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Effects of Size and Surface Charge of Polymeric Nanoparticles on *in Vitro* and *in Vivo* Applications

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Abstract

Biodegradable polymeric materials are the most common carriers for use in drug delivery systems. With this trend, newer drug delivery systems using targeted and controlled release polymeric nanoparticles (NPs) are being developed to manipulate their navigation in complex *in vivo* environment. However, a clear understanding of the interactions between biological systems and these nanoparticulates is still unexplored. Different studies have been performed to correlate the physicochemical properties of polymeric NPs with the biological responses. Size and surface charge are the two fundamental physicochemical properties that provide a key direction to design an effective NP formulation. In this critical review, our goal is to provide a brief overview on the influences of size and surface charge of different polymeric NPs *in vitro* and to highlight the challenges involved with *in vivo* trials.

Keywords

Nanoparticle, Size, Surface Charge, *In Vitro*, *In Vivo*

1. Introduction

Manufacturing effective drug delivery system is a critical challenge in nanomedicine since nanocarriers are expected to reach and accumulate in the site of interest. As a consequence, numerous drug delivery systems have been investigated *in vitro* and *in vivo* to deliver a wide range of drugs and molecules. To conquer the challenge, with the aim of avoiding uncontrolled biodistribution, rapid clearance and systemic toxicities in healthy tissues

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polymeric nanoparticles (NPs) have gained higher interest among all the novel formulations. Research during the past few decades proves their beneficial features in formulation design, characterization, behavior and application [1]. The *in vitro* and *in vivo* fate of NPs is particularly depended on uniformity of particle size and zeta potential. Change in these properties has significant biological implications on cellular internalization, pharmacokinetics, and bio-distribution [2]. These characteristics of NPs facilitate the opportunities for therapeutic application, which can be confirmed by *in vitro* and *in vivo* studies [3]. The aim of most nano-devices is to prevent the degradation of drug followed by higher bioavailability in cellular level and to regulate its pharmacodynamics profile. Thus, the nanomedicine platforms could serve as a drug delivery system that is able to transport a high dose of therapeutics selectively to the desired site of action. Although very few investigations have been performed, most of the articles related to exploring the effects of size and surface charge of NPs have been discussed in this review. This review provides details on the fate of different polymeric NPs and will discuss how the size and surface charge of polymeric NPs are involved in desired effects for both *in vitro* and *in vivo* applications. Moreover, other polymeric NPs using various preparation methods have been also summarized in **Table 1**, which could be considered for further size and surface charge related experiments.

Table 1. Size and surface charge overview of different polymeric NPs.

Polymer type	NPs preparation method	Loaded materials	Stabilizer	Cell line	Size (nm)	Zeta (mV)	References
Chitosan	Chemical reaction	-	-	Caco-2	(418 ± 31)-(531 ± 54)	(13.6 ± 0.8) - (29.4 ± 0.8)	[50]
Chitosan	Ionotropic gelation	Covalent conjugation with fluorescein-5-isothiocyanate	-	A549 cells	195	35.5	[51]
Chitosan (Galactosylated)	Carbodiimide method	DNA	-	HepG2, HeLa	100 - 240	−5	[52]
Chitosan {Arg-Gly-Asp (RGD) peptide-labeled}	Ionic gelation	siRNA	-	SKOV3ip1, HeyA8, A2780, A2780ip2, MOECs	200	−40	[53]
Chitosan, PS, PEG-PLA	Multiple emulsion-solvent evaporation	-	Sodium Cholate	Caco-2, MTX-E12	(196 ± 20)-(290 ± 7)	(−23.9 ± 1.2) - (1.1 ± 1)	[7]
Chitosan-PEG	Ionic gelation	Caspase inhibitor pebptide	-	Mice	150	16	[54]
Gelatin	Desolvation	Anti CD3 mAb	-	HeLa	250 - 300	−20	[55]
Heparin	Coupling reaction through amide linkage	Paclitaxel, Folic acid	-	KB-3-1, Tu212	60	−16	[56]
Hyaluronic acid (Self-assembled)	Chemical conjugation through Carbodiimide method	-	PBS	SCC7, CV-1	237 - 424	-	[57]
Hyaluronic acid-ceramide	Self-assembly	Docetaxel	Pluronic P85	U87-MG, MCF-7, MCF-7/ADR	110 - 140	−20	[58]
Hydrophobically modified glycol chitosan	Self-assembly	-	-	HeLa	359	22	[59]
Mesoporous silica	-	-	-	3T3-L1, HMSCs	108 - 115	(−4.9) - (19)	[60]
Methyl ether poly(ethylene glycol)-modified poly(beta-amino ester)	Solvent casting	Doxorubicin	-	B16F10	62	-	[61]
NAcHis-GC (self-assembled)	-	Paclitaxel	PBS	HeLa, A549	150 - 250	-	[62]
P(MDS-co-CES)	Self-assembly	DNA, Paclitaxel, Indomethacin, Pyrene	-	HEK293, HepG2, MDA-MB-231	(83 ± 1) - (180 ± 2)	(44 ± 2) - (84 ± 5)	[63]
PCL	Solvent displacement	Tamoxifen	Pluronic® F-68	MCF-7	250 - 300	6 - 25	[64] [65]
PCL	Emulsification diffusion	-	-	-	120 - 125	(−32) - (−60)	[66]

Continued

PEG	Top-down strategic PRINT technique	Conjugated antibody	-	HeLa, Ramos, H460, SK-OV-3, HepG2, LNCaP	(267 ± 49) - (292 ± 76)	(−35.6 ± 1.3) - (39.9 ± 1.7)	[67]
PEG-PCL	Dry-down method	-	-	MDA-MB-468, MCF-7	(26.4 ± 0.7) - (60.9 ± 0.7)	(−5.1) - (−7.3)	[68]
PEG-PCL	Solvent evaporation	Paclitaxel	Sodium Cholate	U87 MG, BCECs	<100	(−3.08 ± 0.94) - (−3.28 ± 0.75)	[69]
PEG-PHDCA	Nanoprecipitation	Nile red	Pluronic F68	RBECs	140 - 146	−20	[70]
PEG-PLA	Solvent displacement	-	Solutol® HS 15	HeLa	89.8 ± 4, 96.4 ± 6	32.8 ± 8, −26 ± 1	[71]
PEG-PLA	Emulsion-solvent evaporation	BSA, Coumarin-6	Sodium Cholate	Rat BCECs, Astrocytes	80.4 - 84.4	(−8)-(−17)	[72]
PEG-PLA	Solvent displacement	Coumarin-6	Solutol® HS 15	MDCK	(89.8 ± 4) - (96.36 ± 6)	(−29 ± 7) - (45.46 ± 2)	[73]
PEG-PLA	Nanoprecipitation	Cisplatin	-	A2780	(86 ± 2)	−33 ± 1.2	[74]
PEG-PLGA	Simple emulsion, Interfacial deposition	Paclitaxel	Sodium Cholate	HeLA	190 ± 4.5, and 112 ± 4	−7.76 ± 2.6, −0.556 ± 5.7	[75]
PEG-Trimethyl Chitosan	Self-assembly utilizing electrostatic interactions	Insulin	-	Caco-2	(203.9 ± 7.2) - (376.4 ± 10.3)	(7.4 ± 0.3) - (21.4 ± 0.3)	[76]
PEO-b-PMA	-	Cisplatin, Doxorubicin	-	A2780, A549	(93.8 ± 8.4) - (176.5 ± 6.0)	(−29.2 ± 1.5) - (−2.1 ± 1.0)	[77]
PLA	Diafiltration	Trans-retinoic acid	Poly(vinyl benzyl lactonamide)	Hepatocytes	287.7	-	[78]
PLA	Emulsion-solvent evaporation	Rhodamine, Selectin grafted	Sucrose solution containing Tween 20, PEG-Laurate	HUVEC	173 ± 23, 168 ± 37	−25.7, −19.2	[79]
PLA	Solvent evaporation	Doxorubicin	-	SLK	45 ± 8 - 47 ± 4	-	[80]
PLA, PLGA	Multiple emulsion-solvent evaporation	Plasmid DNA	PVA	MCF-7, PC-3	(270 ± 1) - (1207 ± 30)	(−6.5 ± 2) - (−31.3 ± 2)	[81]
PLA, PLGA	Solvent deposition	Docetaxel	Tween 80	-	(100.7 ± 2.9) - (179.4 ± 1.7), (98.7 ± 1.7) - (172.0 ± 4.9)	(−38) - (−24), < (−10)	[82]
PLGA	Double emulsion-solvent evaporation	DNA	PVA	COS-7, HEK-293	(70±2)-(202±9)	(−20.6 ± 2) - (−20.8 ± 2)	[83]
PLGA	Multiple emulsion-solvent evaporation	BSA	PVA	HASMC	380 - 522	(−0.8 ± 2.3) - (−15.4 ± 0.8)	[84]
PLGA	Emulsion-solvent evaporation	Paclitaxel	PVA	MCF-7 and MCF-7/Adr	216	−8.12 ± 2.8	[85]
PLGA	Double emulsion-solvent diffusion	Cystatin	PVA	MCF-7, MCF-10A neoT, Caco-2, U-937	320 - 360	−25	[86]
PLGA	Emulsion-solvent evaporation	BSA, Coumarin-6	PVA	HUVEC	277 - 372	−10.4	[87]
PLGA	Emulsion-solvent evaporation	Paclitaxel	PVA	A549, H1299, CCL-186	(228.2 ± 0.6) - (330.7 ± 2.9)	(−4.3 ± 0.3) - (−17.9 ± 1)	[88] [89]
PLGA	Emulsion-solvent diffusion	FITC-WGA, FITC-BSA	PVA	A549	246, 356	~(−4)	[90]
PLGA	Solvent evaporation	Camptothecin	PVA	HCT116	(116.5 ± 1.6) - (187.1 ± 1.1)	-	[91]
PLGA	-	Loperamide, Rhodamine-123	-	Tail vein in rats	140 - 180	−20	[92]
PLGA	Interfacial deposition	Paclitaxel	Poloxamer 188	NCI-H69, SCLC	(117 ± 2) - (160 ± 2)	(−33.4 ± 1.8) - (−23.1 ± 3.7)	[93]
PLGA	Direct dialysis	Paclitaxel	Vitamin E TPGS	C6 glioma	(280 ± 28) - (310 ± 28)	(−20) - (−40)	[94]
PLGA	Modified emulsification-solvent diffusion	Docetaxel	PVA	T47D, MCF-7, SKOV3, A549	172 - 178	−12.2 ± 0.6	[95]

Continued

PLGA	Nanoprecipitation	Doxorubicin	BSA	MDA-MB-231	230	−45	[96]
PLGA	Emulsion-solvent evaporation	Indocyanine green, Doxorubicin	PVA	-	(137 ± 2) - (164 ± 2)	(−9.9 ± 0.4) - (−12.3 ± 0.1)	[97]
PLGA	Nanoprecipitation	Curcumin	PVA	A2780CP, MDA-MB-231	(76.2 ± 5.36) - (560.4 ± 10.95)	(−0.06 ± 0.01) - (0.06 ± 0.01)	[98]
PLGA	Emulsion-solvent evaporation	Docetaxel, Poloxamer 188	PVA	MCF-7, TAX30	(217.6 ± 8.6) - (274.7 ± 4.1)	(−23.35 ± 1.17) - (−41.28 ± 2.89)	[99]
PLGA	Emulsion-solvent evaporation, Salting-out	Vincristine, Verapamil	PVA	MCF-7, MCF-7/ADR	98.8 ± 8.4	−0.75 ± 0.12	[100]
PLGA	Nanoprecipitation	Hypericin	PVA	NuTu-19	210.3 - 268.9	(−3.7) - (−7.9)	[101]
PLGA	Solvent diffusion (Nanoprecipitation)	Paclitaxel	PVA	C6 glioma	169.3 ± 4.16) - (182.8 ± 3.78)	(−3.45 ± 0.58) - (−11.72 ± 2.27)	[102]
PLGA	Solvent extraction/ evaporation	Paclitaxel	PVA, Vitamin E TPGS	HT-29	293.6 ± 4.8, 235.7 ± 14.8	−26.05, −35.60	[103]
PLGA	High pressure homogenization	Paclitaxel	PVA	C6 glioma	(245.0 ± 20.4) - (305.4 ± 10.6)	-	[104]
PLGA	Nanoprecipitation	Sialic acid, N-acetylneuraminic acid	Pluronic F68, Polysorbate 80	CD14+ human monocytes	(63 ± 5) - (190 ± 16)	(−23 ± 3) - (−38 ± 3)	[105]
PLGA	Solvent injection	Aromatase inhibitor	-	SKBR-3	170.3 ± 7.6	−18.9 ± 1.5	[106]
PLGA	Emulsion-solvent evaporation	Paclitaxel	-	Ch-hep-3	(119 ± 6.2) - (129 ± 5.2)	(−4.6 ± 0.3) - (−31.5 ± 1.2)	[107]
PLGA	Double emulsion-solvent diffusion	BSA	PVA	MCF-7, MCF-10A neoT, Caco-2	320 - 360	−25	[108]
PLGA	Single emulsion solvent evaporation	Rapamycin	PVA	MCF-7	274 ± 1.6	−13.8 ± 5.1	[109]
PLGA	Modified solvent extraction/ evaporation	Paclitaxel	PVA	Caco-2, SK-BR-3	(293.8 ± 5.7) - (312.3 ± 8.2)	(−35.07 ± 1.68) - (−21.24 ± 2.11)	[110]
PLGA	Double emulsion-solvent diffusion	PE38KDEL, a 38 kDa mutant form of *Pseudomonas* exotoxin A	PVA	D2F2/E2, SK-BR3, D2F2	(108.3 ± 13.9) - (124.2 ± 21.2)	(−36 ± 5) - (12 ± 7)	[111]
PLGA	Emulsion-solvent extraction/ evaporation	Rhodamine	PVA	HEK293, TE671	(400 ± 100) - (550 ± 90)	(−0.96 ± 0.01) - (−2.9 ± 0.2)	[112]
PLGA	Emulsion-solvent evaporation/ extraction	FITC-TT peptide, DQ-BSA	PVA	Granulocytes, PBMCs	202 ± 4, 239 ± 14	(−28.6 ± 0.4), (−44.9 ± 1.8)	[113]
PLGA	Double emulsion-solvent evaporation	siRNA	PVA	DCs	350 - 390	(−13) - (−19)	[114]
PLGA	Double emulsion-solvent evaporation	-	PVA	DCs	(328 ± 30) - (511 ± 26)	(−25) - (−46.4)	[115]
PLGA	Solvent evaporation	Rapamycin, TMRD Dextran	PVA	DCs	150 - 450	-	[116]
PLGA, PLA	Emulsion-solvent evaporation	Dexamethasone, Futamide	PVA		240, 270	19 to 28	[117]
PLGA, PLA	Double emulsion-solvent evaporation	BSA, Coumarin-6	PVA	VSMCs, HASMCs, HA-VSMCs	69 - 98	(−5.3) - (−23)	[118]-[120]
PLGA, PLGA-PEG	Solvent diffusion	Conjugated cyclo(1,12)PenITDGEATDSGC (cLABL) peptide	PEMA	HUVEC	177 ± 11, (202 ± 11) - (268 ± 19)	−40.2 ± 3.7, (−31.4 ± 4.0) - (−8.3 ± 0.9)	[121]
PLGA, PS	Emulsion-solvent extraction/ evaporation	Coumarin-6	PVA, Vitamin E TPGS	Caco-2	(261.6 ± 9) - (295.4 ± 15), 50 - 500	(−36.76) - (18.38)	[27]
PLGA-PEG	Nanoprecipitation	Avidin, Streptavidin, Neutravidin, Coumarin-6	-	N18-RE-105, b.End3, HepG2	(111.1 ± 1.8) - (255.2 ± 6.3)	(−0.497 ± 0.402) - (−32.07 ± 3.19)	[122]

Continued

PLGA-PEG	Nanoprecipitation	Docetaxel	-	LNCaP	80-200	-	[123]
PLGA-PEG	Nanoprecipitation	Cisplatin	-	LNCaP, PC3	(131 ± 0.5) - (172 ± 3.4)	-	[124]
PLGA-PEG	Emulsion-solvent evaporation	Conjugated peptide, Coumarin-6	-	bEnd.3	(104.17 ± 3.45) - (121.46 ± 0.76)	(−24.43 ± 0.22) - (−18.25 ± 0.88)	[125]
PLGA-PEG	Modified double emulsion	Cisplatin	Sodium Cholate	LNCaP	(134.3 ± 5.2) - (159.8 ± 6.2)	(−5.7) - (−9.3)	[126]
PLGA-PEG	Solvent-diffusion	Doxorubicin	PEMA	MDA-MB-231, B16F10, MCF-7	(366.6 ± 3.1) - (423.0 ± 16.6)	(−18.9 ± 2.4) - (−51.7 ± 3.1)	[127]
PLGA-PEG	Nanoprecipitation	Paclitaxel	-	HUVEC	(114 ± 3) - (146 ± 2)	(−0.36 ± 4.3) - (0.12 ± 3.6)	[128]
PLGA-PEG	Solvent evaporation	Paclitaxel	PVA	JC, NCI/ADR-RES or MCF-7	(221 ± 5) - (240 ± 1)	(−18 ± 5) - (−35 ± 5)	[129]
PLGA-PEG	Nanoprecipitation	Docetaxel	-	LNCaP	(153.3 ± 13.9)	−42 ± 1	[130]
PLGA–PEG	Emulsion-solvent diffusion	Docetaxel	PVA	SKOV3	(120 ± 5) - (216 ± 18)	(−6.27 ± 0.95) - (−12.2 ± 0.6)	[131]
PLGA-PEG-Aptamer	Nanoprecipitation	Docetaxel	-	LNCaP, PC3	(160 ± 3.7) - (291 ± 5.2)	(−20) - (−29)	[132]
PLGA-PEI	Solvent displacement	Plasmid DNA	Poloxamer-188	Calu-3	207 - 231	(32.1 ± 6.7) - (58.8 ± 4)	[133]
PLGA-PEI	Solvent evaporation	Paclitaxel	PVA	JC	(228 ± 22) - (237 ± 16)	(−12.1 ± 0.3) - (−24.0 ± 0.5)	[134]
PLGA-TPGS	Solvent extraction/ evaporation	Docetaxel	TPGS	Caco-2, MCF-7	(219.42 ± 5.24) - (253.51 ± 5.38	(−21.87 ± 2.11) - (34.1 ± 4.28)	[135]
PLGA-TPGS	Solvent extraction/ evaporation	Doxorubicin	-	MCF-7, C6 glioma	(324 ± 5) - (359 ± 10)	-	[136]
PLGA-TPGS)	Modified solvent extraction/ evaporation	Docetaxel, Coumarin-6	TPGS	HeLa	(207.15 ± 8.46) - (290.25 ± 7.64)	(−15.22 ± 2.21) - (−32.10 ± 0.65)	[137]
PMB, PMBH	Emulsion-solvent evaporation	Doxorubicin, Paclitaxel	-	HeLa	242 ± 10, 218 ± 9	−2.0 ± 0.5, −2.0 ± 0.6	[138]
Poly (ethylene oxide)-modified poly (beta-amino ester)	Solvent evaporation	Paclitaxel,	-	SKOV-3	60 - 150	40	[139]
Poly (β-amino esters) {Arg-Gly-Asp (RGD) peptide coated}	Electrostatic self-assembly	DNA	-	HUVEC	200	−5	[140]
Poly (β-malic acid)	-	Antisense oligonucleotides	-	U87MG, TG98, MDA MB-231.	6.6 - 24	(−27 ± 1) - (−5.2 ± 0.4)	[141]
Poly (β-malic acid)	-	Antisense oligonucleotides	-	BT-474, SKBR-3, MDA-MB-231, MDA-MB-435, MDA-MB-468, MCF-7	(15.1 ± 1.2) - (22.1 ± 2.3)	(−4.1 ± 0.4) - (−5.7 ± 0.6)	[142]
Poly (γ-glutamic acid)-Poly (lactide)	Emulsion-solvent evaporation	Paclitaxel	-	HepG2	(115.4 ± 4.2) - (263.2 ± 6.8)	(−19.2 ± 2.2) - (−22.5 ± 3.2)	[143]
PS (Carboxyl-modified fluorescent)	Commercial	-	Commercial	HeLa, HUVEC	24 ± 4, 43 ± 6	-	[25] [144]
β-Cyclodextrin (Transferrin conjugated)	Polycondensation	DNA	-	PC-3, K562	100 - 150	15	[145]

Abbreviation: BCEC: Brain capillary endothelial cells; BSA: Bovine Serum Albumin; DCs: Dendritic Cells; HASMCs: Human arterial smooth muscle cells; HA-VSMCs: Human aortic vascular smooth muscle cells; HMSCs: Human mesenchymal stem cells; HUVECs: Human umbilical vein endothelial cells; MOEC: Murine ovarian endothelial cells; NAcHis-GC: N-acetyl histidine conjugated glycol chitosan nanoparticles); P (MDS-co-CES): Poly (methyldiethene-aminesebacate)-co-[(cholesterylox-ocarbonylamidoethyl) methylbis (ethylene) ammonium bromide] sebacate; PBMCs: Peripheral blood mononuclear cells; PBS: Phosphate buffer saline; PEG: Poly (ethylene glycol); PEG-PHDCA: Poly (methoxypolyethyleneglycol cyanoacrylate-co-hexadecylcyanoacrylate); PEMA: Poly (ethylene-maleic anhydride); PEO-b-PMA: Poly (ethylene oxide)-b-poly (methacrylic acid); PLA: Poly (lactic acid); PMB: Poly [2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA); PMBH: Poly [2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA)-co-methacryloylhydrazide (MH)]; PVA: Polyvinyl Alcohol; RBECs: Rat brain endothelial cells; TPGS: Tocopheryl polyethylene glycol succinate; VSMCs: Vascular smooth muscle cells; WGA: Wheat germ agglutinin.

2. Polymeric NPs

For an ideal drug delivery system, recognition of the polymer's potentiality has been evaluated since 1960's [4]. Over the past few decades, two main classifications of polymers have been discovered as synthetic and natural, each with various types and sub-types. Synthetic polymers are chemically synthesized based on repeated structural units, whereas natural polymers are obtained from natural sources. Primarily two types of polymeric NPs have been developed for drug delivery purposes *i.e*. nanocapsules, in which a core of encapsulated drug is surrounded by polymeric membrane or shell; and nanospheres, where drug is distributed/adsorbed throughout a matrix [5]. The most important feature of polymers is the degree of biodegradability, which is an important criterion to differentiate some slowly biodegradable polymers such as polystyrene (PS), poly (cyanoacrelates) (PCA), polyethylenimine (PEI) and poly (methyl methacrylate) (PMMA) [6]-[11]. On the other hand, some synthetic polymers such as poly (ε-caprolactone) (PCL), poly (lactide) (PL), poly (glycolide) (PGA), poly (D, L-lactide-co-glycolide) (PLGA), and some non-synthetic polymers (e.g. chitosan) are categorized as readily biodegradable materials [12]-[16]. Polymeric NPs are capable to maintain high stability in systemic circulation with enhanced half-life, which can be further optimized by controlling the release of therapeutic agents from the NPs. Moreover, polymeric molecules have various solubility profiles in wide range of solvents. This is advantageous for surface modification or functionalization to achieve different purposes of delivery and targeting. Subsequently, both doses and frequency of administration of therapeutic agents can be reduced due to high payloads into nanocarriers, leading to superior efficacy and minimizing the side effects. Besides, polymeric NPs of desired physicochemical properties are capable of preserving their content from hepatic metabolism, enzymatic degradation and rapid clearance. Specifically, the enormous surface area of polymeric NPs is an attractive feature to control the release kinetics, drug loading capacity and administration route, which can regulate the fate of drug into the body [17]. However, only few of them have been approved by health regulatory agencies for human trial to apply for carrying a wide range of diagnostic and therapeutic agents to the desired site of action [18].

3. Effect of Particle Size and Surface Charge Based on *in Vitro* Studies

Different types of NPs have been widely applied as drug delivery vehicles for diagnostic and targeted therapy (active or passive) to achieve maximum cellular uptake and therapeutic bioavailability [19] [20]. Continuous physicochemical changes in the development of polymeric NPs may have substantial implications in the cellular internalization and biological processes [21]. The experiments performed to evaluate the influence of particle size and surface charges of NPs are expected to explain how these physicochemical properties influence the cell uptake through various pathways towards optimum biodistribution.

Cellular internalization or uptake is the most important physicochemical criteria prior to *in vivo* application. Uptake of small molecules by any cells depends mainly on endocytosis among all other mechanisms (**Figure 1**). Endocytosis is the bulk active transport process through lipid bilayer wrapping using energy in the form of ATP to form required vesicles. Two main endocytosis mechanisms are reported as phagocytosis and pinocytosis [22]. Phagocytic cells (e.g. macrophages, neutrophils, dendritic cells, etc.) mediated cellular internalization is mostly involved with engulfing the large particles (>1 μm) [23]. Adsorption or receptor dependent internalization is the main mechanism of pinocytosis, which is mainly related to particle uptake by the cells through different pathways such as macro-pinocytosis, clathrin mediated, caveolin dependent or independent pinocytosis [3]. Size and surface charge of polymeric NPs are likely the preliminary physicochemical variables, which govern the endocytosis dependent cellular uptake. Besides, positive charge of the surface of polymeric NPs may endorse more cellular attachment causing higher uptake either by endocytosis or by direct penetration, since cationic surface of polymeric NPs interacts with anionic terminal of phospholipid, proteins and glycans on the cell surface due to the electrostatic interactions [23].

An interesting experiment by Bhattacharjee *et al*. demonstrated the effects of size and surface charge of fluorescent, monodisperse tri-block co-polymeric NPs based on cellular uptake through different endocytotic pathways [24]. They synthesized polymeric NPs (PNPs) with two different sizes (45 and 90 nm) and surface charges such as neutral (PNP-OH, −4 mV), positive (PNP-NH_2, +22 mV) and negative (PNP-COOH, −19 mV) to observe the *in vitro* cellular uptake into NR8383 (rat macrophage) and Caco-2 (human colonic adenocarcinoma) cells. For size dependent cellular uptake, a relative uptake study was carried out, which revealed the higher intracellular uptake by positively charged polymeric NPs with lower size compared to the other formulations.

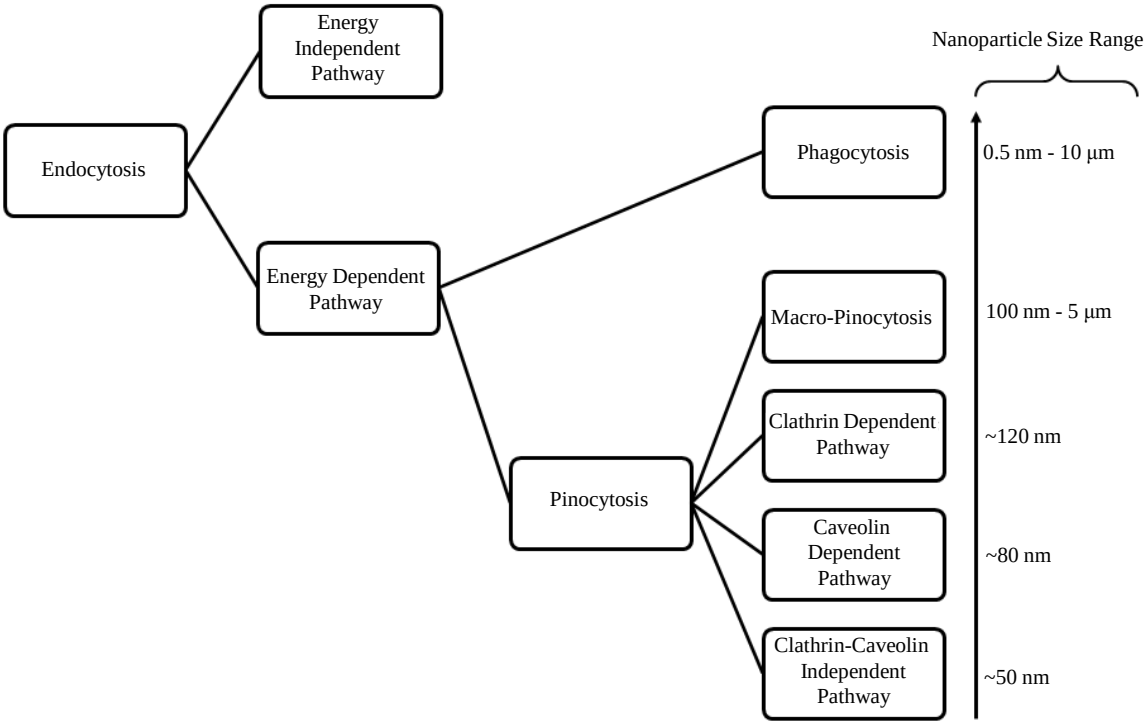

Figure 1. Relative sizes of NPs favorable for ingestion through various endocytotic pathways.

Inhibition of endocytic pathways was adopted to observe the role of endocytosis based cellular internalization of polymeric NPs tracked by two mechanisms such as decreasing temperature to 4˚C of experimental unit and exposing cells with 2-deoxyglucose (2-dOG) and sodium azide (NaN_3). Both inhibitory approaches showed considerably lower uptake, which proved the higher uptake by positive charged polymeric NPs compared to that of neutral to more negative charged polymeric NPs. Followed by the same strategy to block the clathrin and caveolin mediated endocytosis, cells were exposed with hypertonic 450 mM sucrose solution and methyl-beta-cyclodextran, respectively. Meeting the claimed fenestration sizes of these receptors dependent endocytosis, both inhibitions resulted with reduced uptake with smaller size after treating these cells with polymeric NPs, however the uptake was varied with charge variations. For clathrin dependent endocytosis, uptake by both neutral and negatively charged polymeric NPs was higher (65% and 75%, respectively) than positively charged polymeric NPs (less than 38%), however an opposite result was found for caveolin dependent endocytosis.

On the other hand, Lai *et al.* investigated that polystyrene (PS) NPs with smaller size (>42 nm) were successfully internalized into HeLa cells following clathrin and caveolin independent endocytic pathways avoiding endosomal or lysosomal accumulation [25]. Recent studies revealed that positively charged NPs uptake was related to energy dependent process such as proteins dynamin and F-actin but negatively charged NPs were not dependent on dynamin proteins around the cell membrane [26]. Moreover, highly positively-charged NPs could cause perforations in the cellular lipid bilayer to enter the cells by-passing endocytic pathways [23].

Another *in vitro* study for both fluorescent PS NPs and Coumarin-6 NPs in Caco-2 cells by Win *et al.* was performed to assess the effects of different polymeric NPs size [27]. Raw Coumarin-6 could not increase the cell uptake, however fluorescent PS NPs of 100 nm to 200 nm size showed the highest percentage of uptake. Smaller particles (50 nm) showed the lowest uptake and particles as large as 1000 nm showed decrease in uptake, which could be attributed to the uptake by other cellular mechanisms.

Optimization of antigen delivery to human dendritic cells (DCs; antigen presenting cells) is a challenge for advanced vaccine delivery systems. To identify the effects of particle size and surface charge on human DCs, *in vitro* cell uptake study has been investigated by Foged *et al*. in 2005 [28]. They designed the experiment based on wide size ranges (0.1 µm to 4.5 µm) of fluorescent PS NPs with different surface charges (+12.4 to −66.9 mV) after surface modification. Flow cytometric analysis of DCs after 24 hour incubation showed that lower percentage of DCs had taken up 4.5 µm particles (30%); whereas the highest cellular uptake (60%) was ob-

served for 0.5 μm and 0.1 μm sized particles. To optimize the charge dependent interactions, particles with two sizes (0.1 and 1 μm) were modified by attaching variety poly amino acids/proteins covalently utilizing surface amine and carboxyl groups. Sterically same positive and neutral charges particles were obtained using polypeptides poly-l-lysine (PLL) and poly-d-l-alanine (PA), respectively. After 24 hours incubation, only 10% cellular uptake was observed with negatively charged 1 μm size particles, whereas positive charged particles were accounted for 60% uptake. However, around 90% uptake was observed for lower size (0.1 μm) particles with positive charge.

Prior to *in vivo* administration, it is essential to consider the compatibility, safety and biodegradability of the particles with the human blood and cells. To investigate the efficiency of particle size and surface charge in *in vitro* cellular uptake and blood compatibility, recently Dash *et al.* employed chitosan/polyglutamic acid hollow spheres to treat human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (HUASMCs) [21]. Enhanced cellular uptake has been observed with 100 nm neutral charged (−4 mV) in both cells such as 76% in HUVECs and 56% in HUASMCs compared to the other larger as well as pegylated particles regardless of surface charge. However, negatively charged particles showed the least cell internalization in both cases. To measure the effects of particles with erythrocytes of human blood, percentage of hemolysis was accounted towards different sizes and charges of particles. All types of particles were partially associated with very insignificant consequence on hemolysis (1% or less) without considering either size or surface charge. But, highly anionic charged particles of smaller size resulted insignificant delayed clotting time and platelet activation profile compared to larger particles and other types of charged particles.

Testing blood compatibility of polymeric NPs with human blood is another way for finding the probable adverse effects, which may happen after *in vivo* administration. To rationalize the hemo-compatibility test, another research group (Mayer *et al.*) employed PS NPs with variety of sizes and surface charges in different mediums (such as cell culture medium with different FBS ratio, PBS) [29]. To assess the influence of polymeric NPs' size and surface charge on human blood, the aim of study was to monitor the adverse effects by measuring complement activation, induction of coagulation, thrombocyte activation, membrane integrity, granulocyte activation, and hemolysis using flow cytometric analysis. Complement (C3a and C5a) levels detection is a consideration of the body's immune system activation. Cationic amidine PS particles were involved with high C3a generation (150.8%). Irrespective to size and surface charge, no NPs were involved with prothrombin level induction. CD62P/CD42b labelling was employed to investigate the thrombocyte activation, which was tested for both low (0.5 mg/mL) and high (2 mg/mL) concentrations. But no thromocytic damage was observed, which were confirmed by no lactate-de-hydrogenase (LDH) release for any of the particles. The percentage of CD11b expression (marker for granulocyte activation) for particle's different sizes and surface charges was reported in that study. Increased percentage of hemolysis for all types of particles was reported using high concentration of particle treatment with human blood. However, larger particles were found less hemolytic than smaller particles, and the most important point was that no influence was observed for negatively charged 160 nm size NPs on erythrocytes of human blood by treating with lower concentration. Overall, positively charged larger particles were involved with more hemolysis compared to negatively charged particles and the latter ones larger than 60 nm size appeared to be less hematotoxic than smaller particles. One interesting finding was; particles resuspended in cell culture medium with 10% fetal bovine serum (FBS) showed less negative zeta potential or about to close to neutral charge compared to the particles resuspended in phosphate buffer saline (PBS). The presence of salts and proteins in the dispersion cell medium might be accountable for neutralizing surface charge of polymeric NPs.

Upon exposure of different types of PLGA NPs to different experimental media, Mura *et al*. also investigated the possible size and zeta potential variations after resuspension of polymeric NPs in different media with time dependent incubation up to 96 hours at 37˚C [30]. Three types of medium such as water, cell culture medium plus 10% FBS and PBS have been considered for evaluation in this experiment. Among different media, water and cell culture medium containing 10% FBS were not involved with significant variations in particle size regardless of surface charge, however after incubation of PLGA/chitosan (CS) NOS in PBS the size was increased. Furthermore, upon exposure to serum containing cell culture medium, PLGA/CS, PLGA/polyvinyl alcohol (PVA), and PLGA/pluronic F-68 (PF-68) NPs did not show any noteworthy change in zeta potential values.

They also designed *in vitro* model to investigate the toxicity of these prepared three types of NPs with Calu-3 cell line derived from human bronchial adenocarcinoma. This cell line could be a representative bronchial epithelial barrier associated with the discharge of airway mucus substances and the moderation of inflammatory

reaction [31] [32]. Cell viability responses due to NP treatment with higher concentration after 72 hours incubation demonstrated that only PLGA/PF68 NPs showed progressively decreased cell viability compared to other types of NPs.

From other *in vitro* studies, it has also been found that NPs with 40 - 50 nm size range are involved with maximum uptake [33] [34]. However, a recent experiment by Schadlich *et al.* revealed the effect of size for the accumulation of near-infrared (NIR) fluorescent consisting PLA-PEG polymeric NPs in two tumor xenograft models (HT29 colorectal carcinoma and A2780 ovarian carcinoma) utilizing *in vivo* fluorescence imaging technique [35]. NPs of 111 nm and 141 nm size showed higher biodistribution and accumulation in tumors compared to the larger size (166 nm), which was due to rapid clearance of the larger particles by liver.

4. Effect of Particle Size and Surface Charge Based on *in Vivo* Studies

To explore the *in vivo* effects of specifically sized NPs with respect to surface charge, Kulkarni *et al*. injected the fluorescent modified and unmodified PS NPs into Sprague–Dawley rats after physicochemical characterization [36]. Modification of PS NPs was performed by coating with D-α-tocopheryl polyethylene glycol succinate or Vitamin E TPGS, which was able to switch the zeta potentials of different size NPs to less negative charge.

As previously known, circulating mononuclear phagocytic cells in the bloodstream are the key component of reticuloendothelial system (RES). In addition, RES is also composed of matured cells such as macrophages mainly available in lungs, liver and spleen [37]. Studies have shown that the NPs with the size range of 100 to 200 nm could be the optimum range in order to escape the RES recognition [27]. Due to rapid clearance from systemic circulation, mostly uncoated NPs were distributed to those organs such as liver and spleen, where mononuclear phagocytic system is located. Consequently, 100 and 200 nm size fluorescent PS-TPGS NPs resulted in higher fluorescence concentration in blood plasma regardless of their surface charges. Liver and spleen were the main target organs, where a substantial decrease in NP distribution was observed for all sizes of TPGS modified PS NPs, since hydrophilic coated surface (stealth effect) possesses the ability to prevent the NPs from RES capture.

Moreover, He *et al.* in 2010 investigated the effects of size and surface charges on the biodistribution of different sizes of rhodamine B (RhB) labeled carboxymethyl chitosan grafted NPs (RhB-CMCNP) and chitosan hydrochloride grafted NPs (RhB-CHNP) having negative and positive surface charges after intravenous administration into H-22 tumor bearing mice [2]. It was clearly demonstrated that biodistribution of 150 nm size NPs having zeta potential of around −15 mV showed higher accumulation at the tumor site and long residence in blood compared to more negative or positive or even larger particles. Due to inflammation and disorder of endothelium along with high demand of nutrient supply, comparatively larger vascular leakage is found in solid tumors, which can provide more access for extravascular targeting macromolecules [38]. Low anionic charge (−15 mV) bearing RhB-CMCNP-PS particles exhibited higher percentage of distribution in tumor, which might be due to enhanced residing time during systemic circulation [39]. On the other hand, more positive charged NPs could leave the interstitium more competently after arriving the tumor's leaky vasculature leading to be up taken by tumor cells or endothelium adjacent to the endothelium [40]. This phenomenon might be the possible reason why high cationic charge (+35 mV) bearing RhB-CHNP particles showed higher percentage of distribution in tumor. Due to enhanced permeability and retention (EPR) effects, smaller particles might be favorable to target the tumor passively due to higher accumulation. However, blood's complement activation system and blood opsonins have been found to fabricate the size of polymeric NPs to a larger extent (>500 nm) resulting rapid blood clearance [39]. RhB-CMCNP and RhB-CHNP with larger particle size resulted in higher hepatic disposition. Such higher hepatic disposition could be explained by the investigation performed by Liu *et al.* They found that NPs with the size range above 300 nm were inclined to be blocked or captured by RES as well as liver sinusoids [41]. In addition, NPs with the size range from 200 to 500 (nm) was found mainly unaffected by the splenic physical filtration mechanism [42]. This could be attributed to obtain lower percentage of hepatic distribution of RhB-CHNP NPs with the particle size range from 150 to 300 (nm). Besides, no influence was observed in distribution of both RhB-CMCNP and RhB-CHNP particles in kidney owing to their size and surface charge. Due to electrostatic reactivity, positively charge particles had tendency to form aggregates with the cells and proteins present in blood and subsequently the aggregation could be trapped by lung [43]. As this experiment revealed, He *et al.* demonstrated the similar result where more cationic RhB-CHNP NPs distribution was found in lung.

NPs of 10 - 100 nm size is considered as mainly accepted range to design any NP formulation respective to suitable clearance and biodistribution profile before any *in vivo* trial [3]. However, the upper range of particle size is dependent on the interactions with body's immune systems and the lower range is determined by the limit of kidney filtration. Opsonization of larger particles by responsible proteins (e.g. plasma complement, immunoglobulins) in blood compartment is common to develop hypersensitivity response comparatively against larger foreign particles [44] [45]. On the other hand, smaller particles (<5.5 nm) have been found with rapid clearance from the body by kidney's glomerular filtration mechanism [46].

To explore the *in vivo* effects of different size of NPs, Liu *et al*. prepared radioisotope labeled liposomes of different sizes (30 - 400 nm) to inject into the mice models to observe the biodistribution in blood, liver, spleen, and tumor [47]. After four hours of post administration, it was found that about 60% of 100 to 200 nm size particles were found in blood, but only 20% of injected particles with size boundary (>250 nm or <50 nm) were detected in blood. In liver, particle size with 100 nm was associated with 20% accumulation, whereas around 25% distribution in liver was detected for larger particles. In spleen, 40% - 50% of the injected dose was detected for larger size (>250 nm) but the percentage of detection was lower for the particle size range below 100 nm. In 2002, Levchenko *et al.* prepared liposomes of around 200 nm with variety of charged surfaces to evaluate the tissue distribution in mice models [48]. The results from this study showed that the negatively charged liposomes with zeta potential of around −40 mV were involved with higher clearance rate from the blood in comparison to liposomes with neutral zeta potential.

In addition, Yamamoto *et al*. investigated the effect of surface charge of poly (ethylene glycol)-poly (D, L-lactide) block copolymer micelles after injecting into male C57/BL6N mice through the tail vein [49]. They prepared the micelles with both neutral (tyrosine) and negative (tyrosineglutamine) functionalities, which did not show any significant variations in blood clearance kinetics. However, the negatively-charged micelles displayed a significant lower distribution in both liver and spleen after four hours of post intravenous injection. Overall effects of NPs size and surface charge could be summarized in **Figure 2**.

5. Conclusion

In conclusion, polymeric NPs with size range from 10 to 200 nm might not only escape renal filtration and biliary excretion but also accumulate in tumor utilizing EPR effects. Size range above 200 nm may be related to rapid hepatic clearance and RES recognition. Pegylation strategy could be an ideal option to stealth the polymeric NPs for longer residing time during systemic circulation. After *in vivo* administration of cationic

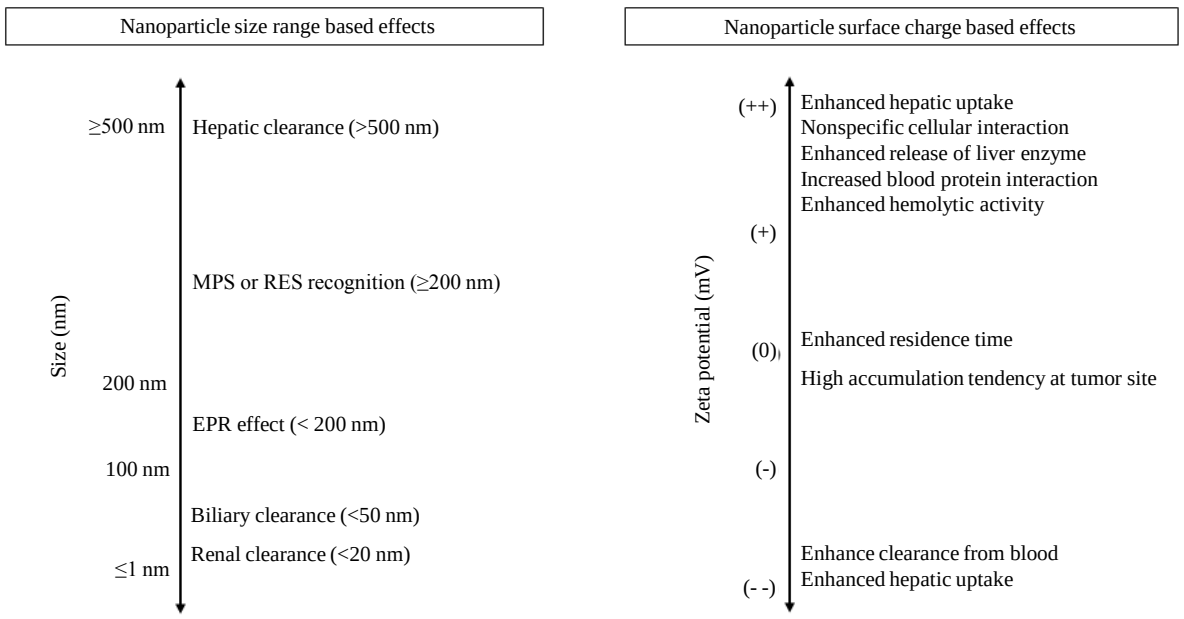

Figure 2. Relative biocompatibility of polymeric NPs based on the effects of size and surface charge. Abbreviation: MPS (Mononuclear Phagocyte System), RES (Reticuloendothelial System), EPR (Enhanced Permeability and Retention).

polymeric NPs, non-specific interaction may occur with non-specific cells or opsonizing protein in blood compartment due to electrostatic bindings, which may involve unexpected cytotoxicity. In order to reduce such non-specific surface reactivity or interaction, relatively less negatively charged anionic (almost neutral) polymeric NPs with desired small size might be more rationale than cationic charged particles for a broad spectrum biological aspect. This review will help researchers to correlate the *in vitro* and *in vivo* effects of polymeric NPs based on particle size and charge. Further investigation and correlation of other physicochemical parameters could be performed on polymeric NPs to understand their biological effects.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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