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A Review

Surface modifications of biodegradable polymeric nanoparticles and their characterization by advanced electron microscopy techniques

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Polymeric nanoparticles have been the focus for nanocarrier preparation in numerous biomedical applications such as cancer treatment, disease diagnosis, vaccination, in the last two decades. They have been variably surface modified using copolymers, Polyethylene glycol (PEG), dextran, cyclodextrin, cytokines, small molecules to improve their efficiency and efficacy. The resulting nano-formulations include polymer-protein conjugate, polymeric micelle, polymer-small molecule conjugate, dendrimer, polymeric vesicles, nano-hybrids, hydrogels *etc*. These may have intrinsic immunogenicity and require accurate characterization in order to improve their pharmacological targeting, pharmacokinetic profiles and to reduce adverse reactions. Therefore, we have reviewed the polymeric nanoparticles and the electron microscopy techniques available for their characterization in the context of their surface modifications and functionalization.

Keywords: Electron microscopic characteristics, Polymeric nanoparticles, Surface modifications

The nanocarriers available for biomedical use include; polymers, lipid carriers (liposomes/micelles), dendrimers, carbon nanotubes, silver, gold nanoparticles[1], quantum dots, organic nanoparticles, liposomes *etc*. Of these, polymers are the most common materials used for nanoparticle (NPs) based drug formulations (Table 1 & Fig. 1). Polymeric nanoparticles (10-1000 nm) are biodegradable, biocompatible, non-toxic, non-immunogenic and water soluble with potential application in tissue engineering, drug and gene delivery, imaging and vaccination strategies. Their action has been studied in cancer therapy at different steps: (i) immunomodulation; (ii) prodrug activation; (iii) anti-sense/ RNAi delivery, (iv) induction of apoptosis *etc*[2-4]. Recently the use of polymeric nanoparticles in vaccine design and delivery has gained interest (Table 2). Hydrogels embedded with nanoparticles are also being extensively studied due to their functional resemblance with the extracellular matrix (ECM)[5].

Polymeric nanoparticles are synthesized by multiple methods and influenced by a number of factors such as polymer DA, DP (degree of acetylation and polymerization), polymer concentration, surfactant used, and degree of crosslinking with surfactant. These result in enormous variation in NPs size, shape and chemical functionality[6]. The polymeric NPs are mainly spherical in morphology and comprise of nanocapsules and nanospheres[7]. They have been categorized based on their origin into: natural polymers such as chitosan[8], gelatin, sodium alginate (Table 1 & Fig. 1) and synthetic polymers like PLA (polylactic acid), polycyanoacrylate, PLGA {poly(lactide-co-glycolide)}, PCL (polycaprolactone), PHBV {poly (3-hydroxybutyric acid-co-hydroxyvaleric acid)}[9], PEI (polyethylenimine). Polymeric NPs are produced by dispersion of preformed polymers (*e.g.*, PLA) in an aqueous colloidal suspension or by polymerization of monomers (*e.g.*, polyalkyl cyanoacrylate)[10] which allows for insertion of drug compounds with greater efficiency[11]. Nanoprecipitation is the commonly used method for preparation of both nanospheres and nanocapsules[12] of around 170 nm dimensions[13].

The morphology (shape and size) of the polymeric nanoparticles is mainly determined by scanning and/or transmission electron microscopy (SEM and TEM) (Fig. 2). SEM generates visual information on external morphology, chemical composition and surface texture which is not quantitative. SEM provides limited information about size distribution of the particles and their pores. TEM is used to determine the size and shape

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Table 1 — Types of Polymeric nanoparticles and their surface modifications

Polymeric NP	Surface modifications	Size of NP (Diameter)	Shape on TEM/SEM	References
Natural Polymers				
Chitosan	LMW-PEI linked chitosan Chitosan-TPP	100–250 nm 100-1200 nm	uniform sphere Spherical	[8, 95-97]
Chitosan based polymeric micelles	Stearyl-grafted chitosan	120-160 nm	Spherical	[56]
Gelatin	Thiolated/ PEGylated/ Cationized Antibody/Peptide/ Carbohydrate/Fatty acid coated	100-200 nm	Nanocapsules, (having a hollow interior that is surrounded by a polymeric shell)	[98-99]
	Gelatin methacryloyl (GelMA) based hydrogels	μm		[100]
Sodium alginate	Alginate-chitosan NPs	50–80 nm	Spherical shape	[101]
Synthetic Polymers				
PLA (polylactic acid)	-None-	100 nm	Dehydrated hard sphere	[102, 9]
	PEI-coated PLA NPs	115 nm	Core shell	[103]
	Lactoferrin (Lf) conjugated PEG-PLA–NPs	131 nm	Spherical	[104]
	Polydopamine-modified (pD-TPGS-PLA/NPs)	205.2 nm	Spherical with smooth surface	[105]
PLGA poly (lactide-co-glycolide),	-None	152.0±58.08 nm	Spherical particles with smooth surfaces on SEM and core-shell structure on TEM	[106]
	Chitosan coated PLGA coated	284 nm	Spherical	[107]
	S2P Peptide-PLGA-Maleimide-PEG-NPs	183.3 nm	Spherical	[108]
	PLGA–PEG–PLGA	275.3 nm	Spherical with smooth surface	[109]
	Gelatin -PLGA composites	160 and 175 μm	microsphere	[99]
PCL(polycaprolactone)				
	Chitosan-PCL-NPs	230 nm	Spherical	[107]
	mPEG-PCL	36 nm	Spherical	[110]
	Polysorbate 80 (PS80)-PCL- NPs	100-200 nm	Spherical shaped with coating	[111]
PHBV {poly (3-hydroxybutyric acid-co-hydroxyvaleric acid)	-None-	243-260 nm	Core shell shaped spherical structure on TEM	[9]
	PEGylated PHB–sorafenib–doxorubicin NPs	199 nm	Spherical	[112]
Hydrogel nanoparticles (HNPs)/nanogels	Injectable and *in situ* gelling hydrogels	80-120 nm	Spherical	[113]
Lipid-polymer hybrid NPs	PLGA–lecithin–DSPE-PEG LPHNs		The lipid component, forms lipid "flowers", with "petals" extruding from the polymer core which exhibits "onion" morphologies, with multilamellar stacking	[73, 74]
Zwitterionic polymers	DSPE-PEG and DSPE-PCB_{20} cationic liposomes	80 nm	Spherical shape	[39]
Branched polymers	PEGMA5/DEA95–EGDMA15–DDT15 branched star shaped copolymers	10–30 nm	Spherical particles	[6]
Glycopolymers	di-block copolymers of PEG- FITC functionalized gold nanoparticles (AuNP)	7 nm	Spherical	[77]
Green NPs	AgNPs	20-50 nm	Spherical	[114]

of nanoparticles. It can measure the thickness of the nanocapsule wall and is used to distinguish between nanocapsules and nanospheres[14]. On TEM, nanospheres have a spherical shape, with a solid polymeric structure, whereas nanocapsules show a thin (about 5 nm) polymeric envelope around an oily core (Core-shell structure) (Fig. 2).

The polymeric nanoformulations undergo surface modifications by various covalent and non-covalent coupling techniques[15] which can extend their half-life, surface charge and improve drug efficacy. For nanospheres, the surface adsorption of drugs allows for a higher proportion of atoms to be in direct contact with solvents. The core-shell structure, in

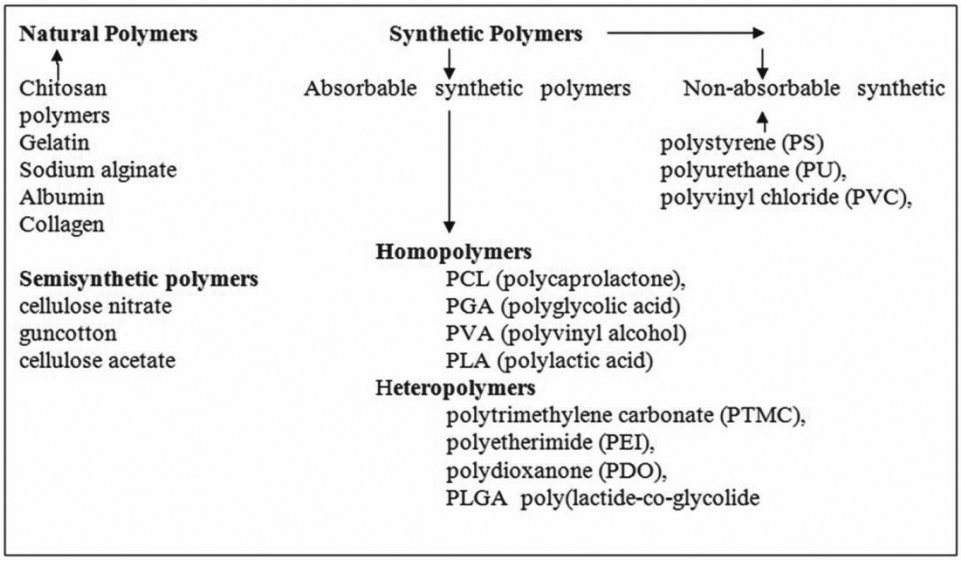

Fig. 1 — Biodegradable polymeric nanoparticles for biomedical use. These include the natural and synthetic polymers

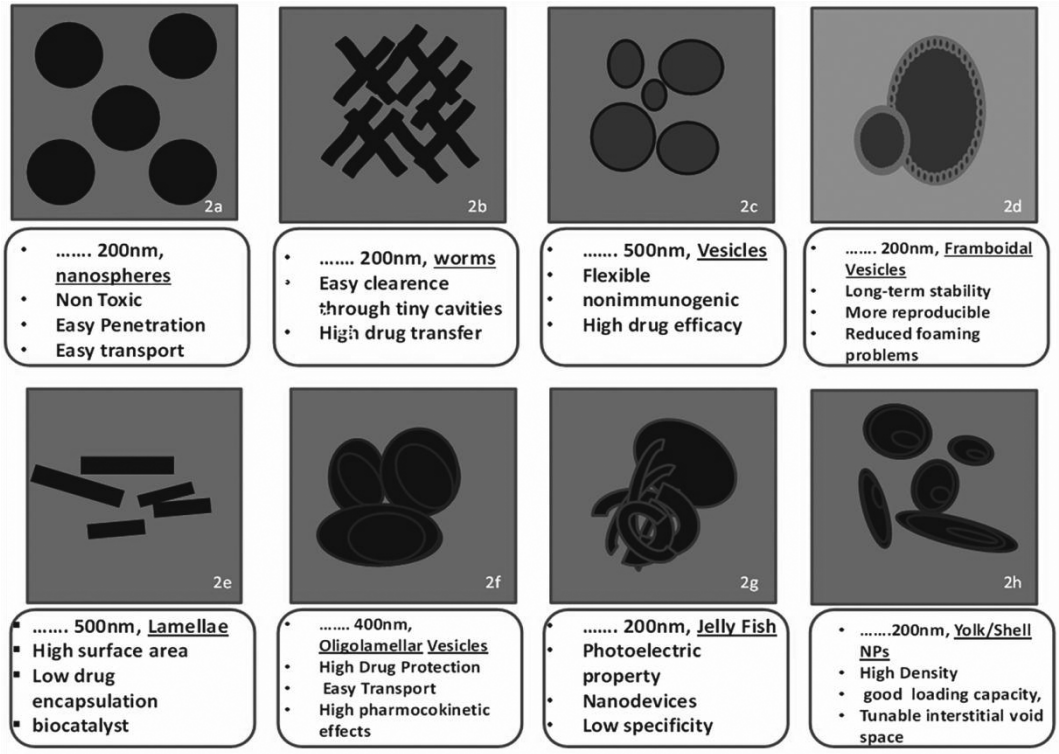

Fig. 2 (A-H) — Shape and size of polymeric Nanoparticles ranging from 200-500 nm on TEM and their advantages

nanocapsules, results in outer surface atoms different from those of the interior core in entrapped drug formulations. Therefore, in nanocapsules, the dual attachment of TNF-α in both the core and the shell of NPs is needed for their strong and specific binding to TNF receptor-expressing cells[16]. The solvent concentration, pH, temperature, and sonication additionally tune the morphology of polymer nanospheres and capsules[10]. The cationically charged polymers (Chitosan and PEI) produce more stable complexes during cellular trafficking[1] with high level of transfection efficacy and are widely used for nucleic acid delivery in a number of target organs[17,18] (Fig. 3).

Therefore, characterization of the nanoparticle morphology, their surface chemistry and growth kinetics by advanced electron microscopic techniques, such as, SEM-EDX/SAM {Energy Dispersive X-ray Spectroscopy (EDX) and Scanning Auger Microscopy (SAM)}, High resolution TEM/SEM (HRTEM, HRSEM), liquid TEM, cryo-TEM, which can characterize the morphology of NPs as well as their elemental-chemical composition are increasing in relevance. The utility of these advanced techniques in polymeric nanoparticle characterization is reviewed in the present paper (Table 2 & Fig. 4).

Types of Polymeric Nanoparticles

The polymeric nanoparticles for biomedical use have been broadly categorized into matrix-like and reservoir-type NPs[19]; (i) matrix-like NPs; Nanospheres (50-300 nm diameter) have a continuous polymeric matrix (drug can be retained inside or adsorbed on the surface) and (ii) reservoir type NPs: nanocapsules (100-300 nm), having central aqueous or oil reservoir and polymerosomes (60-500 nm)[20] (Fig. 2). Recently hybrid polymeric NPs have been designed to improve the circulation stability and for targeted delivery of

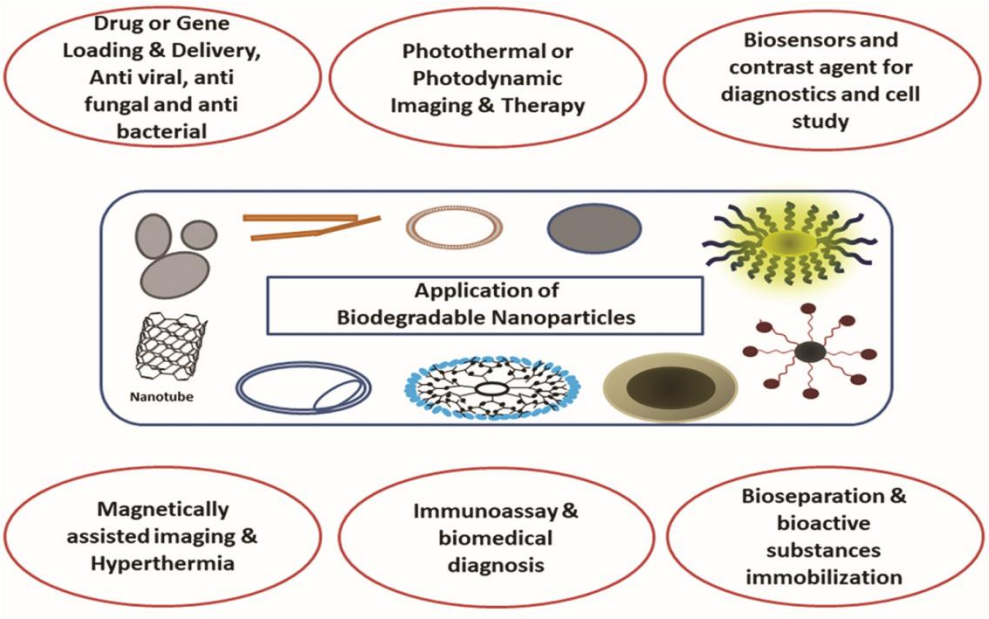

Fig 3 — Biological applications of polymeric nanoparticles

Table 2 — Polymeric Nanoparticles for vaccine delivery

S. No	Polymer	Size (EM/Zetasizer)	Disease	References
1.	PEG-2000	100-200 nm	COVID-19	[115]
2.	PEI-mannose	100 nm	HIV	[116]
3.	PLGA	164 nm	Poultry vaccine	[117]
4.	PLGA	200 nm	Mycobacterium tuberculosis	[118]
5.	PLGA	200 nm	H5N1influenza; HIV Helicobacter pylori	[119]
6.	PCL-Chitosan	208 nm	Hepatitis B	[120]
7.	PEG-PLA-PEG	242 nm	Hepatitis B	[121]
8.	Polyethyleneimine-triethyleneglycol	170 nm	HIV Infection	[122]

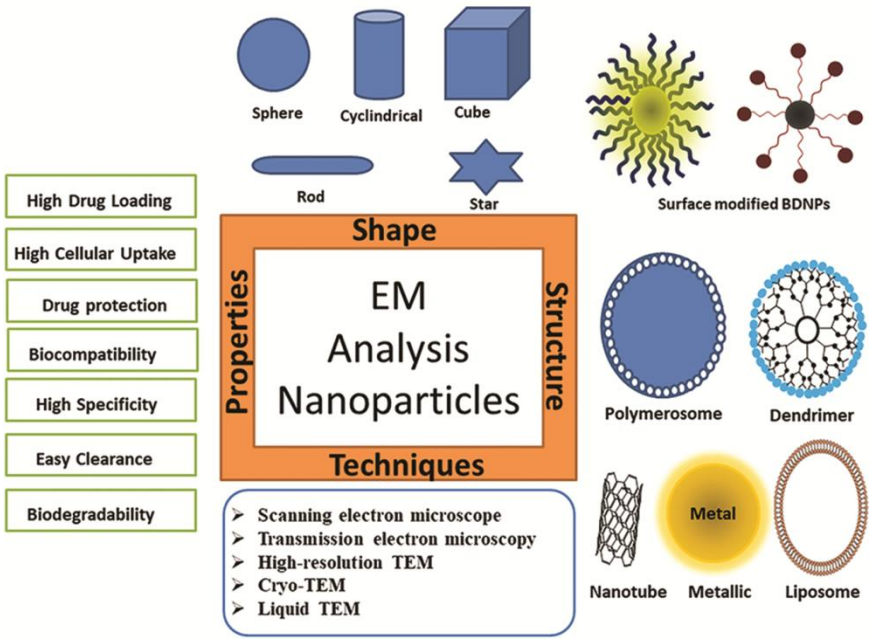

Fig. 4 — Surface modifications of biodegradable polymeric nanoparticles, their properties, morphology and their characterization by advanced electron microscopy techniques

chemotherapeutic agents using polymeric NPs[21]. Currently injectable in-situ gelling hydrogels that form highly branched hydrazone cross-linked (poly oligoethylene glycol methacrylate, POEGMA) are being assessed for biological applications[22,23].

Nanospheres are obtained when the active principle is dissolved or dispersed in the polymeric solution. Nanocapsules are obtained when the drug is previously dissolved in an oil, which is then emulsified in the organic polymeric solution before the internal phase is dispersed in the external phase of the emulsion[24,25]. Non-spherical polymeric nanoparticles with exotic morphologies, such as worms, vesicles, lamellae, framboidal vesicles, jellyfish, and yolk/shell particles, have been prepared by controlled radical synthesis technique, reversible addition-fragmentation chain-transfer polymerization[26-28]. The diagrammatic representation of these morphologies as seen by TEM is given in (Fig. 2).

Surface modifications of polymeric nanoparticles

Polymeric NPs are variably classified on the basis of their surface modifications into; polymer-protein conjugations with polyethylene glycol (PEG)[29] and PEG-alternatives[30] (Table 1), polymeric micelle[31,32], polymer-small molecule conjugation[33], polymeric vesicle[34], dendrimer[35], polymer-polymer NPs, polymer-lipid NPs, polymer-metal NPs (Fig.4).

Polymer-protein conjugations

Protein–polymer conjugates are widely used as therapeutics. These nanosystems are based on drug-loaded polymeric core and are additionally coated by a cross linked bovine serum albumin shell that reduces their interactions with serum proteins and macrophages. Therefore, these surface modified NPs can show potent anticancer activity *in vitro* and *in vivo* while not exhibiting any toxicity to healthy tissue[21]. The other polymeric-protein modified nanoparticles include; Zwitterionic polymers, glycopolymers, hydrogels, green NPs (Table 1).

Zwitterionic polymers

The Zwitterionic polymers include poly (carboxybetaine) (pCB) and poly(sulfobetaine) (pSB)[36]. These have been proposed as PEG alternatives due to their inherently low immunogenicity and high resistance to nonspecific protein absorption and agglomeration[37,38]. Recently, Li *et al*., 2015 developed zwitterionic poly (carboxybetaine) (pCB) modified lipoplexes for the delivery of siRNA therapeutics[39]. These PCB plated lipoplexes showed enhanced tumor accumulation *in vivo* while avoiding the ABC phenomenon. Zwitterionic polymers can undergo pH-responsive surface charge and size variations and spontaneously self-assemble to form micelle-like and inverse micelle-like assemblies depending on the solvent environment[40].

Hydrogels

Hydrogels have structural and mechanical similarity to the extracellular matrix. These branched polymers have secondary polymer chains cross linked to a primary backbone, showing a variety of polymer architectures such as star, H-shaped, pom-pom, and comb-shaped polymers[41]. SEM of the cross linked hydrogels shows a cellular structure with macropores of approximately 1 μm diameter. The topology of the hydrogel depends on the molecular shape (*e.g.* branched or circular polymers) polymer sequence, molecular weight, architecture, and chain connectivity of the precursor polymer[42]. Tuning the precursor polymer branch length and density results in single-component material with superior elasticity and extensibility and formation of three-dimensional cross-linked polymer hydrogels[43].

Green NPs

These nanoparticles show good biocompatibility as they are based on amino acid-based block copolymers and plant extracts. Green synthesis of metal NPs is recently gaining attention as a reliable, sustainable, and eco-friendly technique for synthesizing metal/metal oxides nanomaterials, hybrid materials, and bio inspired materials[44]. For *e.g.*; the silver and gold NPs which were synthesized using chemical methods[45-48] are being synthesized using green methods/sources, like bacteria, fungi, algae, and plant extracts[44,49], resulting in large-scale production with less contamination. These green synthesized-silver nanoparticles are 10-30 nm by TEM and SEM/EDS (Energy-dispersive spectra) revealed that these nanoparticles contain silver in its pure form[50].

Polymeric micelles (PMs)

PMs range from 10 to 100 nm, have a unique core-shell structure and are used for drug delivery of hydrophobic drugs. The inner hydrophobic core incorporates the poorly water-soluble drugs and is surrounded by hydrophilic shell[51,52]. Polymeric micelles are formed by electrostatic interactions, using charged block copolymers of oppositely charged macromolecules, resulting in the formation of micelles[53]. The commonly used core-forming blocks of PMs, include poly(propylene oxide) (PPO) which belongs to pluronics[54], poly(esters) such as poly(lactic acid) (PLA)[55], hydrophobic poly(amino acids)[56], copolymers of lactic acid and glycolic acids[57], poly(caprolactone) (PCL)[58] and chitosan[59]. Poly(ethylene glycol) (PEG) conjugation is mainly used as a hydrophilic block in micelles[60,61] to improve their *in vivo* stability[62], increase the half-live of the drug in the bloodstream, leading to less frequent dosing. PEGylated NPs become hydrophilic and attain near-zero zeta potential. PEGylation minimizes the attachment of serum proteins such as opsonins that confers an increased likelihood of NPs phagocytosis by the mononuclear phagocyte system[29]. However, PEG immunogenicity is a potential drawback. The anti-PEG immune response and formation of anti-PEG antibodies not only limits the efficacy of PEGylated treatment strategies[63] but hypersensitivity reaction to them, can be life threatening in some cases. This is driving the development of PEG alternatives[36], such as; poly (N-vinyl-2-pyrrolidone) (PVP)[64], poly (glycerols), poly (acrylic acid) (PAA)[65]. Modifications of PEG with bottle brush architecture/POEGMA are being evaluated to overcome PEG-associated accelerated blood clearance (ABC) phenomenon[66,67].

Polymer-small molecule conjugate

Polymer-small molecule conjugate used in nanomedicines include; N-(2-hydroxypropyl) methacrylamide copolymer, poly (glutamic acid), dextran, polybutadiene (a bilayer-forming polymer that can be cross-linked for enhanced vesicle stability)[68] and cyclodextrin (CD)-based polymers[69,32]. The drug is (i) covalently bound to the polymer carrier by chemical conjugation (*e.g.*, by hydrazone bond) or (ii) non-covalently entrapped using physical interaction, solubilisation, or polyionic complexation[60] or *via* metal-ligand coordination interactions[70]. The bio adhesive property of CD may facilitate in the drug permeability by increasing contact time of drug at surface of the mucosa. It is therefore being used for pulmonary, oral, ocular drug delivery and theranostics[71].

Polymeric vesicle

Polymeric vesicle also known as polymersomes, are self-assembled from amphiphilic block or graft copolymers to form hollow structures surrounded by a polymeric bilayer membrane or complicated interdigitated and amphiphilic membrane structures[72]. Ye, 2014 developed biodegradable polymeric vesicles as a nanocarrier system for multimodal bio-imaging and anticancer drug delivery. They fabricated poly(lactic-*co*-glycolic acid) (PLGA) vesicles encapsulated with inorganic imaging agents of superparamagnetic iron oxide nanoparticles (SPION), manganese-doped zinc sulfide (Mn:ZnS) quantum dots (QDs) and the anticancer drug busulfan[73].

Dendrimer

Dendrimer are synthetic polymeric macromolecules, composed of branched monomers that are characterized by low polydispersity and good biocompatibility[74]. These are spherical, well-designed branching polymers with interior cavities and abundant terminal groups on the surface which can form stable complexes with drugs, plasmid DNA, oligonucleotides, and antibodies[75]. Dendrimers are made from several different polymers, including polyamidoamines (PAMAMs), Poly (amidoamine-organosilicon) dendrimers (PAMAMOS), Poly(propylene imine) dendrimers (PPI), chiral dendrimers, liquid crystalline dendrimers, – tectodendrimers, hybrid dendrimers (Table 4 & Fig. 4), multilingual dendrimers, micellar dendrimers[35]. Poly (amidoamine) (PAMAM) dendrimers is most commonly used dendrimers. The modifiable surface of the dendrimers allows conjugation with different molecules, like targeting ligands or drugs. Previously, modified PAMAM dendrimers with surface amino groups conjugated to folic acids have been used for the delivery of methotrexate.

Lipid Polymer hybrid

Lipid Polymer hybrid (LPHNP), are hybrid nanoparticles, composed of shell and polymer core which reduce outward diffusion of the encapsulated drug and are emerging in popularity as for drug delivery. Their advantages include controllable ultra-small particle size[48], surface functionality, extremely high surface area to volume ratio, high drug loading, multiple therapeutic drugs, tunable drug release and good serum stability[76]. LPHNP are commonly formulated using-polymers-PLGA, PCL and zwitterionic lipids such as, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), (DOTAP) or 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)[74-76]. Dave *et al*., 2017 prepared LPHNP of Norfloxacin with polylactic acid and soya lecithin which exhibited an average particle size from 178.6 ± 3.7 nm to 220.8 ± 2.3 nm, and a surface charge ranging from +23.4 ± 1.5 mV to +41.5 ± 3.4 mV[80].

Characterization of polymeric nanoparticles based on electron microscopy techniques

Scanning electron microscope (SEM)

SEM analyzes the size, shape and surface morphology of the nanoparticles (Table 3 & Fig. 4). SEM provides finer surface structure images by operating at lower accelerating voltages and is advantageous when compared to TEM and cryo-TEM. Since the penetration and diffusion area of incident electrons is shallow, the number of secondary electrons emitted from the surface is maximized compared to backscattered electrons generated from within the specimen and the surface structures are clearly gained[81]. Ethyl acetate (EA), acetone (ACE), and dichloromethane (DCM) are organic solvents used to produce stable nanoparticles. NPs prepared using these solvents have discrete, spherical morphology with smooth surface and low porosity on SEM imaging[82]. Recent study by Rades *et al.*, 2014, has proven that combination of complementary techniques as SEM, T-SEM, EDX and scanning Auger microscopy (SAM) can be a powerful strategy for comprehensive morphological and chemical evaluation of the properties of nanoparticles[83].

Transmission electron microscopy (TEM)

TEM analyzes the morphologies of polymeric nanoparticles at low-to-medium magnifications (Table 3). TEM produces high-resolution, detailed images of 1 nanometer in size by using high voltages to increase the acceleration speed of electrons, which, pass through the sample and increase the image resolution. TEM resolution is hampered by spherical and chromatic aberrations. TEM investigations are mostly conventional and make use of mass-thickness contrast of selectively stained polymer samples for image formation. These stains include or provide better structural differentiation. Special techniques used for polymer imaging, including electron diffraction, high-resolution TEM, phase contrast transmission electron microscopy, low/high-voltage TEM, and scanning- TEM[84].

Generally, polymeric NPs smaller than about 200 nm diameters, are dispersed onto a carbon-coated grid for TEM inspection. These are composed of only low atomic number elements with similar density. Mass-thickness contrast in chemically untreated polymers can be caused by varying specimen thickness, alternatively, the selective staining of one (or more) of the components by a heavy metal oxide is required for the TEM examination of block copolymer systems. Since the aggregation of NPs can change their physical properties, therefore, TEM has been applied to characterize the dispersion of NPs after their internalization. An additional advantage of TEM is that it allows the assessment of the changes of subcellular structures caused by the NPs[85].

The main disadvantages of TEM are difficulty in quantifying large number of particles, difficulty in characterizing very homogenous samples, researcher training and image artifacts resulting from specimen preparation[85]. Furthermore, traditional TEM cannot be used to study the growth of NPs in solution. It is not possible to directly correlate diameters by TEM in the dry state with hydrodynamic diameters in solution. Therefore, the TEM diameters need close correlation with DLS[6].

High-resolution TEM (HRTEM)

HRTEM uses phase-contrast imaging, and combines both transmitted and scattered electrons to produce the image[86]. HRTEM has become the most common technique to characterize the internal structure of NPs. For *e.g.*, HRTEM has been used to study the effect of ligands in the final structure of NPs. HRTEM can give information regarding NP growth and structure-related properties. However, characterization of NPs is not always feasible by this technique. This is caused by the random orientation of the crystals relative to the electron source, resulting in poor alignment and formation of complex images that cannot be directly used to define the structure[87].

Cryo-TEM

Cryo transmission allows for the specimen of interest to be viewed at cryogenic temperatures (Table 3). Cryo-TEM assesses the morphology, two-dimensional fluidity, lipid shell in nanoparticles in near-unaltered samples in their frozen-native environment by vitrifying them at cryogenic temperatures[88]. The morphology and volume transitions of thermo-responsive core–shell NPs can be imaged by cryo-TEM. cryo-TEM achieves sub-nanomolar resolutions of morphology of the thermosensitive shell without staining[89].

Liquid TEM

In 2003, Williamson *et al.*, developed a TEM liquid cell using epoxy-sealed silicon nitride (SiN) membranes[90]. Liquid TEM allows the characterization of NPs within fluids is under constant movement. It allows for the tracking of the nanoparticle trajectory while this is growing, providing direct observation of the

Table 3 — Electron microscopy of polymeric nanoparticles

Technique	Information obtained	Limitations of Technique	References
SEM	Widely used method to (i) detect and define size and size distribution of NPs, (ii) To visualize NPs in 3D, their dispersion in matrices/supports	conventional SEM imaging mode cannot detect NPs on the back side of the support film The lateral resolution of T-SEM is limited to NP sizes down to 5–10 nm.	[85]
T-SEM-EDX	(i) By using transmission in SEM (T-SEM) surface as well as in depth analysis of NPs is performed, (ii)SEM-EDX/SAM Energy Dispersive X-ray Spectroscopy (EDX), and Scanning Auger Microscopy (SAM) characterizes the elemental-chemical composition of NPs and size of NPs, (iii) It gives precision in lateral dimensions of NPs	Needs a very high-sensitivity EDS detector with a very large active area for unambiguous detection of core-shell characteristics of silica based NPs.	[126]
HRSEM	High-resolution SEM(HRSEM) images the morphology of Au NPs and their dispersion in cells and tissues It can scale down and study the specific spatial arrangements of nanometric elements in their biological context and examine the possible interactions between the two	In biological specimens, metal coating is necessary to decrease charging artefacts. This increases the risk of radiation damage to the samples.	[85,127]
TEM	Most common technique to (i) define NP size, shape, interparticle distance-aggregation state, monodispersity of NPs, (ii) characterize nanocomposites (*eg*. Quantum dots, metals and magnetic NPs) and change in their structure by change in surface charge, (iii) Characterizes Growth kinetics of NPs	difficulty in quantifying a large number of particles or misleading images due to orientation effects, aggregation of NPs during the drying of the colloid suspension	[128-130]
HRTEM	High-resolution TEM (HRTEM) additionally (i) characterizes the crystal structure of nanoparticles, (ii) It distinguishes monocrystalline, polycrystalline and amorphous NPs, (iii) characterize polymer nanocomposites (PNCs), (iv) used to study NP defects	HETEM needs high voltage, the resultant increased temperature, affects the surface quality of the PNC. Therefore, for imaging PNCs- SEM provides 3D image and is preferred	[131,132]
Liquid TEM	Depicts NP growth in real time, Characterises Growth kinetics of NPs	studies single particle motion, super lattice formation	[133]
Cryo-TEM	Characterises Growth kinetics of NPs, their mechanisms, aggregation pathways	It avoids the development of artefacts or destroyed samples	[134]

nanoparticle evolution. However, it suffers from lower image resolution, due to the SiN membrane and liquid layer thickness, which scatters the electron beam[91].

Conclusion

The polymeric nanoparticles are biodegradable and have half-life based on its interaction with biological system. This interaction is defined by their size, morphology and unique set of physical (optical, magnetic, electronic and catalytic) and chemical properties (pH, surface charge)[92-97]. These properties significantly contribute to their pharmacological targeting, their pharmacokinetics in the body, by influencing various physicochemical mechanisms such as their diffusivity, interactions with biological materials, internalization by cells, functionalization *etc*. Thus emphasizing, the need for a meticulous characterization of newly synthesized polymeric nanoparticles by advanced electron microscopy techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Transmission scanning electron microscopy (T-SEM), High-resolution SEM (HRSEM), High-resolution TEM (HRTEM), scanning TEM (STEM), liquid TEM, cryo-TEM.

Conflict of interest

All authors declare no conflict of interest.

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