical	nm to microns	• Extremely scalable, high yield process, lack precise control over size. • Limited in production of variety of shapes. • High lab scale process adaptability. • Cost effective
Layer-by-layer self-assembly	RBC shaped	7 ± 2 μm	• Low scale up, the core need to be removed which may rupture the capsules. • Difficulty of drug encapsulation since the loading is done after the capsule formation. • Cost effective

treatment. Second, formulation development process has to be simple to enhance the scale-up in NCs production and cost–effectiveness. Third, specific targeting approaches are needed in order to enhance the bioavailability and to avoid nonspecific delivery of macromolecules and thus, unwanted side effects. Fourth, comprehensive and robust characterization methods of NC products should be developed to predict their clinical efficacy and safety profiles. Fifth, safety, stability and biocompatibility of the NC systems are needed to be considered. Finally, one needs to look at the upcoming and future trend of personalized delivery systems capable to target site-specific receptors that will significantly impact drug administration.

Although there are formidable challenges to successful delivery of macromolecule drugs, noninvasive especially the oral delivery routes are more appealing in terms of patient preference and compliance. In general, the development of protein and peptide-based therapeutics is an exciting area of research. We are hoping that there will be one common system in the future that can be used for the invasive and noninvasive delivery with a high systemic stability of a variety of macromolecule drugs.

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Executive summary

Macromolecule (protein/peptide) drug delivery

- The therapeutic potential and clinical application of macromolecules is hampered by various obstacles including their large size, short *in vivo* half-life, phagocytic clearance, poor membrane permeability and structural instability.
- Several challenges of delivering macromolecule drugs.

Route of administration for macromolecule (protein/peptide) drugs

- Noninvasive and invasive routes of macromolecule delivery are limited by the presence of several barriers.
- Limitations related to parenteral (invasive) routes of macromolecule administration.
- A list of clinically approved macromolecule formulations delivered via invasive route.
- The advantages and limitations of various noninvasive routes of macromolecule administration.

Formulation development of macromolecule (protein/peptide)

- Nanotechnology-based delivery systems have demonstrated great promise in pharmaceutical applications and can enhance the macromolecule therapeutic efficacy by enhancing stability, providing controlled/sustained or tunable release profile, minimizing the burst release effect, improving the biodistribution and enhancing bioavailability.
- The advantages and limitations of different types of nanotechnology based on macromolecule delivery systems.

The role of nanotechnology in macromolecule (protein/peptide) formulation development

- A summary of different types of nanocarrier (NC) systems and their brief introduction.
- Recent applications of NC systems in macromolecule (protein and peptide) delivery.

Challenges of nanotechnology approaches in macromolecule (protein/peptide) delivery

- A summary of NCs physicochemical parameters and their *in vivo* effects.
- Fabrication techniques on NCs are compared in terms of their applicability on producing different size/shape NCs, process control, scale up and cost–effectiveness.

Future prospects of nanotechnology-based system of macromolecule (protein/peptide) therapeutics

- Efforts should be concentrated on noninvasive and intracellular delivery to overcome the problems associated with invasive routes of macromolecule delivery.
- A long drug circulation time of NCs is highly desired to enhance the therapeutic efficacy of active molecules. Recently, CD47 'marker of self' recognition system has been explored as a key factor toward the long RBC circulation time. Thus, the incorporation of such a 'marker of self' peptide into NCs may improve immune compatibility *in vivo*.
- The formulation development of active molecules through design of experiment approaches may provide a potential way for the efficient delivery of macromolecule drugs.
- The advantages of nanotechnology approaches in macromolecule formulation development may provide solutions to several problems encountered in their delivery. However, there are several challenges those need to be resolved in their clinical applications.

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