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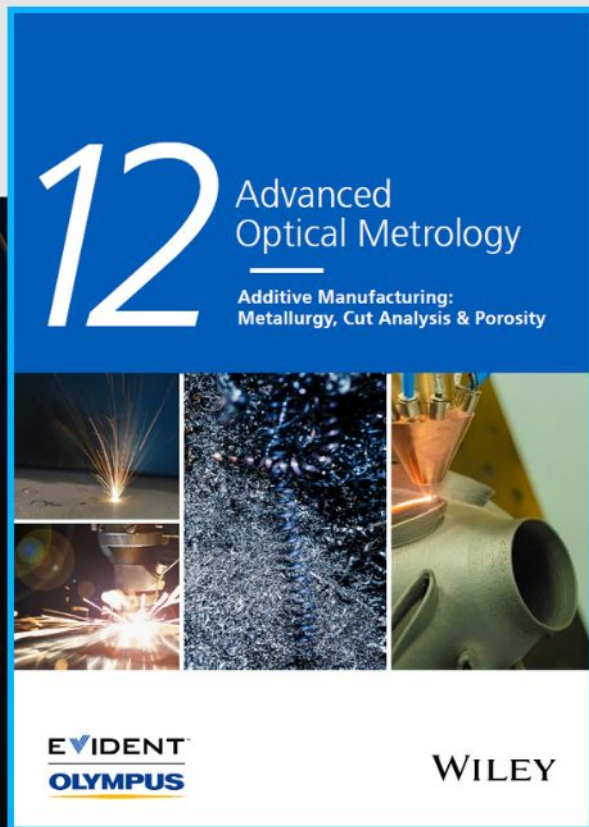
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Colloidal Polyelectrolyte Complexes from Hyaluronic Acid: Preparation and Biomedical Applications

Huu Van Le and Didier Le Cerf*

Hyaluronic acid (HA) is a naturally occurring polysaccharide which has been extensively exploited in biomedical fields owing to its outstanding biocompatibility. Self-assembly of HA and polycations through electrostatic interactions can generate colloidal polyelectrolyte complexes (PECs), which can offer a wide range of applications while being relatively simple to prepare with rapid and “green” processes. The advantages of colloidal HA-based PECs stem from the combined benefits of nanomedicine, green chemistry, and the inherent properties of HA, namely high biocompatibility, biodegradability, and biological targeting capability. Accordingly, colloidal PECs from HA have received increasing attention in the recent years as high-performance materials for biomedical applications. Considering their potential, this review is aimed to provide a comprehensive understanding of colloidal PECs from HA in complex with polycations, from the most fundamental aspects of the preparation process to their various biomedical applications, notably as nanocarriers for delivering small molecule drugs, nucleic acids, peptides, proteins, and bioimaging agents or the construction of multifunctional platforms.

nanocomplexes,^[8,9] nanocomposites,^[10,11] nanoassemblies^[12] and coacervates.^[13,14] Such colloidal systems, especially when prepared from biopolymers, have been extensively investigated due to their promising properties for numerous applications in biomedical fields.^[1] Their merits come from the combination of three aspects: nanomedicine, biomaterials, and green chemistry. With the emergence of nanomedicine, the use of nanocarriers can offer tremendous advantages in drug encapsulation and delivery, namely enhancing the chemical stability of drugs and improving drug solubility and bioavailability.^[15] Due to their small size and the possibility of surface modification with biological ligands or molecular imprinting, nanocarriers can easily cross biological barriers, which have limited pore sizes (fenestrations) under 1 micron in most cases, then target and enter the tissues or cells of

1. Introduction

Polyelectrolyte complexes (PECs), sometimes referred to as polyion complexes, can be spontaneously formed by electrostatic interactions between oppositely charged polyelectrolytes in aqueous media.^[1] Under appropriate preparing conditions and depending on polyelectrolyte natures, such complexes can be elaborated as colloidal particles having submicronic size.^[2,3] Although colloidal PECs are technically comparable to nanogels (NGs) owing to the 3D networks of physically bonded polyelectrolytes in their structures,^[2,4,5] they have also been described with several other terms depending on personal perceptions of researchers, most commonly as nanoparticles (NPs).^[6,7]

interest.^[16,17] Due to these aspects and the possibility of controlling drug distribution and release through particle engineering, nanocarriers can thus reduce systemic side effects and enhance the therapeutic efficacy of drugs.^[15] Nanosystems can also offer unique advantages for biomedical imaging with their ability of sensing, image enhancement, and incorporating concomitantly therapeutic agents for theranostics, that is, simultaneous therapeutic and diagnostic applications.^[18–20] When constructed from biomaterials, especially naturally occurring polysaccharides or proteins, PECs can become significantly biocompatible and biodegradable with much less immunogenicity and toxicity.^[21] Besides such biorelevant aspects, the elaboration of biopolymer-based PECs is also in accordance with green chemistry principles since their preparation process usually requires only gentle mixing of polyelectrolyte solutions at room temperature, which is a simple and rapid procedure without the need for chemical agents, surfactants, organic solvents or high mechanical or thermal energy.^[15]

Beyond the above-mentioned basic advantages, the potential of colloidal PEC systems is increased by the inherent biological and chemical properties of individual constituent polymers. Hyaluronic acid (HA), also called hyaluronan to generally mention both its acid and salt forms, has been one of the most common polyanions among several biopolymers reported so far for the elaboration of colloidal PECs. The significant attention dedicated to HA in biomedical fields is associated with its unique advantages, stemming from not only its outstanding biocompatibility but also interesting biological activities.^[22]

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Consequently, HA has been widely used for elaboration of colloidal PECs with various cationic polymers of different natures: chitosan (CTS) and diethylaminoethyl dextran (DEAE-D) as polysaccharides; poly-L-lysine (PLL) and polyarginine (PAR) as homopolypeptides; zein, protamine (PROT), lactoferrin (LF), whey protein isolate (WPI), feather keratin and bovine serum albumin as proteins; polyethylenimine (PEI) and poly(β -amino esters) (PBAE) as synthetic cationic polymers as well as their derivatives. Although the concept of colloidal PECs from HA has been described in the literature for more than a decade, it was not until 5 years ago did these systems start to be extensively investigated for drug delivery (see **Tables 1** and **2**), while there has been so far no comprehensive review on such specific materials. In this context, the aim of the current review is to summarize the advantages of HA-based colloidal PECs and offer a consolidated overview on the conception and fabrication principles of these systems. Their exploitation with the most recent advancements for biomedical applications, mostly in drug delivery (**Figure 1**), as well as current challenges and perspectives for improving their utility will also be addressed.

2. Advantages of HA as Material for Colloidal PECs

HA is a linear polysaccharide in the form of non-sulfated glycosaminoglycan which comprises repeating two-glycoside units of N-acetyl-D-glucosamine and D-glucuronic acid in its structure (**Figure 1**). It is naturally present in the extracellular matrix of epithelial, neural, and connective tissues of the human body as well as other vertebrates, especially in the umbilical cord, vitreous humor, and synovial fluid.^[67] Given its omnipresence in the human body, HA is extremely biocompatible, biodegradable, and non-immunogenic, rendering it ideal as a material or excipient in biomedical and pharmaceutical domains.^[22] In particular, HA is widely indicated in rheumatology, ophthalmology, or dermatology due to its important biological functions, including tissue moisturizing, angiogenesis, supporting cell migration, and wound healing.^[68] HA has also been known to react with reactive oxygen species (ROS) through the carbons in its glycosidic bonds or the nitrogen atom in its N-acetyl groups,^[69] leading to its potency as a ROS scavenger.^[14,24,69] The protective effects of HA against oxidation stress have also been recently further emphasized with much more complicated mechanisms, which involve numerous cellular signaling pathways.^[70] Commercial HA used to be mostly produced through extraction from animal tissues, mostly from rooster combs.^[67] However, with the current production mainly based on microbial fermentation (e.g., *Streptococcus zooepidemicus*, *Bacillus subtilis*, and *Escherichia coli*) as well as great advancements in extraction and purification methods, HA can be produced on a larger scale, with a larger range of molecular weight (MW), higher purity, lower manufacturing cost, and less environmental pollution.^[71–73]

Containing carboxyl groups (**Figure 1**) with a pKa of around 3, HA can display negative charges in aqueous media and therefore can spontaneously form PECs with positively charged macromolecules.^[74,75] For colloidal PECs with HA in excess, uncomplexed HA should be present on the particle surface to

form an outer shell with a protective effect, which may offer the particles superior properties. For example, due to the cryoprotective effect of HA, such HA shells can improve the stability of PEC particles during lyophilization and reconstitution.^[59,62,76] Indeed, during the lyophilization of PEC particles as well as other nanosystems from biomacromolecules, water molecules are transferred from the liquid phase to the solid phase of ice during the freezing step and then removed by sublimation during the desiccation step, leading to a loss of hydrogen bonds on the surface of these nanoobjects and thus destabilizing their structure.^[77] However, these hydrogen bonds may be preserved by a HA shell on the particle surface as HA is known for its extensive engagement in hydrogen bonding and can form intra- and intermolecular hydrogen bonds on its own.^[78] In addition, the remarkable water binding capacity of HA, its vitrification effect, and its high intrinsic viscosity (i.e., large hydrodynamic volume) allow the HA shell to absorb a great amount of water and maintain them at molecular state in and around the particles to avoid their freezing, constituting thus a thick glassy layer to protect the particles from the external mechanical stress of ice crystals and avoid particle aggregation during cryoconcentration.^[79,80]

Furthermore, HA on the particle surface can significantly enhance PEC biocompatibility, which can be beneficial when polycations are included in their structure. Various polycations (e.g., PEI, PLL, DEAE-D, or CTS) are known to be potentially cytotoxic, which usually depends on exposure time, polymer concentration (C_p), and MW.^[81–83] Such toxicity has been reported to be caused by their interactions with anionic biological structures like cell membranes and their functional proteins, which lead to membrane disruption.^[82] Combining polycations with HA may prevent those adverse interactions and counteract such toxicity due to the exceptional biocompatibility and negative charges of HA. More particularly, HA can enhance the stability of PEC particles in serum as well as reduce their immunogenicity by preventing the adsorption of serum proteins on the particle surface, as known as “protein corona” or opsonization.^[10,12,58,84] Opsonization is one of the most problematic factors which leads to the uptake of nanoparticulate drug delivery systems by the mononuclear phagocytic systems (e.g., macrophages and monocytes) and causes their early elimination from blood circulation before reaching their target sites in vivo.^[85] As these proteins are more likely to bind to highly cationic and/or hydrophobic nanocarriers,^[16,85] the presence of the hydrophilic and negatively charged HA on their surfaces would probably prevent such interactions. Moreover, for the fabrication of nanocarriers from cationic proteins, for example, zein or LF, although they can readily form nanosized particles in water through their inherent hydrophobic interactions, further complexation with HA can result in more compact particles, which can improve drug encapsulation and stability as well as the properties of the complexed proteins.^[6,34,37,38,86,87] Likewise, although HA can also spontaneously form nanocomplexes with cationic drugs like doxorubicin (DOX) or pentamidine for their delivery, further complexation with a polycation should generate better-defined NPs with more useful properties.^[35,88]

The tremendous interest in HA for biomedical applications is also associated with its unique biological behaviors, such

Table 1. Research on colloidal PECs from HA for small molecule drug delivery (references sorted in order of recentness).

Polyanion	Polycation	Drugs	Additives	Studied applications	Publication year	Ref.
PEO-PPO-grafted HA	DEAE-D or PLL	CUR	–	Drug encapsulation	2022	[23]
HA	Zein	Tetrahydrocurcumin	–	UVB-induced skin photoaging treatment in vitro	2022	[24]
HA	BSA	Ibuprofen or picolinic acid	–	Drug encapsulation and controlled release	2021	[25]
HA + pectin	CTS	Lidocaine	–	Drug encapsulation and controlled release	2021	[26]
Thiolated HA	CTS	DOX	–	pH/redox-responsive drug delivery, breast cancer treatment in vitro	2021	[27]
HA	CTS	DNICs	–	Cardiovascular disease treatment in vitro	2020	[28]
HA	PLL	Berberine	–	Wound healing in vitro	2020	[2]
HA	Zein	Quercetin	PEI-coated PLGA NPs	Implantable multiparticle gel assemblies; anti-inflammatory therapy in vitro	2020	[29]
HA	PAR	Cisplatin	–	Ovarian cancer treatment in vitro and in vivo through intraperitoneal aerosolization	2020	[30]
HA	CTS	Tocopherol or cholecalciferol	TPP (optional)	Drug encapsulation	2020	[31]
HA	Feather keratin	DOX	–	pH/redox-responsive drug delivery, colorectal carcinoma treatment in vitro	2020	[32]
HA	Zein	Honokiol	–	Breast cancer treatment in vitro and in vivo	2020	[6]
HA	Deoxycholic acid-grafted CTS	DOX	TPP	Cancer treatment in vitro	2019	[33]
HA	LF	EGCG	–	Encapsulation and antioxidant activity enhancement of EGCG	2019	[34]
HA	PAR	Pentamidine isethionate	–	Lung cancer and breast cancer in vitro	2019	[35]
HA	PLL	DOX or vancomycin	–	DOX: lung cancer and multidrug resistant ovarian cancer treatment in vitro; vancomycin: antimicrobial activity in vitro	2019	[36]
HA	Zein	Quercetagenin	–	Drug encapsulation for oral delivery	2018–2019	[37,38]
HA	Zein	CUR	–	Drug encapsulation for oral delivery	2018	[39]
HIS-grafted HA	HIS-grafted PEI	DOX	Gellan gum	Chemoembolization	2018	[40]
HA	Zein	CUR	–	Colorectal carcinoma treatment in vitro and in vivo	2018	[4]
HA	Thiolated PLL	Chlorin e6	–	pH/redox-responsive drug delivery, breast cancer treatment in vitro	2018	[41]
HA	Lactose-modified CTS	Dexamethasone or fluoresceinamine	–	Drug encapsulation and controlled release, ROS scavenging	2018	[14]
HA	CTS	Mitoxantrone	Methoxy PEG	Improving particle stability and pharmacokinetics in vivo	2018	[7]
HA	CTS	Butyrate	TPP	ROS scavenging	2017	[42]
HA	CTS	Mitoxantrone + verapamil	–	Multidrug resistant breast cancer treatment in vitro	2017	[43]
HA	CTS	CUR	PEG	Glioma treatment in vitro and in vivo	2017	[44]
HA	CTS	CUR	PEG + LF	Glioma treatment in vitro and in vivo	2017	[45]
Gemcitabine-grafted HA	Pt(IV)-grafted CTS	Gemcitabine + Pt(IV)	–	Lung cancer treatment in vitro and in vivo	2017	[46]
Docetaxel-grafted HA	CTS	Docetaxel	–	Breast cancer treatment in vitro	2016	[47]
HA	CTS	Tenofovir	Zn(II)	HIV treatment in vitro	2016	[48]
HIS-grafted HA	HIS-grafted PEI	DOX	–	pH-responsive drug delivery, melanoma treatment in vitro	2015	[49]
HA	CTS	CUR	–	Glioma treatment in vitro	2015	[50]
HA	Galactosylated CTS	DOX	–	pH-responsive drug delivery, liver cancer treatment in vitro	2015	[51]

BSA: Bovine serum albumin, CTS: Chitosan; CUR: Curcumin; DEAE-D: Diethylaminoethyl dextran; DNIC: Dinitrosyl iron complex; DOX: Doxorubicin; EGCG: Epigallocatechin gallate; HA: Hyaluronic acid; HIS: Histidine; HIV: Human immunodeficiency virus; LF: Lactoferrin; PAR: Polyarginine; PEG: Polyethylene glycol; PEI: Polyethylenimine; PEO-PPO: Poly(ethylene oxide)-co-poly(propylene oxide); PLGA: Poly(D,L-lactide-co-glycolide); PLL: Poly-L-lysine; ROS: Reactive oxygen species; TPP: Tripolyphosphate; UVB: Ultraviolet B.

Table 2. Research on colloidal PECs from HA for delivering nucleic acids (NAs) (references sorted in order of recentness).

Polyanion	Polycation	Cargo	Additives	Studied applications	Publication year	Ref.
DOP-grafted HA	PBAE	DNA	–	Cell transfection	2021	[52]
HA	PROT	siRNA + Pt(IV)	Polyglutamic acid	Pt-resistant lung cancer treatment in vitro and in vivo	2021	[53]
HA	Imidazole-grafted PLL	miRNA	–	Triple-negative breast cancer treatment in vitro	2020	[54]
HA	Trimethyl CTS	siRNA	–	Breast cancer, colon cancer, and melanoma treatment in vitro and in vivo	2020	[55]
TCA-grafted HA	PROT	siRNA	–	Colorectal liver metastasis treatment in vitro and in vivo	2019	[56]
HA	PROT	siRNA + EGCG	Cell-penetrating peptide	Drug-resistant triple-negative breast cancer treatment in vitro and in vivo	2018	[57]
HA	PEI-grafted CTS	pDNA	–	Cell transfection	2018	[58]
HA	CTS	pDNA	–	Liver cancer treatment in vitro and in vivo	2017	[59]
HA	CDX-grafted PLL	oligoRNA + DOX	–	Hepatocellular carcinoma treatment in vitro and in vivo	2017	[60]
HA	CTS	miRNA + DOX	TPP	Triple-negative breast cancer treatment in vitro and in vivo	2014	[61]
HA	PEI	pDNA	–	Melanoma treatment in vitro and in vivo	2010	[62]
HA	CTS	pDNA	–	Cell transfection	2009	[63]
HA	PAR	siRNA	–	Melanoma treatment in vitro and in vivo	2009	[64]
HA	PEI	pDNA	–	Cell transfection	2009	[10]
HA	CTS	pDNA	TPP	Cell transfection	2008	[65]
ODN-grafted HA	PROT	ODN	–	Cell transfection	2007	[66]

CDX: Cyclodextrin; CTS: Chitosan; DOP: Dopamine; DOX: Doxorubicin; EGCG: Epigallocatechin gallate; HA: Hyaluronic acid; miRNA: Micro ribonucleic acid; ODN: Oligodeoxynucleotide; PAR: Polyarginine; PBAE: Poly(β -amino esters); pDNA: Plasmid deoxyribonucleic acid; PEI: Polyethylenimine; PLL: Poly-L-lysine; PROT: Protamine; siRNA: Small interfering ribonucleic acid; TCA: Taurocholic acid; TPP: Tripolyphosphate.

as its capacity to recognize CD44 receptors and its specific cleavage by hyaluronidase (HAase).^[57] CD44, the principal cell surface receptor for HA, is overexpressed in various types of cancer cells, namely breast cancer, lung cancer, brain cancer, prostate cancer, pancreatic cancer, and colon cancer.^[89] Likewise, HAase is a family of enzymes catalyzing HA hydrolysis and is also highly expressed at tumor sites, especially in intracellular vesicles of tumor cells.^[90] Such particular distribution

of CD44 and HAase can offer a possibility of drug targeting for oncotherapy, since HA on the particle surface can facilitate their specific uptake by tumor cells through CD44-mediated endocytosis and subsequent particle degradation by HAase for intracellular drug release, which can ultimately enhance drug performance at a local site (Figure 2).^[12,56,57,60,64,91–94] In the same manner, the CD44 targeting effect of HA also allows the fabrication of nanoplatform for cancer bioimaging for diagnosis

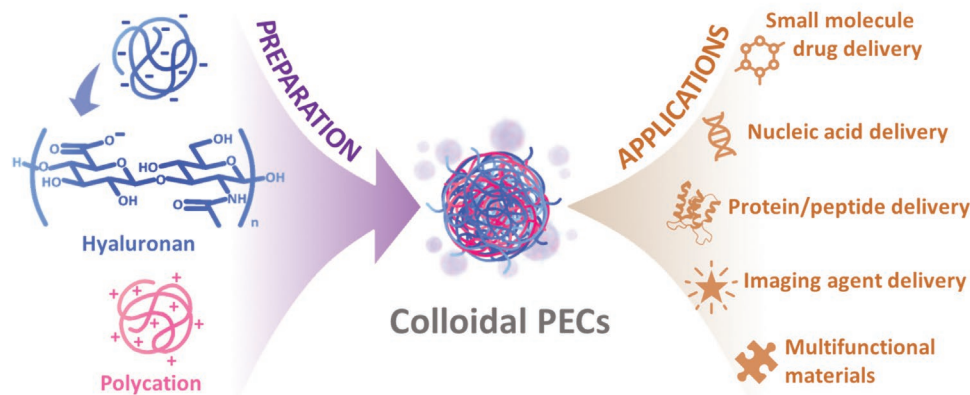


Figure 1. Applications of colloidal PECs prepared from HA and polycations.

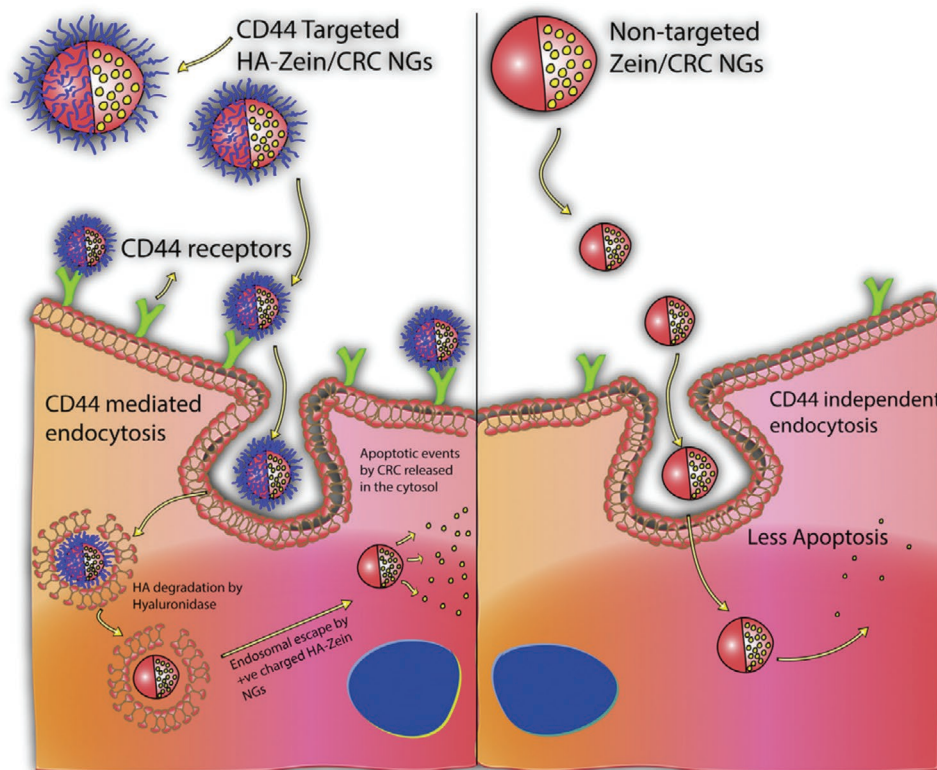


Figure 2. Specific cellular uptake through CD44-mediated endocytosis of the anticancer curcumin (CRC)-loaded HA/zein PEC NGs before their degradation by intracellular HAase for drug release in tumor cells (left) versus non-specific cellular uptake of NGs without HA in normal cells (right). Reproduced with permission.^[4] Copyright 2018, Elsevier.

or theranostics.^[19,20] Furthermore, HA can act synergistically with other biopolymers, namely CTS, in order to improve PEC mucoadhesiveness and offer more advantages for oral or nasal administration.^[26,95,96]

HA can also be chemically modified owing to the ubiquitous distribution of carboxyl, hydroxyl, and acetamido groups across the chain structure (Figure 1), leading to the perspectives of elaborating PECs with more interesting physicochemical and biological properties from HA derivatives.^[97] For instance, the introduction of hydrophobic groups like phospholipids^[98] or alkyl chains^[99] can generate amphiphilic HA derivatives for better encapsulation of hydrophobic molecules. HA functionalization with short chains of amphiphilic and thermoresponsive copolymers can also allow fabricating colloidal PECs with thermo-dependent characteristics and better stability.^[23] PECs prepared from thiolated HA (HA-SH) can be further stabilized by self-crosslinking upon disulfide bond formation to generate redox-responsive systems,^[27] while histidine (HIS) grafted on both HA and the associating polycations can result in PECs with pH-sensitive behaviors.^[49] Bioactive ingredients, including chemotherapeutic agents,^[46,47] nucleic acids (NAs),^[66] and peptide antigens,^[100] can also be directly grafted on HA in PECs in order to improve their therapeutic or biological performance. PECs from HA grafted with functional moieties like bile acids,^[56] dopamine (DOP)^[52] or epigallocatechin gallate (EGCG)^[3] can also offer better stability of macromolecule cargos during their delivery. These examples will be further

described in their applications (Section 4). Collectively, the advantages of HA-based colloidal PECs lie in their material and methodological aspects as well as their final applications, which are summarized in Figure 3.

3. Preparation of Colloidal PECs from HA

From the above-mentioned advantages, HA should be among the most potential polyanions of choice to elaborate colloidal PECs for biomedical applications. Before the preparation, the properties of PEC particles should be understood in order to fabricate PEC systems with specifically pertinent properties for intended applications. To that end, the following sections will explain the formation mechanism of colloidal PECs, how their final characteristics should be anticipated for intended applications, and the effects of various parameters during PEC elaboration on their characteristics.

3.1. Formation Mechanism of Colloidal PECs

The general mechanism of PEC formation from two water-soluble polyelectrolytes is illustrated in Figure 4. Complexation between oppositely charged polyelectrolytes can take place autonomously in aqueous media with an increase in entropy upon the release of counterions and water molecules as the

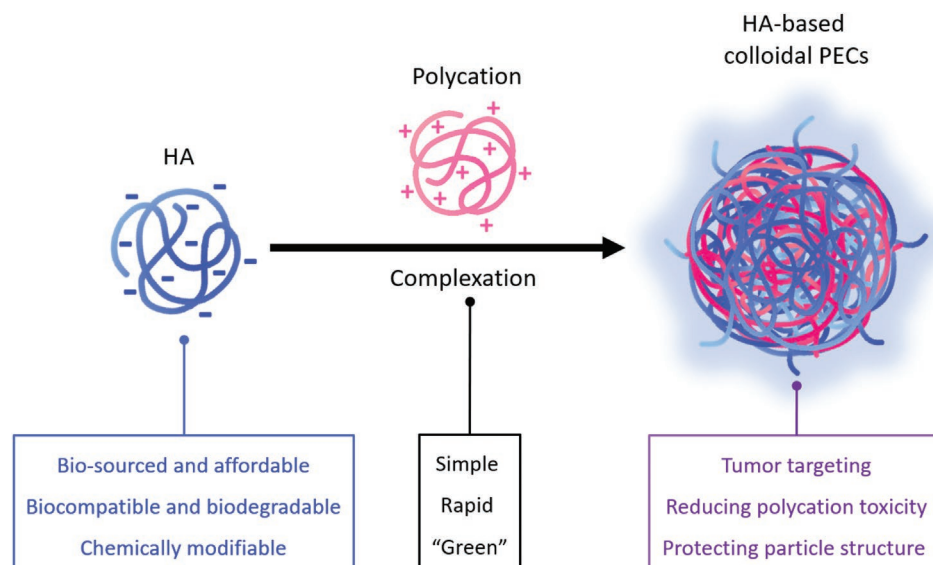


Figure 3. Main interests of colloidal PECs from HA in biomedical fields.

main driving force.^[101] The interactions involved in the formation of PECs are mainly electrostatic interactions (Coulomb force). The primary structures of PECs, which are formed at the first step of electrostatic association, can be classified as “scrambled egg” model or “ladder-like” model according to relative arrangement of polymer chains (Figure 4).^[102] However, PEC structures in practice may not strictly follow either of such models but rather a combination of both, with the former being closer to the reality.^[103,104] Double strain segments in these primary PECs are electrostatically neutralized, thus relatively less hydrophilic and can segregate to form secondary PECs, involving a combination of hydrophobic interactions, electrostatic interactions, hydrogen bonding, and van der Waals force.^[102,103,105,106]

As long as the complexation parameters are appropriately controlled, which will be discussed in Section 3.3, final PECs can be formed as colloidal particles with a core-shell structure,

that is, a neutral PEC core surrounded by a highly charged and more hydrophilic shell of uncomplexed polyelectrolyte segments.^[25,108] More specifically, for colloidal PECs from HA and positively charged proteins with strong intramolecular hydrophobic interactions, for example, in HA/zein PECs, the final particles should have a highly hydrophobic innermost core composed of mainly proteins, followed by a layer of HA/zein PECs in the strict sense and an outermost layer consisting of mainly uncomplexed HA (Figure 5).^[37,109]

3.2. Conception of Colloidal PECs from HA

Final characteristics of biomedical nanocarriers should be envisaged depending on intended applications. Among the physicochemical characteristics of colloidal systems, particle size is of utmost importance for their performance in vivo.

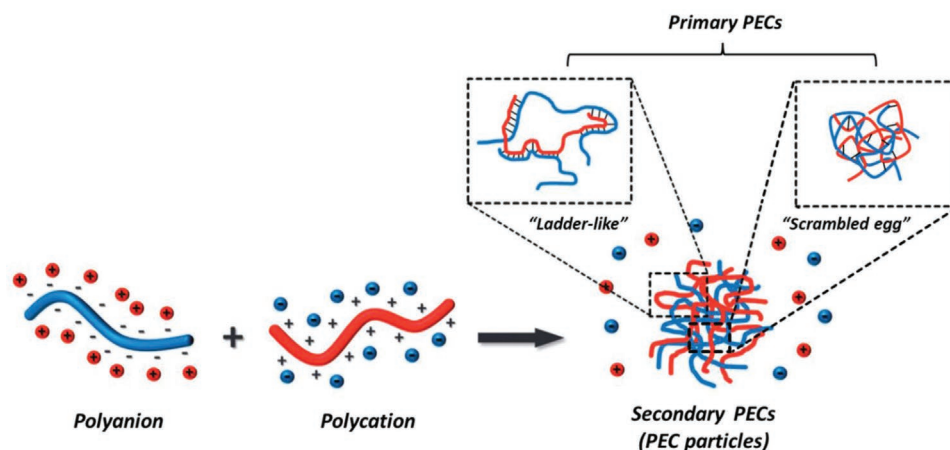


Figure 4. Mechanism of PEC formation from two water-soluble polyelectrolytes (redraw from ref. [107]): primary PECs can display “ladder-like” structures (orderly ion-pairing) and/or “scrambled egg” structures (random ion-pairing). The segregation of neutralized segments in primary PECs can lead to secondary PECs in the form of PEC particles. Counterions are liberated from PECs as the electrostatic complexation occurs.

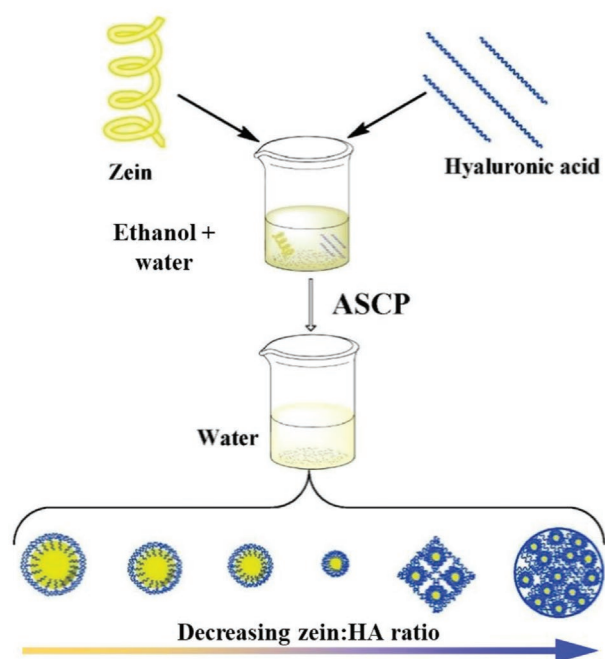


Figure 5. Schematic illustration of colloidal HA/zein PECs prepared by antisolvent coprecipitation (ASCP) method and their final structures in line with decreasing zein:HA ratio. Adapted with permission.^[37] Copyright 2019, Elsevier.

The particle size should be small enough to i) be transported through biological barriers since they usually have limited opening or pore sizes (fenestrations), which can largely vary between 1 and 1000 nm depending on tissue type and pathological condition,^[110] ii) avoid being recognized and cleared by monocytes and macrophages and iii) be uptaken by target cells if intracellular drug delivery is necessary.^[16] A particle size of 100–200 nm may facilitate particle transport through the gastrointestinal tract or the blood-brain barrier.^[111] For nanocarriers targeting tumor cells, which have been the most common subject of research in nanomedicine, the ideal particle size is reported to be around 70–200 nm to ensure tumor penetration and cellular uptake.^[110,112] A too large size (e.g., >500 nm) may facilitate particle clearance by monocytes and macrophages and/or prevent the uptake of these particles by the target tumor cells.^[112,113] Meanwhile, particles with a too small size (e.g., under 20 nm) are more prone to rapid renal clearance,^[16,110] or cannot offer enough energy to bend cell membranes and enter the target cells.^[85,111] In addition, reduced particle size leads to a larger total surface area, which may cause faster and less controlled drug release.^[114] In terms of in vitro stability, for colloidal systems in general, a highly large particle size are prone to sedimentation while smaller particles are more prone to flocculation or agglomeration due to high total surface energy.^[115] In the specific case of HA-based PECs, there has been so far no thorough study on the effects of their particle size on their in vitro stability and in vivo behaviors. In general, most of the colloidal PECs from HA studied for biomedical applications in the literature have their average particle size in the range of 100–400 nm with a polydispersity index (PDI) under 0.3 to ensure its relative homogeneity.^[2,4,7,29,35,36,43,116] These character-

istics are most commonly evaluated by dynamic light scattering (DLS) and can be further confirmed by transmission (TEM) or scanning (SEM) electron microscopy.^[74,105]

Surface charge is also an important characteristic of nanocarriers for in vivo applications. As mentioned in Section 2 above, particles having a negative net surface charge are more biocompatible than positively charged particles as they are less likely to electrostatically interact with the negatively charged cell membranes.^[54,117,118] The presence of the negatively charged HA on PEC surface can therefore enhance their biocompatibility. Umerska et al. found a threefold higher IC_{50} of CTS in cationic HA/CTS PEC NPs (zeta potential of +20 mV) compared to free CTS ($IC_{50} = 84 \mu\text{g mL}^{-1}$ versus $26 \mu\text{g mL}^{-1}$), whereas anionic HA/CTS PEC NPs (zeta potential of –19 mV) showed no cytotoxicity on the whole range of CTS concentration studied.^[117] However, due to electrostatic repulsion, anionic nanocarriers may be less ready to cross biological barriers (e.g., mucous membranes) and be internalized by targeted cells as compared to their cationic counterparts.^[85] Meanwhile, highly cationic NPs have been known to be more rapidly cleared from in vivo circulation by monocytes and macrophages than highly anionic NPs, while NPs with neutral or slight negative charges would show a more prolonged circulation.^[116] The net charge on the particle surface is represented by zeta potential, which can be measured by electrophoretic light scattering (ELS).^[119] Technically, nanocarriers with zeta potentials between –10 and +10 mV are regarded as approximatively neutral, while a zeta potential less than –30 mV or higher than +30 mV indicates a highly anionic or cationic surface, respectively.^[119] In terms of colloidal stability in vitro, it is a matter of common consensus that the absolute value of zeta potential should be greater than 30 mV to ensure sufficient electrostatic stabilization and avoid phase separation, unless steric stabilization is applied.^[120] Due to the advantages of HA discussed in Section 2, most of the HA-based colloidal PECs reported in the literature have HA in excess on the particle surface, thus a negative zeta potential with a common range between –10 and –50 mV.^[2,4,7,29,35,36,43,116]

Hydrophobicity is another important aspect. Stiff and hydrophobic NPs are more prone to opsonization and elimination by the mononuclear phagocytic system.^[16,85] The most classical approach to overcome this problem is coating the particle surface with a hydrophilic polymer like polyethylene glycol (PEG),^[85] but this may not be necessary for PECs having HA on the surfaces since HA can offer the same advantage with a high hydrophilicity as discussed in Section 2. The common problem of HA-based PECs is a too hydrophilic structure, as they are formed mainly through electrostatic interactions and hydrogen bonding of hydrophilic polymers. Highly hydrophilic nanocarriers may show low encapsulation and fast release of drugs due to large pore size,^[121] as well as poor tissue penetration and cellular uptake as many biological barriers are basically hydrophobic.^[122] This explains the emergence of using HA derivatives with amphiphilic or hydrophobic grafts for improving the performance of PECs as earlier described (Section 2).

Particle geometry can also affect the in vivo behaviors of nanocarriers. Discoidal particles are known to have unique tumbling and margination dynamics in blood circulation, which favor more vessel wall interaction than spherical particles, as well as improved particle binding and endothelium

adhesion.^[16,123,124] Particles with greater curvature are more likely to be uptake by macrophages.^[16] Filamentous particles may also show long-circulating lifetimes compared with spherical counterparts.^[125] The morphology of HA-based colloidal PECs in the literature is commonly verified by TEM or SEM and presents mostly spherical or poorly defined shapes,^[36,43,50] with no attempt so far to control this parameter or study its effects.

Taken together, depending on the intended applications, especially the administration route and the targeted tissues or cells, the desired properties of final colloidal PECs should be anticipated before their fabrication. In particular, a target range should be set for quantifiable characteristics like particle size and zeta potential to ensure both PEC stability and performance. In order to obtain such desired characteristics, it is imperative to understand their structure and investigate the impact of different parameters on their properties during the preparation process, which are described in the next sections.

3.3. Parameters Impacting the Formation of Colloidal PECs

The sections below give a detailed description of parameters having influence on PEC characteristics, including material parameters (MW and charge density of polyelectrolytes), formulation parameters (total polyelectrolyte concentration, charge ratio, pH, ionic strength, and adjuvant ions), and technical parameters (addition mode, addition order, stirring settings and complexation temperature). It should be remembered that all of these parameters act interdependently, either synergistically or contradictorily to govern PEC properties. The resulting system is therefore a compromise between these factors and in no case should they be considered separately.

3.3.1. Polyelectrolyte MW

HA used for fabricating colloidal PECs has a common MW between 20 and 1000 kDa (Table 3). As a general trend, an increase in MW of either HA or the complexing polycations can foster electrostatic complexation and thus reinforce the stability of PECs.^[126] Kayitmazer et al. found an enhancement of coacervation between CTS (260 kDa) and HA when the MW of HA is increased from 50 to 750 kDa, which was evidenced by a decrease in pH_{ϕ} (critical pH above which PEC particles are formed) from 2.7 to 2.2 (Figure 6B).^[74] Such results were attributed to less entropy loss when HA of higher MW is transferred from diluted to concentrated regime.^[74] Logically, HA with a too low MW may be unable to form stable PECs with polycations, as shown with HA of 29 kDa in HA/PAR PECs reported by Oyarzun-Ampuero et al. (ref. [127]—Table 3). Increasing polymer MW can lead to larger particle size of PECs with larger distribution, or even aggregation at macroscopic level (precipitation or macrogelation) if their MW is too high (i.e., above 1000 kDa), as shown in Table 3 (refs. [2,39,43,128,129]). However, a MW slightly under 1000 kDa is still susceptible to cause macro-aggregation if the MWs of both the complexing polymers are comparable, for example, HA of 830 kDa in complex with DEAE-D of 700 kDa (ref. [105]—Table 3). Likewise, a

MW higher than 1000 kDa can still form homogenous colloidal PECs when the partner polyelectrolyte has a much smaller MW, for example, HA/zein PECs with HA MW of 2000 kDa and zein MW of 22 kDa show a particle size of nearly 300 nm (ref. [39]—Table 3).

Specifically, the effect of HA MW should be more obvious when HA is the main polyelectrolyte in PECs.^[102] For instance, an increase in MW of HA from 270 to 830 kDa can cause an important increase in particle size (1.6-fold) and polydispersity index (PDI) (twofold) of HA/DEAE-D PECs having negative to positive charge ratio ($n-/n+$) of 10, while only small increases by around 1.2-fold are observed with $n-/n+ = 0.1$, that is, with HA being the minor polymer.^[105] Similar observation was also reported when comparing HA/CTS PECs having HA:CTS ratio of 1:2 *m/m* and 2:1 *m/m* (ref. [43]—Table 3). Figure 7 depicts the general effects of polyelectrolyte MW on PEC structure, as well as those of charge density, C_p , charge ratio, and ionic strength, which are described in the next sections.

Regarding surface charges, higher MW of HA may cause more steric hindrance for their compaction in the PEC core and thus more exposure of their charges on PEC surface, leading to a lower zeta potential.^[9,43,127,130] This can be observed from the decreasing trend of zeta potential when HA MW is increased in HA/PLL, HA/zein, HA/PROT, and HA/CTS PECs (refs. [2,39,43,128] in Table 3, respectively). As a result, for negative particles, such decrease in zeta potential may lead to better colloidal stability due to stronger electrostatic stabilization. In an experiment regarding lyophilization of plasmid deoxyribonucleic acid (pDNA)-loaded HA/CTS PEC NPs, Sato et al. observed large increases in particle size after freeze-drying and rehydrating PEC NPs with a HA MW of 400 kDa (from 340 to 1200 nm) or 600 kDa (from 320 to 710 nm), while a HA MW of 1300 kDa resulted in less particle size increase (from 470 to 670 nm).^[59] In this work, the high exposure of the high MW HA on the PEC surface may also explain the increase of 4–5-fold in cellular uptake efficiency when the HA MW is increased from 400 kDa to 1300 kDa, probably due to better interaction between HA and CD44 receptors.

3.3.2. Charge Density

The effect of charge density on HA-based colloidal PECs is ascribed for both the absolute charge density on each polyelectrolyte and the relative arrangement between their charges. In principle, when the charge densities of polyelectrolytes in PECs increase and/or become comparable to each other, their electrostatic attraction should be stronger and subsequently increase phase separation, from microscopic (increased PEC particle yield or particle size) to macroscopic level (precipitation) (Figure 7).^[74,104,131] For PECs from HA, the effects of charge density are usually studied in the case of HA/CTS PECs. Unlike the natural HA which has a fixed distribution of carboxyl groups, amine groups on CTS result from the deacetylation of natural chitins, which leads to CTS of different grades in terms of degree of deacetylation (DD).^[126] CTS with a high DD has therefore a high number of amine groups. As a result, an increase in DD, or a decrease in degree of acetylation (DA) in other words, can lead to a higher charge density and allow better coacervation

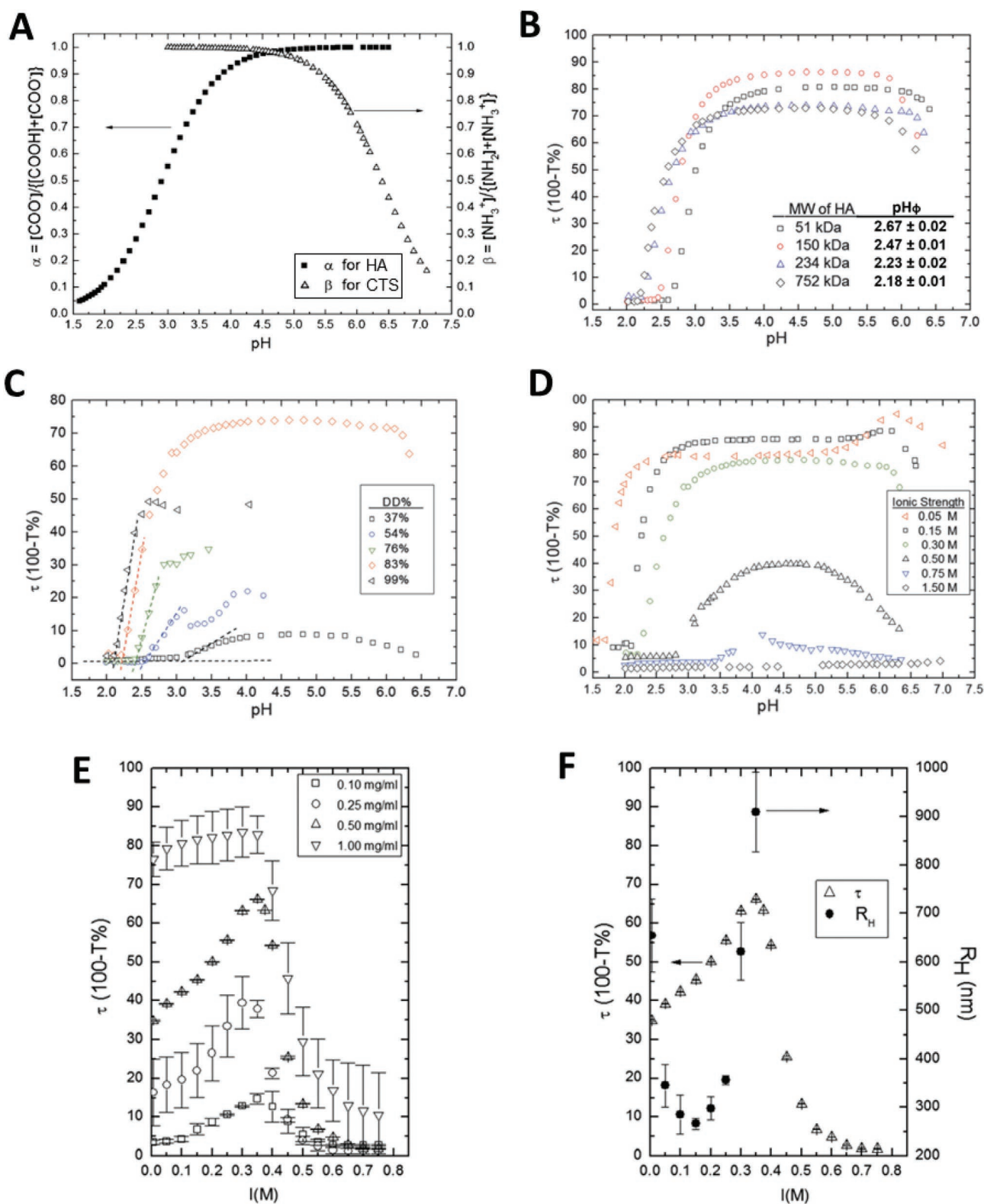


Figure 6. Characterization of colloidal HA/CTS PECs in varying conditions (Adapted with permission.^[74] Copyright 2015, Royal Society of Chemistry.): A) ionization rate of HA (α) and CTS (β) as a function of pH. B-D) Turbidity, i.e., $\tau(100-T\%)$, as a function of pH with variation of B) MW of HA, C) degree of deacetylation (DD) of CTS, and D) ionic strength represented as NaCl concentration. E, F) Turbidity and radius of hydration (R_H) as a function of ionic strength (I) at E) different polymer concentrations (C_p) or F) C_p fixed at 0.5 g L^{-1} . Standard parameters are $\text{MW}_{\text{HA}} = 234 \text{ kDa}$, $\text{MW}_{\text{CTS}} = 260 \text{ kDa}$ with $\text{DD} = 83\%$, $C_p = 0.5 \text{ g L}^{-1}$, HA:CTS ratio of 1:1 m/m , $I = 0.3 \text{ M}$, and $\text{pH} = 3$. pH_ϕ denotes the pH above which the turbidity is abruptly increased, which translates the formation of PEC particles as HA is sufficiently ionized.

Table 3. Notable results on HA-based PEC characteristics with different polymer ratios, concentrations, and molecular weights.

System	−/+	C _p [g L ^{−1}]	MW _{HA} [kDa]	MW _{PC} [kDa]	Average D [nm]	PDI	ζ [mV]	Ref.	
HA/PAR	5:6 <i>m/m</i>	0.5	29	5–15		Not formed		[127]	
			165		128 ± 8	0.15 ± 0.05	+31.3 ± 1.0		
HA/PLL	2:3 <i>n/n</i>	1.25	200	4.7	200 ± 5	0.16 ± 0.02	−38.8 ± 0.1	[2]	
			700		207 ± 9	0.21 ± 0.02	−40.9 ± 2.5		
			1200		278 ± 6	0.27 ± 0.18	−44.2 ± 2.2		
		1.8	200		255 ± 28	0.47 ± 0.06	−51.1 ± 2.6		
			700		244 ± 16	0.43 ± 0.05	1.2 ± 0.1		
			1200		Aggregation				
	5:6 <i>n/n</i>	2	200	245 ± 4	0.09 ± 0.02	−38.8 ± 0.5			
			700	280 ± 9	0.26 ± 0.01	−55.4 ± 2.8			
			1200	Aggregation					
	HA/zein	1:5 <i>m/m</i>	4	100	22	180–190	ns	−32 to −38	[39]
1000				235–245		ns	−30 to −36		
2000				285–295		ns	−30 to −34		
HA/DEAE-D	0.1 <i>n/n</i>	0.5	270	700	300–350	0.19–0.23	+45 to +55	[105]	
	0.8 <i>n/n</i>				160–200	0.06–0.1	+30 to +33		
	1 <i>n/n</i>				Aggregation				
	1.25 <i>n/n</i>				160–170	0.11–0.14	−29 to −35		
	10 <i>n/n</i>				300–320	0.33–0.44	−46 to −50		
	0.1 <i>n/n</i>	1			770–830	0.75–0.87	ns		
	0.8 <i>n/n</i>				190–240	0.09–0.14	ns		
	1.25 <i>n/n</i>				200–220	0.14–0.15	ns		
	10 <i>n/n</i>				570–650	0.41–0.53	ns		
	0.1 <i>n/n</i>	0.5	830		Aggregation				
	0.8 <i>n/n</i>				Aggregation				
	1.25 <i>n/n</i>				Aggregation				
	10 <i>n/n</i>				Aggregation				
HA/PROT	3:1 <i>m/m</i>	1	176	5.1	70–130	0.10–0.13	−35 to −45	[128]	
			257		70–130	0.16–0.19	−55 to −65		
			590		60–90	0.25–0.30	−85 to −105		
			2900		Aggregation				
			2		257	110–170	0.1–0.2	−70 to −80	
HA/CTS	1:2 <i>m/m</i>	2	10	100	280–320	ns	+27 to +33	[43]	
DA 14%			50		250–300	ns	+30 to +35		
			120		400–450	ns	+30 to +35		
			500		450–550	ns	+20 to +37		
	2:1 <i>m/m</i>		10		300–350	ns	−15 to −25		
			50		380–320	ns	−10 to −30		
			120		200–220	ns	−18 to −20		
			500		950–1050	ns	−35 to −45		
	HA/CTS	1:3 <i>n/n</i>	2	39	90	450–470	ns	ns	[126]
DA 28%	1:2.5 <i>n/n</i>			400–420	ns	ns			
	1:2 <i>n/n</i>			320–340	ns	ns			
	1:1.5 <i>n/n</i>			240–260	ns	ns			
HA/CTS	0.05 <i>n/n</i>	1	120	50	Not formed			[50]	

Table 3. Continued.

System	−/+	C_p [g L ^{−1}]	MW _{HA} [kDa]	MW _{PC} [kDa]	Average D [nm]	PDI	ζ [mV]	Ref.
DA 10%	0.12 <i>n/n</i>					Not formed		
	0.2 <i>n/n</i>				166 ± 1	ns	24.7 ± 0.3	
	0.31 <i>n/n</i>				113 ± 4	ns	26.4 ± 1.3	
	0.47 <i>n/n</i>				130 ± 2	ns	23.2 ± 0.7	
	0.71 <i>n/n</i>				220 ± 3	ns	20.2 ± 0.9	
	1.1 <i>n/n</i>				>1000	ns	−7.9 ± 1.6	
	1.89 <i>n/n</i>				>1000	ns	−11.6 ± 3.0	
	4.25 <i>n/n</i>				358 ± 11	ns	−18.7 ± 2.4	
	6.61 <i>n/n</i>				264 ± 7	ns	−21.1 ± 0.9	
	13.69 <i>n/n</i>				238 ± 4	ns	−21.8 ± 0.3	
HA/CTS	1:2 <i>m/m</i>	0.7	90	90	200–250	0.16–0.20	+25 to +30	[129]
DA 63%					170	200–250	0.14–0.18	+18 to +22
					310	400–650	0.17–0.35	+22 to +30

−/+ : Polyanion/polycation ratio, in weight (*m/m*) or in mol of charges (*n/n*); C_p : Total polymer concentration; CTS: Chitosan; D: Diameter; DA: Degree of acetylation of CTS; DEAE-D: Diethylaminoethyl dextran; HA: Hyaluronic acid; MW: Molecular weight; ns: Not specified; PAR: Polyarginine; PC: Polycation; PDI: Polydispersity index; PLL: Poly-L-lysine; PROT: Protamine; ζ : Zeta potential.

between CTS and HA, thus fostering phase separation as well as aggregation rate during PEC formation.^[74,126] This can be seen from the enlargement in the pH range for effective PEC formation and the increase in suspension turbidity when DD of CTS is increased from 37% to 99% in the work of Kayitmazer et al. (Figure 6C).^[74] Concerning the relative arrangement between two complexing polyelectrolytes, Le et al. reported that HA forms more stable PECs with PLL than with DEAE-D, as both HA and PLL have their charges distributed evenly on the polymer chains, which may allow more optimal ion-pairing than in the case of HA/DEAE-D PECs, where DEAE-D has random charge distribution.^[23] It should be noted that since biopolymers usually have weak charges, their ionization rate also depends on media pH. A change in pH may lead to an increase in ionization rate, therefore higher charge density as well as enhance the rigidity of polymer chains and increase thus the yield or particle size of resulting PECs, or vice versa.^[132] The effects of pH on PECs are further described in Section 3.3.5.

3.3.3. Total Polyelectrolyte Concentration

The most common range of total C_p for obtaining colloidal PECs from HA is 0.5–2 g L^{−1} (Table 3). Generally, with a fixed polyanion/polycation ratio, an increase in C_p would enhance PEC formation, that is, larger size and higher yield of PEC particles or macro-aggregation due to the involvement of more polymer chains (Figure 7). Kayitmazer et al. observed higher turbidity of HA/CTS PEC suspensions when C_p is increased from 0.1 to 1 g L^{−1} (Figure 6E).^[74] Umerska et al. reported an increase in particle size and turbidity for PECs from HA/PROT^[128] (Table 3) and HA/CTS^[117] when C_p is increased from 1 to 2 g L^{−1}. Greater particle size and PDI are also observed when C_p becomes higher in the systems of HA/PLL

and HA/DEAE-D PECs (refs. [2] and [105] in Table 3, respectively), or other HA/CTS PECs.^[50,126] Logically, an increase in C_p can also facilitate macro-aggregation if the polyelectrolyte MW is relatively high, for example, when C_p is increased from 1.25 to 1.8 g L^{−1} in HA/PLL PECs with HA MW of 1200 kDa (ref. [2] in Table 3), or even lead to a macrogel at extremely high C_p , for example, HA/CTS PECs at C_p of 40 g L^{−1}.^[133] In general, acceptable characteristics of polysaccharide-based PECs can be guaranteed by using dilute polymer solutions, usually corresponding to a C_p under 1 g L^{−1}.^[65,132]

3.3.4. Charge Ratio

In aqueous media with a given ionic strength, the molar ratio between negative and positive charges (*n−/n+*) of two complexing polyelectrolytes decides the sign and strongly affects the magnitude of the net surface charge of PECs, thus influencing their structure and stability. A *n−/n+* which is far from the stoichiometric ratio (*n−/n+* = 1) can lead to a great mismatch in ion-pairing and generate particles with more unpaired charges, thus a greater net charge. This would result in looser, more expanded, and more polydisperse structures of PEC particles, with a more pronounced and highly charged shell from uncomplexed polyelectrolyte segments.^[105,116,117] The presence of such uncomplexed segments on particle shell can be evidenced through zeta potential: a negative zeta potential indicates uncomplexed polyanion chains on PEC surface, while a positive value indicates an excess of polycations.^[35] When *n−/n+* is closer to 1, more effective charge compensation would generate more compact and hydrophobic PEC particles with a lower net charge. This can be seen from the decrease in particle size, PDI and absolute zeta potential as *n−/n+* is varied from 0.1 to 0.8 or from 10 to 1.25 in HA/DEAE PECs (ref. [105]—Table 3),

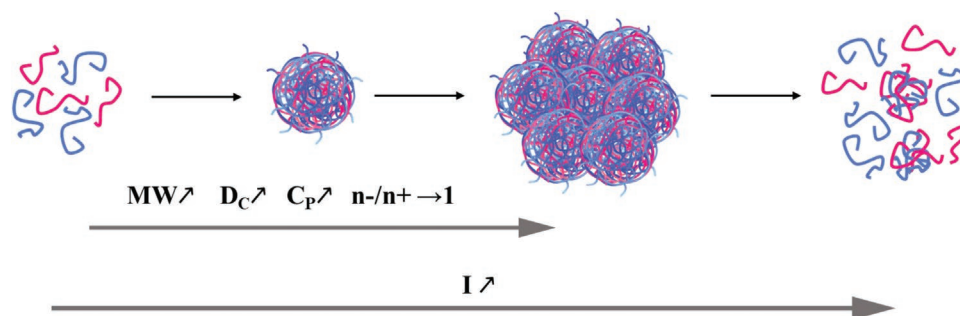


Figure 7. Effects of polymer molecular weight (MW), charge density (D_c), total polymer concentration (C_p), charge ratio ($n-/n+$), and ionic strength (I) on PECs from polyanions (blue) and polycations (red). (\nearrow : increase, \rightarrow : toward).

and from 1:3 to 1:1.25 (ref. [126]—Table 3) or from 0.2 to 0.31 (ref. [50]—Table 3) in HA/CTS PECs.

However, when $n-/n+$ is sufficiently close to 1, the low net surface charge of PECs may not allow enough electrostatic stabilization, thus can facilitate their agglomeration to form larger particles as illustrated in Figure 7, and eventually precipitation.^[6,63,117,134,135] This can be seen at $n-/n+ = 1$ for HA/DEAE-D PECs (ref. [105]—Table 3) and $n-/n+$ between 0.47 and 6.6 for HA/CTS PECs (ref. [50]—Table 3). Meanwhile, if $n-/n+$ is extremely far from 1, PECs may not be formed due to the insignificant amount of either polycation or polyanion, as reported at $n-/n+$ under 0.12 for HA/CTS PEC in ref. [50] in Table 3. This effect can be compensated by increasing C_p or MW.^[105,127] It is thus necessary to set an upper and lower limit for $n-/n+$ in order to ensure sufficient polyanion and polycation for PEC formation depending on polymer nature and preparation condition.^[74] The critical $n-/n+$ values for effective formation of PEC particles can be determined with an abrupt change in transmittance or viscosity of PEC suspensions upon $n-/n+$ evolution: when the microscopic phase separation corresponding to particle formation occurs, the transmittance would be reduced due to the increase in size and number of particles while the viscosity would also be decreased because there are less free polymer chains in the continuous phase.^[74,25] In some cases, the effect of $n-/n+$ can be important enough to outweigh the other factors, for example, for HA/zein PECs where an increase in the concentration of HA with an unchanged concentration of zein can result in PEC particles with a smaller shape despite an increase in the overall C_p , probably because of the strong ability of HA in crosslinking zein to generate more compact particles.^[4]

Taken together, for obtaining colloidal PECs with good colloidal stability, $n-/n+$ should be optimized to be far enough from 1 to ensure sufficient electrostatic stabilization (namely zeta potential over ± 30 mV^[6,117]) and avoid large aggregates but not too far from 1 to avoid poorly defined particles with large size or ineffective PEC formation. It should also be remembered that $n-/n+$ can change as a function of polyelectrolyte ionization rate and thus be affected by media pH (see Section 3.3.5).

3.3.5. pH

Since charges in HA as well as other biopolyelectrolytes are mostly constituted by weakly charged groups (i.e., carboxyl and amine groups), pH can govern their ionization rate and subse-

quently the complexation efficiency and thus phase behaviors of PEC systems.^[32,74] Generally, pH should remain between the pKa values of HA ($pK_a = 3$) and polycations to ensure their adequate charges for complexation.^[74] A pH higher than 4.5 is ideal to ensure the complete deprotonation of HA (Figure 6A) in order to form stable PEC particles.^[25] In most cases, both HA and polycations are readily dissolved in deionized water and sufficiently ionized to generate PECs without further pH adjustment,^[36,105,116,127] except for CTS which requires a starting pH under 5.5 to ensure its dissolution.^[136] After PEC formation, a gradual change from acidic to alkaline conditions will increase the deprotonation rate of polyelectrolytes, thus increasing $n-/n+$ and reducing the zeta potential, and vice versa.^[34] This can lead to the transformation in PEC morphology according to the effects of $n-/n+$ as described above in Section 3.3.4. In other words, an extremely low or high pH can render the charge density of respectively HA or polycations inadequate for effective charge compensation, which can lead to more expanded structures (i.e., larger particle size) or even disintegration of PECs, while either more compact particles or macroscopic aggregates can be observed when the actual $n-/n+$ approaches the stoichiometric ratio with a net surface charge closer to zero.^[34,137,138] Particularly, a pH under 2 would deionize HA nearly completely and cause disintegration of PECs, which is proven by the change from turbid suspension to transparent solution of HA/CTS PECs when pH is decreased below 2 in the work of Kayitmazer et al. (Figure 6A–D).^[74] These results also show that the favorable pH range for efficient PEC formation depends also on other parameters, namely a wider range if polyelectrolytes with higher MWs are used (Figure 6B). The effect of pH is critical to consider when colloidal PECs are formulated for biomedical applications since particle stability should be maintained in the strict pH ranges corresponding to physiological conditions,^[24,126] for example, 7.35–7.45 in extracellular fluid (including blood plasma),^[139] 4.1–5.8 on skin surface,^[140] 1.0–2.5 in gastric fluid and 6.5–7.5 in the intestines.^[141]

3.3.6. Counterions

Also due to the weak nature of ionic interactions in PECs, the presence of salts, typically NaCl, can lead to charge screening of the polyelectrolytes by counterions and thus profoundly affect the formation and stability of PECs. For colloidal PECs, an increase in ionic strength can cause higher charge screening

on particle surface, which can be seen through a reduction in the absolute value of zeta potential and lead to higher PEC yield due to particle aggregation or even macroscopic precipitation due to weakened interparticle electrostatic repulsion (weaker long-range repulsive force).^[74,106] Such effects were observed in the work of Kayitmazer et al., where the increase of NaCl concentration from 5 mM to 0.3 M led to a higher turbidity and a larger particle size of HA/CTS PECs, while the decrease in turbidity at NaCl concentration beyond 0.3 M was attributed to the sedimentation of macroaggregates (Figure 6E,F).^[74] In case of HA being complexed with proteins, a salting-out effect may also enhance hydrophobic interactions as supplementary stabilization to favor the formation of PEC particles. This is suggested by Zhong et al. after remarking a larger particle size of PECs from HA and WPI with more hydrophobic interactions within these PECs when NaCl concentration is increased from 0 to 30 mM.^[142] However, an extremely high ionic strength can prevent PEC formation or cause PEC disintegration because the electrostatic attraction between oppositely charged polyelectrolytes (short-range attractive force) is also reduced as charge screening takes place at molecular level.^[87,143] Changes in phase behavior as well as average particle size of colloidal PEC systems are thus the net result of the two contradictory effects of charge screening at particle and molecular levels. Therefore, particle size evolution can be non-monotonic during the increase of salt concentration. For instance, in the above-mentioned work regarding HA/WPI, when NaCl increases beyond 40 mM, PEC hydrophobicity and particle size start to decrease since WPI is more likely to dissociate from PECs.^[142] Similarly, the particle size of HA/DEAE-D PECs was reported to go up when NaCl concentration increased from 1 mM to 0.1 M but then decreased abruptly when the salt concentration reached 1 M.^[105] Such effects of ionic strength are also illustrated in Figure 7. As with pH, ionic strength is also an important parameter for evaluation when PEC systems are designed for in vivo applications since the physiological ionic strength (i.e., NaCl 0.9% or 0.15 M) is usually sufficiently high to destabilize HA-based colloidal PECs.^[105,126]

The effects of counterions on PECs can also depend on counterion nature and some of them can even stabilize PECs against physiological ionic strength. For HA/CTS PECs, compared to Na⁺, Ca²⁺ may have no different effect on complexation rate but can result in a more porous and swelling structure of PEC particles with higher water content, since the chaotropic property and larger hydration number of Ca²⁺ would not only increase the solubility and thus the hydrophilicity of polyelectrolytes but also make itself as well as its neighboring water molecules preferably remain in PECs rather than be expelled during the complexation.^[13] Meanwhile, Zn(II) has been shown to stabilize HA/CTS PECs against physiological ionic strength.^[48,126] Wu et al. found that the addition of ZnCl₂ or ZnSO₄ at 1.5 mM before HA/CTS PEC formation (pre-stabilization) results in PEC NPs with good stability regarding particle size and zeta potential in PBS at both 4 and 37 °C for at least 1 month, while NPs without Zn(II) are disintegrated immediately upon submersion in PBS.^[126] The stabilizing effect of Zn(II) in such case is supposed to stem from its chelation with CTS as ligands, in which Zn(II) adopts a tetra-coordinate mode with primary amines and hydroxyl groups of CTS.^[144] Nevertheless, the coordination

between Zn(II) and the oxygen-containing donor groups of HA can also be considered as another mechanism.^[145] The order of Zn(II) addition also affected the stability of these PECs, since NPs with Zn(II) added after the complexation (post-stabilized NPs) were stable in PBS at 4 °C but unstable at 37 °C. However, the work did not clarify the reason behind such difference between post-stabilized and pre-stabilized NPs, as well as between the two storage temperatures. Indeed, in the case of post-stabilization, the penetration of Zn(II) cations into the NP core may be limited by positive charges of the NP surface, as well as the increased hydrophobicity of these NPs at 37 °C since many CTS-based complexes can show thermoresponsiveness due to the thermo-dependent hydrophobic interactions between CTS chains.^[146,147] The salt form of Zn(II) had also a certain impact, as ZnCl₂ showed better stabilizing efficiency than ZnSO₄.^[126] However, the work did not clearly describe the mechanism behind such results. This might be explained by the participation of sulfate in PECs as divalent crosslinking ions through electrostatic interactions with cationic charges of CTS, which can limit the exposure of these charges on particle surface and reduce their electrostatic stabilization.^[148]

Besides Zn(II), tripolyphosphate (TPP) can also be used as an adjuvant ion to stabilize HA/CTS PEC NPs in physiological salines.^[31,33,42,61,129,149,150] The stabilizing effect of TPP stems from the crosslinking of CTS through electrostatic interactions between anionic charges of phosphate groups in TPP and amine groups in CTS.^[151] The effects of glutamate as a counteranion on HA/CTS PECs have also been described. The chaotropic nature with dispersed charges of glutamate can increase considerably the solubility of CTS and renders glutamate less likely to screen the positive charges of CTS than chloride ions, allowing therefore a wider n^-/n^+ range for effective formation of colloidal PECs.^[13] It was also reported that glutamate would remain in PECs rather than be released and thus reduce the zeta potential of PEC particles through its negative charges.^[117]

3.3.7. Mixing Method

When PECs are produced by simply mixing two polymer solutions, addition mode (rapid one-shot or slow dropwise addition) and addition order (HA in polycations or vice versa) can influence the properties of the resulting PECs.^[50,152] Dropping slowly the major polyelectrolyte into the other can result in large aggregates or macroscopic phase separation in the system, because at a certain point during such a gradual addition, the actual n^-/n^+ can reach the unity and the aggregation of neutral particles may take place at a much greater speed compared to that of n^-/n^+ evolution and thus leading to aggregation, which may be merely irreversible even with a final n^-/n^+ far from the stoichiometric value if there is no further external interference.^[105,130] Accordingly, slow addition of the minor polymer in the major polymer can generate finer and more homogeneous PEC particles, which would give the same particle structure as that of one-shot dropping. Yang et al. reported a high turbidity (absorbance largely varied between 0.3 and 1.2) for PEC suspensions of HA/CTS with HA:CTS ratio under 1:1 prepared by adding dropwise CTS to HA solution, indicating the formation of large aggregates, while the opposite order resulted in either

absence of PEC particles or colloidal PECs having relatively small size (130–170 nm), making up transparent suspensions with a low absorbance which stays under 0.05.^[50] Concerning the one-shot method, the effect of addition order should be insignificant since the target $n-/n+$ can be immediately reached without sufficient time for particle aggregation to occur.^[105] For instance, HA/DEAE-D PECs at $n-/n+$ of 0.8 have relatively small and similar particle sizes (150–200 nm) upon one-shot mixing regardless of addition order and dropwise addition of HA in DEAE-D solution, while dropwise addition of DEAE-D to HA leads to large particle size over 400 nm.^[105]

Despite being of minor importance, stirring rate, stirring time, and complexation temperature might also have certain effects on physicochemical properties of colloidal PECs between HA and polycations. In an experimental design study, formation of smaller and more monodisperse particle size of HA/CTS PECs was reported to be favored by a middle stirring rate, as homogeneous distribution of both polyelectrolytes in the reaction media might not be guaranteed with either a low stirring rate (e.g., under 300 rpm) due to insufficient kinetic energy or a too high stirring rate (e.g., above 1300 rpm) which may cause bubbles and solution splashing.^[47] Another experimental design study concerning HA/CTS PECs prepared with three different stirring times (10, 95, and 180 min) suggests that a longer stirring time can result in more monodisperse PEC particles as shear can be offered during a sufficient time to generate rigid and stable particles.^[137] In case of PECs from thermosensitive polymers, for example, PECs between HA and elastin-like polypeptide, temperature should be sufficiently high (e.g., above 30–40 °C) to render their coacervation effec-

tive to generate particles.^[153] Otherwise, temperature variation in a common range (4 to 60 °C) usually has little effect on the formation and/or the stability of colloidal PECs.^[47,50,105]

Besides the classical mixing method which requires adequate stirring, colloidal PECs can also be produced using electrospray ionization technique. This was applied in a work of Simonson and co-workers, where HA solution was propelled as nanosized droplets from the outlet of an electrically-charged capillary into a bath containing PLL solution without agitation, followed by centrifugation of the resulting dispersion to obtain HA/PLL PEC NGs (**Figure 8A**).^[36] Such colloidal PECs could thus be produced rapidly by electrospray method with relatively small and monodisperse particle size as well as good colloidal stability (**Figure 8B,C**).

4. Applications of Colloidal PECs from HA

The following sections will provide a global view on various applications reported so far of HA-based colloidal PECs with a focus on recent advances within the last 5 years. These systems have been widely developed to deliver small molecule drugs (**Section 4.1**), NAs (**Section 4.2**), and peptides or proteins (**Section 4.3**) for therapeutic purposes. Imaging agents can also be delivered by colloidal HA-based PECs for bioimaging and diagnosis, possibly in combination with the above-mentioned therapeutic agents for theranostics (**Section 4.4**). Some nanoparticulate HA-based PECs which are not necessarily loaded with other bioactive or bioimaging cargo can also be employed as multifunctional platforms, for example, carriers for HA delivery, emulsion stabilizers, or building blocks for biomedical micro- or macro-materials (**Section 4.5**). Notably, a large number of those studies concern oncologic applications due to the rising need for cancer diagnosis and treatment, for which colloidal HA-based PECs can offer tremendous advantages as mentioned in **Sections 1 and 2**.

4.1. Small Molecule Drug Delivery

Colloidal HA-based PECs have been described as nanocarriers for delivering small molecule drugs in numerous works, which are listed in **Table 1**. Many of them show positive results in vitro and/or in vivo regarding improvement in therapeutic efficacy of the drugs of interest, most of which are anticancer agents since their delivery is usually problematic due to poor aqueous solubility, poor stability, low bioavailability, and high systemic toxicity owing to lack of cellular selectivity and thus needs more improvement.^[154] Some works did not include in vitro or in vivo studies on therapeutic performance but still reported good drug encapsulation and/or controlled release with satisfactory physicochemical stability, showing thus their potential as promising nanocarriers for drug delivery. It should also be mentioned that among the works presented in **Table 1**, drug-loaded colloidal PECs of Hsu et al.^[40] and Freitas et al.^[29] were further incorporated in secondary carriers as multifunctional platforms rather than employed directly in their colloidal form for their applications, for which a more detailed description will be provided in **Section 4.5**.

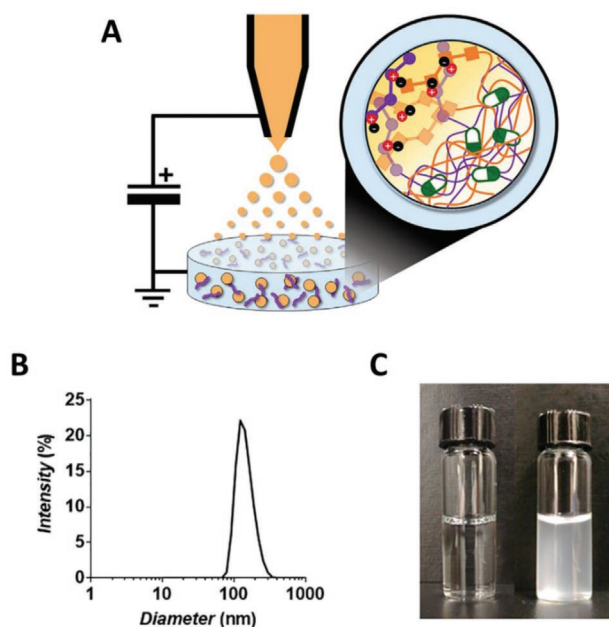


Figure 8. A) Schematic illustration of using electrospray for preparing colloidal PECs from HA (yellow) and PLL (purple): drugs can be incorporated in either starting solution for their encapsulation in PEC particles. The obtained HA/PLL PEC suspension shows B) relatively monodisperse particle size and C, right) good colloidal stability with homogeneous turbidity as compared with the C, left) transparent aspect of the starting PLL solution. Reproduced with permission.^[36] Copyright 2019, Elsevier.

During the formulation and preparation steps of colloidal PECs, different factors (e.g., C_p , mixing ratio, ionic strength, starting drug amount, addition order of drugs, stirring time, and dialysis time) should be studied and optimized as they can affect drug encapsulation and release rate.^[43,50,142] The effective encapsulation of drugs in PECs, that is, in molecular or amorphous state, can be verified through differential scanning calorimetry (DSC) or X-ray diffraction (XRD) techniques.^[7,43,50] For encapsulation of a positively charged drug like DOX, a high $n-/n+$ ratio which leads to negatively charged particles should allow a better encapsulation rate due to electrostatic attraction between particles and drug molecules.^[27] For hydrophobic drugs, adding drugs before the complexation can offer better drug encapsulation when compared to incubating the fully formed particles with the drugs since the highly charged and hydrophilic shell around a PEC particle can inhibit the adsorption of hydrophobic molecules, as proven in the case of pyrene encapsulation in HA/DEAE-D PECs.^[105] However, being also a hydrophobic drug, DOX was reported to be better encapsulated in HA/PLL PEC particles after their formation, since including DOX in the starting polymer solution can cause precipitation.^[36] Yang et al. also found that adding curcumin (CUR) before the complexation between HA and CTS can lead to micro-scaled rather than nanosized PECs.^[50] In order to limit the precipitation of such hydrophobic drugs and maintain their molecular state in a sufficient time during the complexation or the co-incubation for an effective inclusion of drug molecules in PEC particles, organic cosolvents like ethanol or acetone can be used, which should be thereafter removed by evaporation or dialysis.^[23,50] Specifically, to encapsulate hydrophobic bioactive polyphenols like CUR and quercetin in HA/zein PECs, the complexation should take place in an ethanol-water mixture due to the low aqueous solubility of both zein and the active compounds, followed by ethanol evaporation in order to obtain the final drug-loaded PECs (antisolvent coprecipitation method, Figure 5).^[37–39,109] For PECs from HA and proteins, encapsulation of a hydrophobic drug like CUR can also be improved with an adequate NaCl concentration due to enhanced hydrophobic force with “salting-out” effect, as reported for colloidal PECs from HA and WPI.^[142] Physical coating of PECs with PEG might also reduce the leakage of hydrophobic drugs and therefore enhance their encapsulation, as suggested by Xu et al. in their work regarding CUR encapsulation in HA/CTS PECs.^[44] By using HA functionalized with thermosensitive amphiphilic polyethers, that is, poly(ethylene oxide)-*co*-poly(propylene oxide) (PEO-PPO), Le et al. also found that the encapsulation of CUR in PECs can be improved by controlling complexation temperature.^[23] Covalent conjugation of drug molecules on parent polymers of PECs as prodrugs instead of physical entrapment method can also improve drug solubility and encapsulation, pharmacokinetic properties, and therapeutic efficacy while reducing their systemic side effects, for example, HA/CTS PECs with covalently bonded gemcitabine and platinum (IV) on HA and CTS respectively^[46] or with docetaxel grafted on HA.^[47] Another finding worth mentioning is that for HA/CTS PECs loaded with tocopherol (also known as vitamin E), which is a lipophilic compound, using TPP for further particle stabilization as mentioned in Section 3.3.6 can lead to particles having a better controlled structure but may reduce the encapsulation rate of tocopherol.^[31]

The stability of colloidal PECs in biological fluids, as for other nanoparticulate systems, is greatly affected by the high ionic strength and proteins in these fluids since the former can lead to PEC aggregation or disintegration as described in Section 3.3.6, while the latter can form a “protein corona” (opsonization) on the particle surface that would possibly modify their stability with undesirable effects, such as aggregation or phagocytosis by immune cells.^[30,155,156] Evaluating the particle size in biological fluid can allow verifying their stability in such conditions. Considering some inconveniences of DLS in observing particle stability in undiluted biofluids, Shariati et al. reported the use of fluorescent single-particle tracking as an alternative but more pertinent method to ascertain the stability of HA/PAR PEC particles in undiluted ascetic fluid.^[30] Such good stability of HA-based colloidal PECs can be attributed to the protecting effect of HA against protein adsorption.^[10] PEGylation, one of the most common methods to produce “stealth” NPs in nanomedicine, can also be applied to protect colloidal PECs from opsonization, therefore improving their circulation time in vivo and bioavailability of the loaded drugs, as evidenced with PEGylated HA/CTS PECs for mitoxantrone delivery.^[7] In addition, complementary counterions have been employed to stabilize drug-loaded PEC particles against physiological salines as mentioned in Section 3.3.6, for example, Zn(II) ions for stabilizing tenofovir-loaded HA/CTS PECs^[48] or TPP for HA/CTS PECs encapsulating DOX.^[33] The amphiphilic PEO-PPO grafts on constituent polymers can also stabilize PEC particles against physiological ionic strength through supplementary intraparticle hydrophobic interactions.^[23] Moreover, when prepared from thiol-disposing polymers, for example, HA-SH, PLL-SH, or feather keratin, PEC particles can be further stabilized by self-crosslinking through the formation of the cleavable disulfide bonds as a result of redox reaction between thiol groups, either spontaneously with oxygen readily present in the media or more favorably upon adding an oxidizing agent like hydroxy peroxide, which may ultimately enhance drug encapsulation and PEC stability against high ionic strength.^[27,32,41]

Regarding drug release, PECs can offer a controlled release which follows an initial burst release phase and might depend on the studied pH.^[4,50] In case of DOX, which becomes more positively charged and thus more hydrophilic at lower pH, a mild acidic condition as in tumor microenvironment (pH between 6 and 7) or intracellular vesicles (pH between 4.5 and 6.5) can trigger drug release from PECs, since the protonation of both polyanions and polycations can favor PEC swelling for drug diffusion and the increase in positive charges can extrude drug molecules from PECs through electrostatic repulsion, allowing thus a pH-responsive behavior for controlling drug release.^[24,51] Conjugation of HIS on parent polymers was also reported to enhance PEC stability and the encapsulation rate of DOX through hydrophobic interactions and favor drug release in acidic conditions owing to the pH-dependent hydrophilic-hydrophobic balance of HIS, where the protonation of HIS moieties at low pH can reduce their hydrophobic interaction with more positive charges and lead to the expulsion of DOX from the particles.^[49] Moreover, self-crosslinking through disulfide bonds in HA-based PECs can result in not only better PEC stability as previously mentioned but also a dual-responsive

behavior for triggering drug release in tumor intracellular microenvironment, since the extremely high concentrations of both HAase and glutathione (GSH) at these locations can cause respectively HA degradation and disulfide bond cleavage to destabilize PECs and release the loaded drugs.^[27,32,41]

In terms of drug targeting, besides the CD44-targeting effect of HA, further addition of other ligands can improve the tumor targeting effect, for example, LF attachment on PEC particle surface through electrostatic interactions with HA can offer these particles with better permeation through the blood-brain barrier, leading to a dual-targeting effect for treating brain tumors,^[45] whereas grafting with galactose moieties can further improve hepatoma-targeting capacity through the recognition of galactose receptors on hepatocytes.^[51] Co-delivery of anticancer drugs with other therapeutic agents can also be applied for additional benefits, for example, HA/CTS PEC NPs for attacking multidrug resistant breast tumor cells by co-delivering mitoxantrone, a nonspecific anticancer drug, and verapamil, a calcium antagonist which can effectively reverse drug resistance in order to enhance the cytotoxic effect of mitoxantrone.^[43] Administration route can also affect the therapeutic efficacy of drug loaded PECs, for example, delivering cisplatin-loaded HA/ PAR PEC nanocarriers intraperitoneally by pressurized local aerosolization demonstrated a more homogeneous distribution of particles with a deeper drug penetration at targeted tumor sites and thus better antitumor efficacy compared to the classical intravenous (IV) injection.^[30]

HA-based colloidal PECs have also been developed to deliver small molecule drugs for non-oncologic therapy. Simonson et al. suggested that HA/PLL PEC NGs are promising for improving the antibiotic potency of vancomycin against both Gram-negative and Gram-positive bacteria, as the minimum inhibitory concentration (MIC) of vancomycin delivered by such NGs in treating *E. coli* and *S. aureus* was respectively four-fold and 15-fold lower when compared to free vancomycin.^[36] The mechanism proposed by the authors for this effect is that HA can preferentially recruit the bacteria pathogens to the NG surface since this polysaccharide is a carbon source for their essential activities, while the subsequent electrostatic interactions between PLL and bacterial cell wall can permeabilize the latter and therefore bacterial cells are more exposed to the drug. Encapsulation of berberine in HA/PLL PEC NGs for wound healing with satisfactory in vitro results was also described by Amato et al.^[2] Meanwhile, Wu et al. reported the delivery of tenofovir, a human immunodeficiency virus-1 (HIV-1) reverse transcriptase inhibitor, by colloidal HA/CTS PECs stabilized with Zn(II) ions and decorated with anti- $\alpha 4 \beta 7$ immunoglobulin as a ligand for targeting HIV-infected cells.^[48] The work showed good biocompatibility of such PECs and their ability to improve the inhibitory effect of tenofovir on HIV-1 infection, in which the contributing roles of Zn(II) ions and the targeting ligand were evident. HA/CTS PEC NPs were also elaborated by Aken-tieva et al. to deliver dinitrosyl iron complexes (DNICs) as a nitric oxide (NO) donor for treating cardiovascular diseases.^[28] Their studies demonstrated the capability of HA/CTS PEC NPs to incorporate and stabilize DNICs, then increase and prolong NO generation from such complexes and subsequently improve the viability of cardiomyocytes in vitro. In another work, HA/zein PEC NPs were reported for topical delivery of

tetrahydrocurcumin (THC) to alleviate Ultraviolet B (UVB)-induced skin photoaging, since the particles can be uptaken by UVB-exposed keratinocytes through CD44-mediated endocytosis, where the ROS produced in UVB-induced inflammation can promote the degradation of HA to liberate both THC and low MW HA, which can act synergistically in turn as scavengers of ROS to reduce oxidative damage and cell apoptosis.^[24] Indeed, the protective effects of HA against radiation damage may stem from not only its chemical reactions with ROS but also a biological mechanism involving a complexity of cellular signaling pathways.^[70]

4.2. NA Delivery

NA delivery has been applied in gene therapy technologies as an emerging approach for treating genetic diseases, especially cancers.^[157,158] Classical gene therapy involves transporting coding deoxyribonucleic acids (DNAs) into diseased cells in order that their expression can correct defective genes, replace absent genes or provoke antitumor reactions.^[157] Gene silencing, which is a more recent technology for gene therapy, is done by introducing specific non-coding ribonucleic acids (RNAs) like small interfering RNAs (siRNAs) or microRNAs (miRNAs) into targeted cells so as to suppress the translation and thus the expression of targeted genes.^[158] Numerous cationic polymers (e.g., PROT, CTS, PEI, PLL, and PAR) have long been known as cellular transfection agents due to the capability to form electrostatic complexes with NAs, which can neutralize their strong negative charges and thus facilitate their intracellular delivery.^[159] However, such binary complexes can exhibit insufficient compaction and low stability as well as nonspecific interactions with biological molecules due to extensive positive charges and high toxicity,^[82,160,161] leading to the need for further complexation with a biocompatible polyanion like HA to form ternary complexes disposing better properties.^[53] As for small drugs-loaded PECs, HA can also contribute to the physicochemical stability of NA-loaded PECs in serum.^[58,162] Furthermore, the presence of HA as a protecting shell can prevent the aggregation of NA-loaded PEC particles and the inactivation of NAs after lyophilization and rehydration in order to ensure an unimpaired transfection efficiency.^[62] Accordingly, high transfection efficiency was reported in multiple studies regarding colloidal PECs from HA for delivering different types of NAs, including siRNAs, miRNAs, oligoRNAs, and linear or plasmid DNAs (pDNAs) (Table 2).

During PEC formulation, the proportion of every component, that is, HA, polycations, and NAs, are highly important as they can affect the affinity of NAs to PECs and thus both their loading and release rate. A too low proportion of polycations or a too high proportion of HA might prevent efficient incorporation of NAs in PECs due to insufficient positive charges to form complexes with NAs. Accordingly, evaluating the amount of free NAs remaining in the formulation through gel electrophoresis can be realized to find the optimal proportion of these components.^[53,162] For example, Ma et al. reported that the amount of HA/PROT PECs with HA:PROT ratio of 1:1 *m/m* should be at least 20 times higher than that of siRNA in order to include all siRNA in the complexes.^[53] Meanwhile, Kim et al.

found that the optimal PEC:siRNA mass ratio is at least 12:5 when using PECs of HA/PAR (1:10 *m/m*) but this should be increased to at least 24:5 when HA:PAR ratio is increased to 1:1 *m/m*.^[64] However, an excessively high proportion of polycation may result in large aggregates, for example, HA/PLL/miRNA PECs with N/P ratio (molar ratio of amine groups on PLL to phosphate groups on miRNA) of 20 were reported to show a high particle size of 450 nm, which is much greater than the particle size of 120 nm obtained with N/P of 10 and 5.^[54] The MW and the proportion of HA are also important and can have various impacts on gene delivery efficacy. Sato et al. reported that HA has a protective effect on NA-loaded PEC NPs during lyophilization and rehydration, where a higher MW of HA can strengthen such effect to improve the transfection efficiency due to a better exposure of HA on the particle surface as mentioned in Section 3.3.1 and thus better interaction with CD44 receptors.^[59] Meanwhile, although HA can improve cell targeting through CD44 receptors, a too high proportion of HA in PECs may increase excessively negative charges in the complexes and therefore limit NA loading or cellular uptake due to stronger electrostatic repulsion between PECs and NAs or cell membranes respectively.^[54,64,118] In return, once uptaken by cells, HA can accelerate the expression of the transported genes by enhancing transcriptional activity in the transfected cells through certain cellular signaling pathways.^[59,163]

In order to improve the stability of NAs in colloidal HA-based PECs, further modifications on PEC particles can be applied. Similar to small drug molecules, oligodeoxynucleotides (ODNs) can also be conjugated to HA in PECs through the cleavable disulfide bonds, which might allow better incorporation and stabilization of ODNs while still ensuring their intracellular release in the presence of GSH.^[66,164] Functional additives can also be introduced through either physical attachment or chemical grafting. For oral delivery, for example, in targeting colorectal liver metastasis, the low oral stability of NAs can be a great challenge and might be overcome by using HA grafted with bile acids like taurocholic acid (TCA) in PEC nanocarriers. In a work of Hyun et al. regarding siRNA oral delivery for treating liver tumor, it was shown that inclusion in HA-TCA/PROT PECs can better protect the siRNA from gastrointestinal pH and nuclease than complexing with PROT alone.^[56] Furthermore, these PECs were also shown to enhance intestinal absorption as well as increase and prolong liver biodistribution of siRNA. Such advantages may stem from the enterohepatic circulation of TCA as a bile acid: TCA in the gastrointestinal tract can bind to apical sodium bile acid transporters (ASBT) proteins in the small intestine for intestinal absorption of TCA, then be transported to the liver for recycling and reintroduced back into intestines with minimal clearance in the whole process (around only 5%).^[165] However, the contribution of TCA in such improvement was not convincingly proven as these studies did not include HA/PROT PECs as a referent sample.^[56] Another example is DOP grafted on HA for complexation with the biodegradable synthetic polycation PBAE and DNA to form DNA-loaded nanocomplexes in a work of Guo and co-workers,^[52] who took advantage of the bioadhesive abilities of DOP through interactions with cell membrane and the high expression of DOP receptors on tumor cell surfaces in order to favor the tumor cellular uptake of such nanocomplexes.

Colloidal HA-based PECs were also studied for co-delivery of NAs and chemotherapeutic small molecules, which may offer synergistic effects. Ma et al. developed HA/PROT PEC nanocarriers for co-delivery of Pt(IV) as a platinum prodrug with survivin siRNA, which can reduce survivin overexpression and reverse Pt resistance to improve its cytotoxic effect in drug-resistant lung cancer cells.^[53] In this work, Pt(IV) was used as a prodrug instead of Pt(II) (i.e., cisplatin) in order to avoid their interactions with siRNA, whereas polyglutamic acid was used not only as an additional anionic coating agent to increase the biocompatibility of the system but also as a layer of drug reservoir with covalently bonded Pt(IV), which offers a slower release of Pt(IV) compared to siRNA, allowing the reversal of drug resistance by siRNA to take place before the cytotoxic Pt(II) is produced and exerts its function. HA/PROT NGs were also described for the co-delivery of EGCG and siRNA.^[57] EGCG is a natural compound exhibiting anticancer activity and NA stabilizing effect due to their noncovalent interactions, while siRNA can be useful for downregulation of drug resistance-related factors to allow better response to EGCG. The decoration of PEC NGs with tumor-homing cell-penetrating peptides was also employed in this work in order to improve particle accumulation in the tumors (**Figure 9**). In another work, coadministration of miRNA with DOX by TPP-stabilized HA/CTS PECs was also demonstrated by Deng et al.^[61] Grafting cyclodextrin (CDX) on polycations in complex with HA is another approach which may allow not only a concurrent delivery of both NAs and hydrophobic chemotherapeutic compounds owing to the inclusion of the latter in CDX moieties, as reported for co-delivering oligoRNA and DOX with PECs from HA and CDX-grafted PLL,^[60] but also a better biocompatibility and higher transfection rate when compared to non-modified polycations, for which the mechanism remains undiscovered.^[162,166]

Compared with oncotherapy, non-oncologic therapy has been less described for applications of NA delivered with colloidal HA-based PECs. An example is PEC NPs from HA and PEI-grafted CTS oligosaccharide (CSO) loaded with siRNA for the treatment of endometriosis.^[167] In this work, HA/CSO-PEI/siRNA particles showed better selectivity to endometriosis lesion when compared to CSO-PEI/siRNA complexes, probably due to the targeting effect of HA through CD44 receptors on these cells. The former also exhibited no toxicity on reproductive organs while favoring both a reduction in endometriosis lesion size and degeneration of ectopic endometrium in rat models. Colloidal PECs from HA and oleoyl-carboxymethyl-CTS were also developed to deliver pDNA as an oral vaccine for fish,^[168] where the incorporation of HA was reported to enhance the uptake of the nanocomplexes by Caco-2 cells and improve the immune response in carps immunized with the DNA-loaded PECs through oral route, which was evidenced with a higher antigen-specific antibody titer.

4.3. Peptide and Protein Delivery

As for NAs, proteins as well as peptides are also biomacromolecules which can be delivered by HA-based PECs with improved stability. In contrast to NAs, which dispose strong negative charges, proteins can be either negatively or positively charged

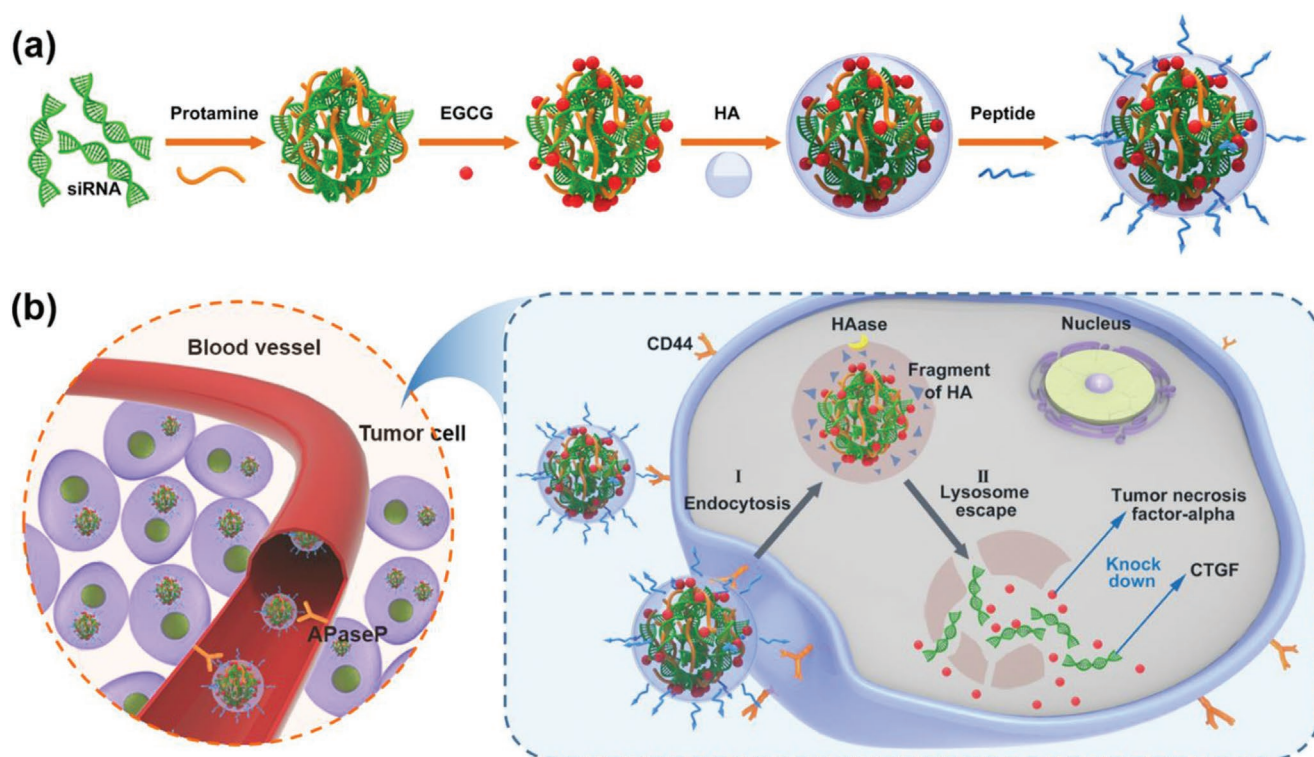


Figure 9. NGs from self-assembly of siRNA, PROT, EGCG, HA, and tumor-homing cell-penetrating peptides. a) Fabrication scheme and b) Antitumor mechanism: NGs are accumulated in blood vessels of tumors through specific recognition of peptide ligands on the NG surface by aminopeptidase P (APaseP) on tumor blood vessel wall. The NGs are then bound to CD44 receptors on tumor cells before cell internalization, which is followed by NG degradation by HAase in lysosomes to liberate EGCG and siRNAs in order that EGCG can reduce the generation tumor necrosis factor- α to exert its antitumor functions while siRNA can downregulate the expression of connective tissue growth factor (CTGF) to reduce chemoresistance against EGCG. Reproduced with permission.^[57] Copyright 2018, American Chemical Society.

depending on pH, and thus their associations in HA/polycations PECs would be mainly based on their electrostatic interactions with polycations or HA respectively, which can dictate the structure and properties of protein-loaded PECs. Umerska et al. reported that the effective inclusion of salmon calcitonin (sCT) in HA/PROT nanoplexes is due to electrostatic interactions between HA and the positive charges of sCT.^[128] Therefore, a higher proportion of HA can improve the loading rate of sCT and ultimately result in negatively charged PECs with sCT located nearer to the particle surface rather than the core, as the latter is rich in highly cationic PROT which may propel the protein toward the particle surface. It should be noticed from this study that even though HA and sCT can readily form electrostatic complexes, the use of PROT as a polycation is still necessary since the relatively weak complexation between HA and the protein alone is not sufficient to form well-defined and stable particles, but an excessive presence of PROT might prevent sCT loading due to electrostatic repulsion and destabilize the system. In coherence with its relative emplacement near the particle surface, sCT showed great effects of on both the particle size and surface charges as well as a release pattern starting with an initial burst followed by a prolonged release. Similar findings on protein displacement and release pattern were also described for positively charged HA/CTS PEC NPs loaded with the negatively charged insulin.^[150] On the contrary, proteins with the same charge sign as that of PEC shells

are more likely to be compacted in the innermost core rather than near the surface of PEC particles, which can be evidenced through the negligible effects of protein loading on particle size and net surface charge in the case of insulin loaded in negative HA/CTS PECs^[132] and green fluorescent protein (GFP) loaded in negative HA/PLL PECs.^[36] This may lead to a very different release pattern of proteins from PECs compared to that of small molecule drugs, as Simonson et al. reported that the release pattern of GFP from their negative HA/PLL PEC NGs started with an initial slow-release phase before the rapid release in the second phase corresponding to the degradation of the PEC core, while it is the opposite for the release of DOX from the same PEC particles.^[36] Despite such a difference in release manner between the two types of cargo, both of them were effectively delivered into the studied cancer cells by CD44 targeting and exerted the desired effects, as evidenced by the cytotoxic assay in the case of DOX and intracellular fluorescence evaluation in the case of GFP. In another work, Liang et al. reported PEC NGs from PEI and EGCG-grafted HA loaded with lysozyme as a model protein or granzyme B as a cytotoxic protein with anti-cancer effects,^[3] where the role of EGCG grafting is to facilitate the binding of protein cargos in PEC NGs through physical interactions and result in NGs with smaller size, better homogeneity, and higher stability. The optimized systems showed promising results with a targeted cellular uptake and significant cytotoxic effects on CD44-overexpressing colon cancer

cells. Nevertheless, as lysozyme disposes positive charges and was thus included in PECs through its association with HA,^[69] an excessively high proportion of HA-EGCG can reduce protein release and lower the therapeutic efficacy.^[3] Collectively, the distribution of loaded proteins inside PEC particles and also their release pattern can be different case by case depending on protein nature and particle design.

Colloidal PECs from HA were also investigated in several works for oral protein delivery, which is usually challenging due to the low stability of proteins in the gastrointestinal tract as well as their low permeation through the mucous layer and enterocytes. Logically, most of these works focused on PECs from HA and CTS due to their synergistic effects in enhancing the mucoadhesive property of drug delivery systems.^[95] In a work of Cui et al. regarding insulin oral delivery with HA/biotin-grafted CTS/insulin ternary nanocomplexes, HA can enhance the permeation of the studied systems in the mucous layer while the grafted biotin can enhance the uptake of insulin-loaded nanocomplexes in enterocytes through their biotin receptors, leading to an improvement in hypoglycemic effect in vivo, which can be optimized with a medium MW of HA and the highest substitution degree of biotin within the studied range.^[9] Oral delivery of insulin with HA/CTS nanocomplexes was also reported by Sladek et al. but with a slightly different delivery mechanism, where the role of PECs was to protect insulin in digestive media and facilitate its passage through mucus but not through the epithelial wall due to scarce epithelial uptake of these nanocomplexes. Therefore, insulin should be released in the mucus and penetrate in its free form through epithelium, which can be assisted by sucrose laurate as a permeation enhancer.^[132] The group also found that insulin in plain HA/CTS/insulin PECs is indeed more prone to degradation by digestive enzymes than free insulin despite the compact inclusion of insulin within the PEC core. Such a lower stability of insulin in PECs was attributed to insulin restructuring upon complexation and can be avoided by coating PECs with a specific commercial enteric coating polymer.^[132] On the contrary, Umerska et al. reported that HA/PROT nanocomplexes can limit the digestive degradation of sCT even though the protein was found to be located near the PEC surface.^[128]

HA-based nanoparticulate PECs have also been investigated for delivering antigenic peptides or proteins as a vaccine. Specifically, intranasal delivery of ovalbumin was reported in some research regarding PECs from HA/poly-L-lysine-co-poly-lactide^[170] and HA-SH/trimethyl CTS-SH.^[96] In the former work, the PECs of interest could effectively deliver ovalbumin into dendritic cells to upregulate cytokine production in vitro and enhance the production of IgG antibody in vivo upon intranasal administration in mouse models, which was attributed to the mucosal adhesion property of HA as well as its ability to enhance specific uptake of PECs through CD44 receptors on mucosal epithelium and immune cells. Regarding ovalbumin-loaded HA-SH/trimethyl CTS-SH PECs, Verheul et al. reported that the presence of thiol groups allowed the self-crosslinking of PECs with two main advantages: i) better physicochemical stability of PECs during post-particle chemical modification (PEGylation) or under physiological saline condition and ii) enhancing the adjuvant effects of PECs to improve ovalbumin-induced immunological response.^[96] However, the work also

showed that PEGylation offers no additional benefit or even abolishes the positive effect of PEC crosslinking on immunogenicity when the particles are delivered intranasally, which was contradictory to many previous works and could be attributed to the change in particle surface charge after the PEG-grafting reaction. HA/CTS as well as chondroitin sulfate/CTS PEC particles were also described in the work of Dacoba et al. on delivering the peptide antigen PCS5 as an anti-HIV vaccine,^[100] where it was found that the covalent conjugation of the antigen on material polymers and their coadministration with polyinosinic:polycytidylic acid as an immunostimulant can both improve the immunological response, that is, activation of antigen-presenting cells in vitro.

4.4. Imaging Agent Delivery

Imaging agent delivery has also been reported among the applications of colloidal PECs from HA and polycations, especially HA/PROT PECs. Xu et al. developed PECs from PROT in complex with HA and HA-SYL3C aptamer conjugation for delivering fluorescent catalytic hairpin assemblies (CHAs) as a novel type of nanoprobe for in situ detection of living circulating tumor cells (CTCs) in undiluted blood.^[171] The mechanism of this approach is that once the catalytic hairpins are uptaken and released within CTCs, miRNA-21 readily present in these cells will activate the amplification of CHAs, which will restore and intensify their fluorescence. As HA and SYL3C aptamers on the PEC surface can bind respectively to CD44 receptors and epithelial cell adhesion factors on CTCs, they would provide the nanoprobe with dual targeting capability in order to efficiently deliver the CHA systems into these cells. HA/PROT PECs were also employed by Xia et al. for delivering GSH-coated gold nanoclusters (AuNCs) as a theranostic agent into tumor cells.^[11] Their results showed that the stable complexes were formed based on electrostatic interactions between carboxyl groups of both HA and GSH on AuNC surface and amine groups of PROT as well as their hydrophobic interactions. The cytotoxic activity of AuNCs is based on the concept of photodynamic therapy, in which AuNCs are photosensitizing agents which can be transformed to excited state by irradiation and then react with intracellular triplet oxygen ($^3\text{O}_2$), that is, ground state oxygen, to produce cytotoxic singlet oxygen ($^1\text{O}_2$).^[172,173] In parallel, the return of AuNCs to their lower energy state can also emit fluorescence. Due to the above-mentioned interactions among HA, PROT and GSH, the nanocomposites demonstrated enhanced fluorescence emission and better singlet oxygen generation when compared to plain AuNCs, respectively leading to better imaging results and greater cytotoxic effect on tumor cells in vitro after the selective cellular uptake which was favored by specific binding of HA to CD44 receptors. In another work, Sim et al. reported the delivery of ferumoxytol-loaded HA/PROT nanocomplexes (HAPF) to natural killer cells (NK cells) in order to obtain HAPF-labeled NK cells, which can be subsequently administered to solid tumors for adoptive cell transfer therapy (Figure 10).^[8] Labeling NK cells with HAPF has two advantages: i) the possibility of exploiting magnetic resonance imaging (MRI) for guiding the transcatheter delivery of NK cells to tumors and ii) magneto-activation

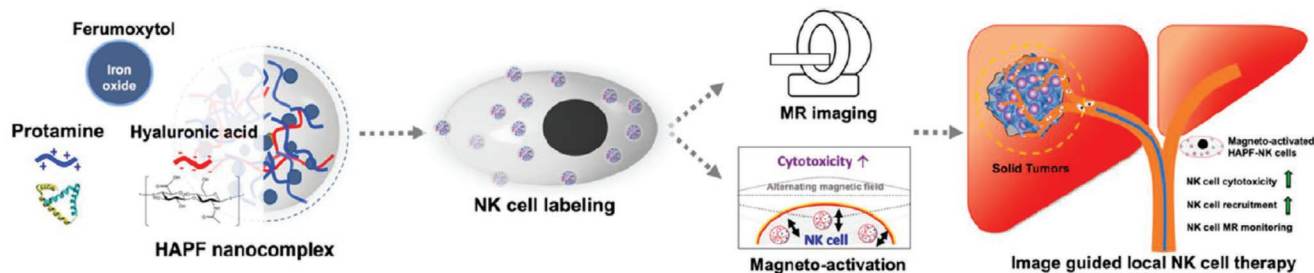


Figure 10. Schematic illustration of NK cell labeling with ferumoxytol-loaded HA/PROT PECs for MRI-guided local transcatheter delivery of NK cells in liver tumors and NK cell magneto-activation for antitumor effects. Reproduced with permission.^[8] Copyright 2021, American Chemical Society.

of NK cells,^[174] which means that applying an alternating magnetic field can lead to mechanical movements of HAPF in NK cells and subsequently activate their cytolytic function through a mechanical-sensing mechanism to attack tumor cells. The efficient attachment of HAPF to NK cells was ascribed to the presence of both HA and PROT in HAPF, leading therefore to the high labeling efficiency of NK cells as observed in the study. For proof-of-concept, the authors reported the successful transcatheter intra-arterial local delivery of HAPF-labeled NK cells in a hepatocellular carcinoma rat model with good accumulation of NK cells at the tumor site and significant suppression of tumor growth.

PECs between HA and disulfide crosslinked PEI were also utilized for theranostic purposes, for example, the delivery of hausmannite (Mn_3O_4) and hematite (Fe_3O_4) NPs as MRI contrast agents in combination with DOX and pDNA encoding Bcl-2 shRNA as therapeutic agents.^[12] The obtained nanoassemblies showed excellent MRI contrast by reducing artifact signals and significant toxicity on cancer cells by the synergistic effect of both pDNA and DOX, while the CD44-targeting effect of HA was necessary for the selective tumor cellular uptake of such nanocomplexes. In another work, near-infrared (NIR) fluorescent indocyanine green (ICG) as a fluorescent probe was delivered to tumor cells by using HA/PBAE PEC NGs, which could be effectively internalized within these cells through CD44-mediated cellular uptake and thereafter disassembled under the highly acidic condition of intracellular vesicles in order to liberate ICG from its quenched state and strongly recover its NIR fluorescence.^[175]

4.5. Multifunctional Platforms

With respect to dermatologic application of HA, the research group of Tokudome reported the use of nanoparticulate HA-based PECs as a novel approach for effective HA delivery into the skin.^[116,176] In a first approach, HA/PROT PEC NPs were investigated and showed the capability to effectively penetrate into the dermis layer of the skin both in vitro and in vivo while free HA could only reach the stratum corneum, that is, the outermost layer of the epidermis. Such studies further showed that after the penetration of HA/PROT PECs into the dermis, HA could be liberated in its free form and diminish the transepidermal water loss caused by UV irradiation.^[116] Following this work, the group continued the research with HA/PLL PECs in simulated skin environment in order to elucidate the skin pen-

etration pathway of HA-based PEC NPs,^[176] where the obtained results suggested that the better skin penetration of such NPs compared to free HA is due to the less hydrophilic characteristics of the particles as compared to free HA, which facilitates their penetration through intercellular lipids rather than trans-cellular pathway, while the subsequent release of free HA from these particles may be the result of PEC disintegration due to the high ionic strength under physiological conditions. It could be seen that for such topical administration, the disassembly of PECs under physiological salinity was indeed beneficial and necessary for HA release, in contrast with many works previously mentioned where it is a problem to overcome.

In regard to HA/CTS PECs, HA/CTS coacervates were shown to be promising scaffolds for cartilage tissue engineering since bone marrow stem cells can be encapsulated in such systems with long-term cell viability and well-spread cell morphology.^[13] In addition to drug delivery capability, coacervates from HA and lactose-modified CTS can also scavenge ROS produced by neutrophils during inflammatory processes since both HA and CTS have been shown to possess ROS scavenging effect.^[14] All of those results have highlighted the interest of HA/CTS coacervates for treating joint inflammation.^[14] HA/CTS PEC NPs were also reported for the fabrication of polysaccharide composite films by particle casting in a work of Yamazaki et al.,^[177] where the obtained films showed satisfactory mechanical properties with good adhesion to CD44-overexpressing cells and revealed therefore great potential as a material for biomedical and drug delivery applications.

Some nanocomplexes from HA and cationic proteins have also been described as emulsion stabilizers with more interesting properties than the proteins alone, for example in studies regarding HA/LF PECs.^[86,178] Such complexation was reported to enhance the thermal stability and antioxidant property of LF, while the emulsifying capability can only be improved upon covalent crosslinking between HA and LF.^[86] In a work of Freitas et al., anionic HA/zein PEC NGs were exploited as building blocks for fabricating colloidal gel assemblies through the electrostatic interactions between the former and cationic PEI-coated poly(D,L-lactide-co-glycolide) (PLGA) NPs (Figure 11).^[29] This concept was developed based on the usefulness of such a multiparticle assembly, including i) moldability to generate solid implants with desired shapes and ii) autonomous multiparticle shedding, that is, spontaneous and gradual release of the constituent NPs and NGs into the adjacent microenvironment to exert specific functions on neighboring cells. For proof-of-concept, HA/zein NGs encapsulating quercetin as a model

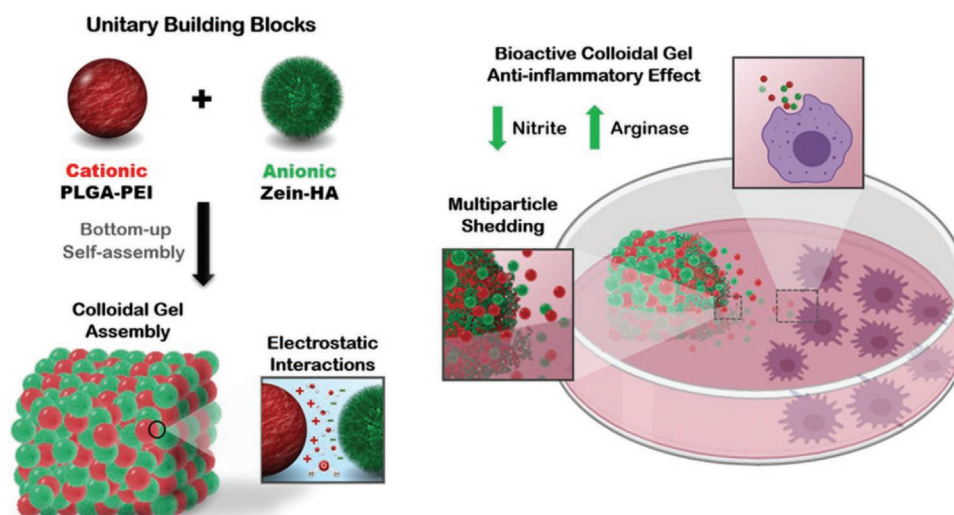


Figure 11. Bottom-up self-assembly of nanostructured colloidal gels through electrostatic interactions between PEI-coated PLGA NPs and HA/zein PEC NGs. Quercetin-loaded particles can be thereafter shed from the gel assembly and uptaken by neighboring macrophages to reduce their production of nitrite as a proinflammatory biomarker and increase the expression of arginase as an anti-inflammatory biomarker. Reproduced with permission.^[29] Copyright 2020, American Chemical Society.

drug were used to obtain the quercetin-loaded multicomponent gels for delivering this drug to macrophages *in vitro*, which showed thereafter high drug loading with significant improvement in anti-inflammatory activity of macrophages. Hsu et al. also described the inclusion of DOX-loaded nanoparticulate PECs from HIS-grafted HA and HIS-grafted PEI in Gellan gum microspheres for chemoembolization.^[40] Chemoembolization is a process widely used for treating liver tumors, in which anticancer agents at a high concentration are delivered into the tumors through a small catheter along an artery, which is then blocked off (embolized) by an embolizing agent (e.g., micro-particles) in order to deprive the tumors from blood supply and keep a long-term high concentration of the drugs in the tumors.^[179] The above-mentioned studies showed that the unitary PEC NPs can facilitate DOX delivery into liver cancer cells, while the microspheres can serve at the same time as an effective embolic agent and a reservoir for releasing the PEC NPs to cause necrosis in the embolized regions *in vivo*, showing thus the potential of this system for application in transcatheter arterial chemoembolization to treat liver tumors.

5. Current Limitations and Future Perspectives

Despite the huge biomedical potential revealed through tremendous advancements in research, HA-based colloidal PECs are regarded as relatively new systems and receive less attention than their counterparts from other polysaccharides, for example, CTS or alginate (ALG). PubMed database (PubMed.gov) shows only 185 articles related to HA-based PECs, while 915 and 354 articles are found for CTS-based and ALG-based PECs, respectively. In terms of clinical trials, according to ClinicalTrials.gov, there has been so far no clinical trial of HA-based colloidal PECs or even HA-based NPs in general, while there are at least 8 and 1 clinical trials regarding CTS-based and ALG-based NPs, respectively. Needless to say, due to the lack of clin-

ical evidence, no HA-based nanoformulation has reached the healthcare market, while commercialized HA-based products approved by FDA (US Food and Drug Administration) regard only HA concentrated solutions (e.g., Healon and Triluron) or HA-based films (e.g., Seprafilm) according to FDA Medical Device Database. These observations show that many critical aspects regarding HA-based colloidal PECs remain to be thoroughly understood before they can be recognized as safe and efficacious for human application. One big gap in the literature for HA-based colloidal PECs concerns their behaviors *in vivo*, in terms of both therapeutic efficacy and toxicity. Namely, very few works on HA-based colloidal PECs for small molecule drug delivery in Table 1 include *in vivo* results, rendering their therapeutic potential less convincing since the *in vitro* data may not reflect exactly the *in vivo* pathological conditions. The pharmacokinetic profile and therapeutic efficacy *in vivo* of the carried drugs should be therefore assessed. Nanotoxicology is another important issue receiving inadequate attention, which may prevent future clinical applications of HA-based colloidal PECs as well as all nanoformulations in general.^[180] Most of the biocompatibility studies for HA-based colloidal PECs have been realized through cytotoxic assays on healthy cells *in vitro* without considering their interactions with biological barriers and biomacromolecules during *in vivo* circulation. In this context, as mentioned in Section 3.2, the transport through biological fenestrations *in vivo*, the possibility of opsonization, the uptake by the mononuclear phagocytic systems, and the *in vivo* circulation time of HA-based PEC particles as well as the effects of particle characteristics (namely size, surface charge, and hydrophobicity) on these aspects are of pivotal importance and should thus be evaluated in order to verify the biocompatibility and optimize the *in vivo* stability of these nanocarriers.

Although both CD44-targeting effect and HAase-induced degradation of HA can improve tumor-targeting drug delivery as mentioned in the advantages of HA-based colloidal PECs (Section 2), the latter effect seems to receive much less attention.

HA-based colloidal PECs are often implied to be degradable by HAase without supporting empirical results.^[7,41,57] Indeed, the degradation of HA by HAase is not always the case for HA in complex state. Le et al. reported that HA/PLL PEC particles with PLL in excess ($n-/n+ < 1$) are stable against HAase, which is attributed to the protective effect of PLL on the particle surface.^[23] More interestingly, HA-based nanostructures may not only be stable against HAase but also inhibit this enzyme from degrading free HA, as reported by Duan et al. with micelles from HA grafted with poly(γ -benzyl-L-glutamate) as a potential HAase inhibitor for biomedical applications.^[181] Therefore, HAase-induced degradation should not be generalized for all HA-based colloidal PECs and needs to be evaluated case by case, which can verify their potential as a HAase-responsive system for tumor targeting or a HAase-inhibiting agent. Furthermore, when HA-based colloidal PECs are degradable by HAase, one may doubt whether such a degradation can be catalyzed by extracellular HAase in tumors and liberate free HA fragments that compete with PECs for interactions with CD44 receptors.^[182] This question is highly relevant as it can reduce the cell targeting effect of PECs and should be therefore verified. Additionally, free HA has been known for its low stability in the presence of multiple factors, including high temperature,^[183] extremely acidic or alkaline conditions,^[184] UV irradiation, and oxidizing species^[69,185] or ultrasound.^[186] Electrostatic complexation with polycations may alter the stability of HA against these factors, which should be an essential topic of investigation and potentially leads to unprecedented applications of HA-based PECs.

From several works regarding HA-based colloidal PECs mentioned in Section 4, it is undeniable that their most prominent potential concerns cancer treatment. However, anticancer applications of HA-based colloidal PECs have been mostly limited to the delivery of chemotherapeutic agents and NAs, while depending on CD44-receptor binding and responsiveness to tumor microenvironment factors like acidic pH, HAase, and glutathione for enhancing targeting effects. With the flexible utility of HA-based colloidal PECs, they should be applied to more advanced concepts of anticancer therapy. As can be seen in Section 4.4, the work of Xia et al. with intracellular delivery of gold nanoclusters (AuNCs) is seemingly the only one using HA-based colloidal PECs for cancer treatment through ROS generation.^[11] Intracellular delivery of Fe₃O₄^[8,12] or Mn₃O₄ NPs^[12] by means of HA-based colloidal PECs was also reported but only for bioimaging. It would be therefore interesting to verify their potential to treat tumor through ROS production, since Fe₃O₄ and Mn₃O₄ NPs have also been known for applications in chemodynamic therapy based on Fenton reactions, that is, interactions between metal-based nanomaterials and H₂O₂ abundantly present in cancer cells to generate cytotoxic hydroxyl radicals (\cdot OH).^[172,173,187] Furthermore, the recent emergence of photoresponsive^[188,189] and thermoresponsive^[190–192] HA derivatives may allow the elaboration of much more intriguing HA-based colloidal PECs with multi-stimuli-responsiveness, which can be applied for photo-triggered and/or thermo-triggered drug release and allow thus spatiotemporal control of drug delivery to target tumors.^[182]

Compared with oncologic applications, vaccine delivery and bacterial infection treatment are also topical and prominent

subjects but have been much less investigated for HA-based colloidal PECs. From little but encouraging results reported in Section 4.3 on the immunologic adjuvant properties of colloidal PECs from HA/poly-L-lysine-co-poly lactide,^[170] HA-SH/trimethyl CTS-SH^[96] and HA/CTS,^[100] more studies should be dedicated to HA-based colloidal PECs as a potential vaccine adjuvant. Particularly, while the works above concern only peptide or protein vaccine delivery, HA-based colloidal PECs have also demonstrated their capacity to deliver different types of RNA and therefore can be studied for RNA-based vaccines, which are emerging as a new vaccine generation.^[193] Those studies would not only shed more light on the immunological effects of HA-based colloidal PECs, which remain barely understood, but also be highly practical in the race to find the safest, most efficacious, and most economical vaccines against newly emerging human viruses, like SARS-CoV-2 and new Monkeypox virus variants. Likewise, HA-based colloidal PECs have not been widely studied for antibiotic delivery. From the bacterial targeting effect of HA suggested by Simonson et al. after their studies on HA/PLL colloidal PECs for treating *E. coli* and *S. aureus*^[36] (Section 4.1) and several recent works which reveal remarkable interest in HA-based nanoformulations for infection treatment,^[194,195] antimicrobial applications of HA-based colloidal PECs should be considered for more serious investigation, especially in the current era where more powerful therapeutic platforms are needed to cope with increasing antibiotic resistance.

6. Conclusion

HA-based colloidal PECs can be fabricated with a simple, rapid, and “green” process based on self-assembly of HA and polycations through electrostatic attraction. These systems combine several advantages from green chemistry, nanotechnology, and the exceptional characteristics of HA, namely excellent biocompatibility, flexibility in chemical modification, and specific biological properties including the CD44-binding capability and HAase-induced degradation. Despite a technically simple process of preparation, designing the structures of colloidal PECs to fit their intended applications and accordingly choosing the right parameters to achieve such desired characteristics are not straightforward. The performance of colloidal PECs should be dictated by their size, surface charge, and hydrophobicity, while these characteristics are governed by a complexity of interdependent parameters, including polyelectrolyte characteristics (MW and charge density), formulation composition (C_p , mixing ratio, pH, and counterions) and preparation technique (addition order and addition speed). It is therefore necessary to optimize these parameters and find the best compromise in order to obtain PEC particles with satisfactory properties. Through numerous research works, colloidal HA-based PECs have been proven as potential materials for biomedical platforms and drug carriers with continuous advancements in drug and nanocarrier stabilization as well as biological selectivity. Nevertheless, some fundamental aspects of these structures remain to be deciphered, namely their in vivo fate and toxicity, as well as the stability of HA in complex state. In addition, novel HA derivatives with stimuli-responsive characteristics can be applied in

the future to elaborate more high-performing PECs for targeted drug delivery. Last but not least, many potential applications of HA-based colloidal PECs remained insufficiently exploited and should be therefore studied more closely, especially for applications in chemodynamic therapy, vaccine delivery, and bacterial infection treatment.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

colloids, drug delivery, hyaluronic acid, nanomedicine, polyelectrolyte complexes

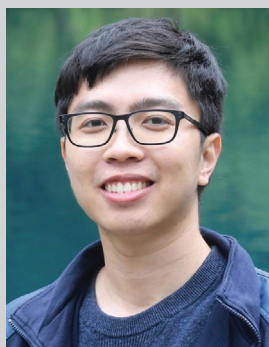
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