Review Article

Strategies for the Suitability of Self-assembled Supramolecular Polymersomes in Drug Delivery and Diagnostics

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ABSTRACT

Amphiphilic copolymers which can self-organize into distinct nano vesicles comprising of hydrophilic core and a hydrophobic bilayer membrane with innumerable morphologies have wide applications ranging from cell mimics to diagnostics. The flexibility to control the size, shape, morphology, functionality and surface properties makes polymersomes widely acceptable. The suitability of these systems for various biomedical applications exhilarated because of its robust responsiveness to both internal and external stimuli at the site of interest. The biodegradability with lesser toxicity adds up to its acceptability as an innate cargo molecule. A large share of research works published on the polymersomes in recent years showcase this vesicle as a future device of choice for targeted drug delivery and bio imaging. But the effectiveness of these systems meticulously relays on the precise engineering of physical, morphological and surface characteristics supported by thorough understanding on the various aspects of its designing. With an intention of accelerating multiple dimensions of polymersome research, this article focuses on recapitulating those controllable essential attributes of polymersomes. It also reveals how changes in these variables contribute to polymersomes becoming an important potential freight moiety for a variety of biomedical relevence.

Keywords: Polymersomes, PEG, Amphiphiles, Payload, Stimuli.

INTRODUCTION

Synthetic analogues and models of cell membranes using natural and artificial moieties have greater importance in bioscience. Since many years the researchers are enthusiastically exploring this territory to expose an ideal alternative to the natural cell membrane, which can compartmentalize to different intracellular organelle like components. These cellular mimics can be successfully used for fruitful targeting of therapeutic agents, improving the frameworks for tissue/regenerative engineering and offering superior precision with uncompromised quality in diagnostics. But many developed models and systems faced problems with low efficiency, significant toxicity, polydispersity and poorly understood delivery mechanisms. Various strategies have been proposed to counteract these drawbacks. The efficiency of these systems relay on the techniques for the fabrication of moieties with lessened toxicity, inclusion of cell targeting strategies, surface guarding, additional transport provinces for effective and definite delivery, and enhanced chemistry for syntheses of polymers with exceptional surface characteristics.[1]

Alternative cell membrane assembly can be prepared from lipids, amphiphilic block polymers and surfactants. Liposomes prepared from lipids of various functional groups have greater resemblance to the bi-layered cell membrane.[2] A lot of importance was given to research in development of liposomes, but these systems were proven to be sensitive and unstable, despite of its greater biocompatibility. So it become essential to extend the research to find more

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suitable, easily achievable bio-membrane alternatives. This has fuelled the research in the direction of polymer-based self-assembled nanostructures.

Copolymers and block polymers prepared by various polymerization techniques caught up the attention of the scientific world because of the ease of preparation and its ability to form well-defined periodic self-assembled structures with controllable nano size.[3] In recent times a lot of research work published on functional block polymers and its allies. Polymersomes are one of those versatile and useful engineered hollow vesicular host moiety comprising of hydrated hydrophilic core surrounded by a bi-layer membrane shell. The self-association of block polymers in aqueous solution deliberately produces nano-sized particles, which are comparable to liposomes but with enhanced stability. A structural representation of polymersomes is shown in the Figure 1. Hydrophobic part of the amphiphilic block polymer become the middle portion of the vesicle and act as a protective shield that separates the hydrophilic core from the outside environment. The lower permeability of this rigid physical barrier is capable enough to insulate the entrapped moiety from the external biological environment. The accurate and deliberate responsiveness of this vesicle to various stimulus, both *in vivo* and *ex vivo* makes this system superior for targeted drug delivery and bio imaging. Its greater bio compatibility, increased blood circulation time and reduced cell toxicity added appeal to its acceptability.

The size, surface characteristics, rigidity and the degree of responsiveness to various stimulus can be modified by altering the ratios of hydrophilic-to-hydrophobic polymers and preparation methods.[4–7] Both hydrophilic and hydrophobic molecules can be encapsulated in to the aqueous core and the middle lipid loving broad layer of polymersomes respectively. Polymersomes are having exceptional potential to incorporate wide range of therapeutic moieties like proteins, enzymes, deoxyribonucleic acid (DNA) ribonucleic acid (RNA) and low molecular weight drugs.[5-6]

On the view of the vast potential of polymersomes and the increasing interest on this self-assembled nano carrier, this review is intended to furnish an extensive report on the various strategies adopted for the development of suitable of polymersomes for drug delivery and other bio medical imaging applications.

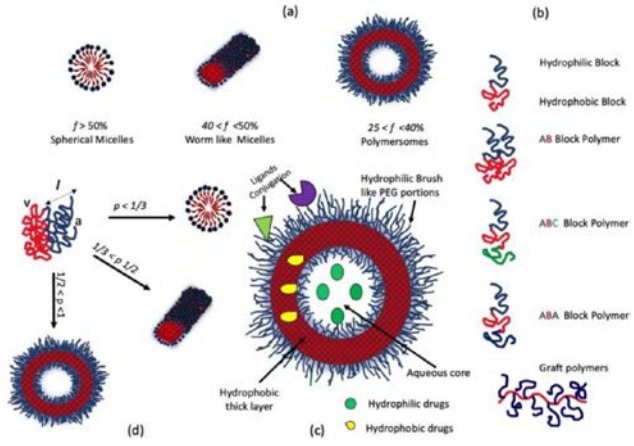

Figure 1: Different aspects of polymersomes self-assembly. Figure 1(a): Relationship between shape, degree self-assembly with *f*(hydrophilic); 1(b): Types of block polymers; 1(c): Structure of polymersomes and the incorporated materials; 1(d): Relationship between shape, degree self-assembly with *p* value.

STRATEGIES FOR THE SUITABILITY OF POLYMERSOMES FOR DRUG DELIVERY AND DIAGNOSTICS

In order to assign polymersomes for various biological applications, a thorough understanding of various aspects of its designing and engineering is mandatory. Under this section we will be extensively discussing the various strategies for the polymer selection, preparation, size reduction, surface engineering, functionalization and important modes of release from the polymersomes. A diagrammatic representation of different strategies of polymersome engineering is given in Figure 2.

Selection of the Block Polymers

Polymersomes can be prepared from a wide variety of diblock, triblock or graft copolymers Figure 1(b). The incorporation and release of the drugs from the polymersomes are primarily depend on the ratio of the

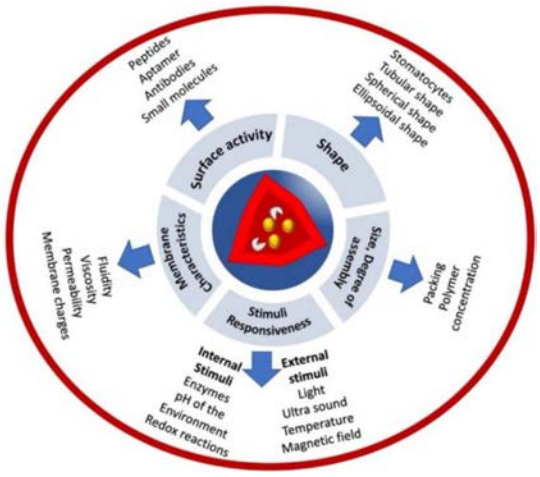

Figure 2: Different strategies of polymersomes design and engineering.

hydrophilic and hydrophobic portion of amphiphilic polymers. The most widely used hydrophilic polymer block is poly ethylene glycol (PEG). The suitability of PEG is because of its high flexibility and water solubility. Poly ethylene oxide (PEO), poly glutamic acid (PGA), dextran, poly ethylene glycol (PEG), poly(2-methacryloyloxy-ethyl phosphorylcholine (PMPC), polyacrylic acid (PAA), polyvinyl pyridine (PVP), are some of the other hydrophilic block polymers commonly used.[8-9]

Biodegradable and biocompatible straight chain polyesters and their analogues like poly caprolactone (PCL), poly(lactic-co-glycolic acid)(PLGA), poly lactic acid(PLA) are usually chosen as hydrophobic block part. Non-biodegradable hydrophobic blocks polymers like poly(dimethylsiloxane)(PDMS), poly(styrene), poly(ethylethylene)(PEE), poly(2 cinnamoyl- ethyl methacrylate) (PCEMA), poly(propylene oxide)(PPO), poly(di-methylsiloxane) (PDMS), poly(butadiene) (PBD) can also be used. The selection of this hydrophobic part is very crucial as it determines the release profile, circulation time and the successful targeting. The thickness of the hydrophobic layer is tunable and controllable. It usually ranges from 8-22 nm depending on molecular weight of copolymer and the polymer block ratio.[10] This complex bilayer, which can be ten times thicker than liposomes, ensures physical stability, flexibility, and decreased leakiness of the incorporated payloads.[8,11]

Degree of Self-assembly

The self-assembly of the amphiphilic polymer into supramolecular structures is dependent on the concentration, molecular weight and geometrics of the block polymers. The non-covalent collaborations of the hydrophobic block are also a significant contributor of this sophisticated process. The produced structure can vary from micelle to lamellae and vesicles. The identification and characterization of the generated structure is indispensable for the process to continue. The characterization of the self- assembly of the polymer is done by *f* (hydrophilic). Generally an amphiphilic block polymer with *f* (hydrophilic) 25-40% yields polymersomes. Spherical micelle and lamellae are obtained with *f* (hydrophilic) > 50%, with 40-50% *f* value worm like micelles are more likely.[12-13] Di block polymers with *f* (hydrophilic) < 20% and high molecular weight hydrophobic blocks have a tendency to retain its rigid solid consistency. With an *f* (hydrophilic) value of 20-42%, it is expected to acquire more fluid constancy and production of spherical micelle is achievable with *f* value of >42%.[11,14] Another parameter used for predicting the structure is the packing parameter (p = v / al) where, (v) is the volume of the hydrophobic block, (a) the contact area of the head group, and (l) is considered as the length of the hydrophobic block. If the p value is $1/2 < p < 1$ the assembly would be polymersomes. If the value is between 1/3 - 1/2 cylindrical micelles are formed and p value of < 1/3 will yield spherical micelles. The formation mechanism itself has an impact on the shape and process of self-assembly. The diagrammatic representation of various aspects of self-assembly is given in the Figure 1(a)-(d). Polymersomes can form through one of the two following mechanisms.

a) Mechanism I: The self-assembly of the block polymer starts with formation of spherical micelles followed by the bilayer lamellae or sheets resulting the completely closed spherical vesicles. This process is driven by the edge energy and yields comparatively smaller vesicles.[11]

b) Mechanism II- The slow growth in size of circular micelle in to bigger ones due to the diffusion of solvent is followed. This results a decrease in bending energy which subsequently leads to the increase in diameter of the polymersomes, resulting a bigger vesicles.[11]

Polymer Topology and Membrane Characteristics

Polymer topology and membrane characteristic play crucial role in the polymersome's efficiency and performance. Vesicles fabricated from biodegradable amphiphilic graft polymers showcase improved resistance to adsorption of protein and other macro molecules. Wang Y *et al.* designed poly (lactide- *co*-diazdomethyl trimethylene carbonate*)- g* -polyethylene glycol [P(LA-co-DAC)-g-PEG] polymer for producing polymersomes with improved circulation time and stay in the body.[15] Kishimura A *et al.* reported that chimeric biodegradable polymersomes prepared from asymmetric triblock copolymer polyethylene glycol-*b*-poly(caprolactone)-*b*- poly(diethyl -amine) ethyl methacrylate (PEG-*b*-PCL-*b*-PDEA) has a loading capacity of 99%. This was attributed by the powerful hydrogen bonding and electrostatic association between PDEA portion and the associated drug moiety. It is beneficial for incorporation of lysosomes, immunoglobulins, bovine serum albumin (BSA) delivery protein, ovalbumin etc.[16] Laskar P *et al.* prepared highly cationic polymer vesicles with cationic co polymer formulated from N-N (dimethyl amino) ethyl methacrylate (DMAEM) and PEG. This system is well suited for delivery of human serum albumin and gene materials (DNA and RNA) with excellent stability and sustained action. This moiety can favour the stimuli responsive release especially by an acidic pH.[17]

Membrane property like fluidity and thickness of the hydrophobic layer purely depends on molecular weight of the hydrophobic parts and the number of units of blocks in the amphillic co polymer. As the molecular weight increases the membrane fluidity decreases. Fluidity problems are more prevalent in longer hydrophobic blocks. The viscosity and fluidity of the vesicular preparation can be altered by UV irradiation of cross linkable functional groups containing butadiene and methacrylate. The movement of molecule across the membrane is also affected by the relative hydrophilicity of the polymers. The membrane permeability and porosity can be manipulated by carefully selecting copolymer blocks. The di polymer blocks with poly ethylene glycol -*b*- polybutadiene (PEG- *b*-PBD) and triblock polymer, polyethylene glycol-*b*- polypropylene oxide-*b*- polyethylene glycol (PEG-*b*-PPO- *b*-PEG) is reported to have improved permeability for molecules of size < 5 KDa. The diffusivity and solubility of the entrapped molecules in the hydrophobic portion of the polymersome also have an effect on the permeability of the membrane. Charged molecules exhibit slower permeability than the neutral molecules. The membrane permeability of polymersomes to small molecules can be controlled by inserting ionizable functional groups within the hydrophobic block.[18-20]

The surface charge on the vesicle contributes to the successful interaction of the polymersomes with the negatively charged cell membrane (zeta potential ~ −20 mV). A better endocytosis is expected when the polymersome membrane is asymmetrically charged. Asymmetric polymersomes can be fabricated with the help of triblock polymers containing two chemically-unlike hydrophilic block skirting with a hydrophobic block. By modifying the volume fraction of poly(acrylic acid) with respect to poly(ethylene oxide) in a poly(ethylene oxide)-*b*-poly(caprolactone)-*b*-poly(acrylic acid) triblock polymer, can yield asymmetrically charged polymersomes. A near IR fluorophore labelled negatively-charged polymersomes may be prepared for bio imaging of blood vessels and cell micro environments using a diblock polymer of poly(acrylic acid)-*b*-poly(1,4-butadiene) (PAA-*b*-PBD).[18,21]

Shape of the Polymersome Vesicles

The shape of the vesicles that are formed is also important in terms of practicality which is promising for the design and development of adaptive artificial biomimetic systems. The transport across various membranes in the body is greatly affected by the shape of the polymersomes. Because of its thermodynamic stability, self-assembly of the copolymer into a spherical form is most probable. But the transition of spherical structure into non-spherical structure like tubular, ellipsoidal, and stomatocytes is possible with the help of different chemical and physical methods (pH, osmosis, temperature, gas, redox). Polymersomes display morphological diversity with its tailor-made chemical design. Vesicles with non-spherical assembly having close similarity to the biological cells and organelles. Due to the higher surface area, polymersomes shows higher adhesion to the cell surface. Ellipsoidal polymersomes are found to have improved binding capacity with target cells and higher diffusivity than the spherical moiety. A combination of alkyl substituted, poly (2-hydroxyethyl aspartamide) (PHEA)with poly (ethylene glycol) (PEG) can yield ellipsoidal polymersomes.[22] Tubular polymersomes can be prepared by film rehydration method by mixing aqueous solvent with solvent casted thin film of amphiphilic copolymer under stirring. For example, the poly(2-(methacryloyloxy)ethylphosphorylcholine)-*b*-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-*b*-PDPA) can yield ellipsoidal polymersomes. The elongation transition may be induced on a polymersomes fabricated by biodegradable copolymer poly(ethylene glycol)-*b*-poly(D,L-lactide)(PEG-*b*-PLA) by subjecting it to a dialysis technique using an aqueous solution of osmogen (sodium chloride). Large elongated nanotube can be produced by using 10-50 mM sodium chloride. A 100 mM sodium chloride can yield elongated ribbons like polymersomes. The dialysing temperature is also have an effect on the shape of vesicles. At a temperature 4°C, there is clear transition of spherical to tubular polymersomes. But at 25°C the transition is very minimum and the polymersomes remain as spherical entity. A further increase in temperature up to 30-35°C facilitated the formation of elongated ribbon structures.[18,23] Even though the tubular polymersomes holds larger luminal volume which justifies greater drug load, it exhibits different internalization kinetics with initial swift binding followed by a slow internalization. This relaxed internalization of tubular polymersomes is credited to the amplified energy need for endocytosis of the moiety. Consequently the tubular polymersomes become less efficient than its spherical counterparts.

In recent times a lot of interest is shown on non- spherical polymersomes because of its higher capacity for cell targeting. A bowl shaped polymersomes "Stomatocytes" fabricated with polyethylene glycol- *b*- poly polystyrene (PEG-*b*-PS) showed precise and effective penetration through the cell barriers. A mixture of poly(ethylene glycol)-*b*-polystyrene and poly(ethylene glycol)-*b*-poly(ε-caprolactone) is used to produce biodegradable

stomatocytes. The transformation of micelles into stomatocytes is depends upon the ratio between organic solvent and water used during the formulation. They can successfully entrap highly sensitive enzymes that catalases with 100% efficiency and retention of activity. As a result, depending on the polymer's application, it's critical to refine its form. Non-spherical polymersomes should be used when cellular or tissue adhesion is required, but for an efficient cellular uptake spherical counterparts could be the wiser choice.[24]

Methods of Preparation of Polymersomes

Most liposome preparation techniques can also be used to make polymersomes. The size, type, yield and polydispersity of the vesicles are mainly depend on the method of preparation. The procedures of preparation can be broadly classified as solvent displacement methods and solvent free methods.

All film rehydration methods are considered as solvent free methods. The block polymers are dissolved in any suitable organic solvent which is then poured over any solid surface followed by the complete evaporation of solvent. The produced thin film is swollen immediately after the addition of the aqueous solvent resulting the formation of vesicles. Mechanical stirring, or sonication can be used to improve the speed of vesicle formation. The straight solvent hydration methods face problem with polydispersity of vesicles. Hence different post conditioning treatments should be followed to achieve a uniform distribution. Sequential extrusion, gel-assisted hydration or electro formation can be used.[25] Another solvent free method which is in lime light is the polymerization-induced self-assembly (PISA).[26] In this method the nano particle assembly pass through various stages of transition ranging from spheres to vesicles. The achievement of the vesicle formation is depends on the polymer concentration and the degree of polymerization.

In solvent displacement method, the amphiphilic polymers are dissolved in suitable organic solvents which is then carefully mixed with an aqueous solvent, followed by subsequent elimination of the organic solvent. This technique is excellent for the efficient entrapment of water-soluble biologicals, but may face some problem for the organic solvent eviction. Solvent injection is another method which uses solvent displacement principle. The homogenization is proceeded with extrusion and organic liquid removal is executed by dialysis, centrifugation and vacuum. More controlled and uniform lamellae can be prepared by adopting emulsion phase transfer method and microfluidics.[2] Highly specific compound mixtures are needed for the removal of sugars, surfactants, non-amphiphilic polymers and polyelectrolytes attached to the vesicles. These impurities can affect some of the properties of the polymersomes. As a result, more attention should be paid to the elimination of these impurities.

Controlling the Size of Polymersomes

For cellular uptake the size of the particle should be limited to 50-60 nm. The packing of the amphiphilic polymer chain determines the size of the polymersomes. The total polymer concentration also has an influence to the size of polymersomes. Size reduction to the desired range can be done with the help of controlling the mixing rate, extrusion/sonication and filtration. An increase in the mixing rate with total flow rate of 5-10 mL/min can navigate the polymers to get in to water phase at a faster rate and self-associate into very small polymersomes (50-75 nm). A lower mixing rate with a flow rate less than 1 mL/min impair the diffusion of polymer in to water resulting large polymersomes with size 100 nm in diameter. Subjecting the vesicles to cross-flow filtration followed by differential centrifugation is another prominent approach for the size reduction.[13,18] Application of centrifugal force facilitates the sedimentation of bigger vesicles as pellets and smaller polymersomes are retained in the supernatant. A careful sequential stepwise application of centrifugal force, collection and processing will ensure effective size separation. Shear force extrusion can be followed for reducing the size without affecting the thickness of the layers. Extrusion can be a superior method of size reduction as it produces a narrow size distribution of polymersomes. Size reduction by sonication largely depends on the rigidity of the bilayer membrane which intern depends on the water content of the polymersomes. After a very short incidence of sonication of (30s), polymersomes of size >450 nm containing less than 33% water effectively reduced its size into <100nm. However, when polymersomes containing 66% water were subjected to sonication for more than 15 min, they failed to reduce it size significantly. Alternatively an application of ultrasound, will result in a larger size distribution.

Encapsulation of Biomolecules in to the Polymersomes

The encapsulation of the payload in to the polymersomes can be done by active and passive loading methods.[27] For passive loading hydrophilic molecules are added to the aqueous phase during the vesicles preparation. Hydrophobic molecules can be

encapsulated actively by including it in to the organic solvents with amphiphilic polymers. With the help of electroporation, sequential addition of incompatible multicomponent payloads to the vesicles may be performed. With this single drop injection, precise incorporation of drugs to each vesicle is anticipated.

Surface Functionalization of Polymersomes for Targeted Drug Delivery

Polymersomes are commonly administered through intravenous route. In the blood stream they will be exposed to opsonization and dilution. So the chances of dissociation or aggregation of the self-assembled structures are very likely which intern lead to the premature release of the payload *in vivo*. Even though the thick brush like layers of polyethylene glycol Figure 1(c) on the surface is offering resistance to opsonization, certain surface treatments are always necessary to improve the stability and circulation time of vesicles in the blood stream. Covalent crosslinking of hydrophobic and hydrophilic portions of polyethylene glycol with fumaryl chloride shows minimum stability issues *in vivo*.[18,28] Another method to improve the stability is the fabrication of thiol containing cross linked polymersomes by the treatment of bi-sulphide group using cysteamine within the bilayer of triblock polymers. They can offer tenacity to dilution and protects the release of the encapsulated bio molecules.

Surface functionalization also has greater application for the amicable drug targeting to various biological cells, organs, membranes, tumors and invading bacteria cells. Alibolandi M *et al.* designed polymersomes using dextran- *b*-poly(lactide-co-glycolide) (DX -*b*-PLG) with small molecule folic acid. Intern they are loaded with docetaxel, an anticancer drug.[29] As the folate receptors are over-expressed in breast and liver cancer, surface functionalization with folic acid displayed 1.7-fold higher accumulation in the tumor cells. Functionalization with cell-permeable peptide trans activating transcriptional activator (TAT) in porphyrin fluorophores conjugated poly(ethylene glycol)- *b*-poly(1,4-butadiene)(PEG-*b*-PB) polymersomes can experience a remarkable uptake by the dendritic cells in cancer treatment.[30] Similarly, Epithelial cell-adhesion molecule (EpCAM) is highly expressed in non-small cell lung cancer (NSLCL). The specificity of the 19-mer EpCAM RNA to bind with the extracellular portions[31] of the epithelial cell adhesion molecule allowed Alibolandi M *et al.* to develop poly(ethylene glycol)-*b*-poly(lactide-co-glycolide) (PEG-*b*-PLG) polymersomes loaded with doxorubicin. It was reported that the existence of EpCAM aptamers on the polymersomes increased the uptake by A549 and SK-MES-1 lung tumor cells.[32]

Peptide conjugated polymersomes can easily cross the blood-brain barrier because of it's specificity towards BBB surface protein 1, trisialganglioside (GT1b) and monosialotetra- hexosylganglioside (GM1) receptors. A synthesised polymer poly[2- (methacryloyloxy)ethyl phosphylcholine] was used after capping with maleimide functional group which can further treated with Angiopep-2 peptides.[33] These peptide can bond with protein 1 of BBB and taken up to the brain cells easily. Surface modification of polymersomes with antibodies such as and transferrin (T*f*) and lactoferrin (L*f*) has been testified to improve the targeting of therapeutic agents to the brain. Pang *et al.* developed biodegradable conjugated transferrin polyolefin polymersomes loaded with doxorubicin, (T*f*-PO-DOX) to improve the DOX delivery into brain tumor cells.[34] Vesicles fabricated by multiple glucose units conjugated polymers can be used to target fim H protein-producing bacteria.

Surface functionalization of polymersomes for bio imaging

Polymersomes are proven to be an excellent tool for successful bioimaging and various diagnostics. Several imaging agents such as magnetic particles, fluorescent dyes, radionuclides, quantum dots can magnificently been amalgamated with polymersomes. Enhanced visualization of soft tissues can be assured by the fabrication of dual-imaging polymersomes prepared from poly(acrylic acid-co-distearin acrylate) poly(AAc-co-DSA), by parallelly incorporating magnetic gadolinium (Gd(III) and hydrophobic flurophene (Cy5.5). The Gd(III/ Cy5.5-loaded polymersomes have exhibited superior payload detention and lesser dilution effect when injected intravenously. An exceptional contrast in magnetic resonance imaging of the targeted tumor site is obtained by these polymersomes delivery.[35] Polymersomes tagged with pH sensitive Nile blue based fluorescent / colorimetric biosensors can be utilized for imaging liver cells.[35] Polymersomes prepared with poly (L-glutamic acid)-*b*-poly(ε-caprolactone)(PGA-*b*-PCL) conjugated with folic acid-were also experimented to detect and image tumor cells. Brinkhuis RP *et al.* studied the capability of iodine-lush polymersomes for single-photon emission computed tomography (SPECT/ CT) radioisotope therapy and imaging of 4T1 murine in breast cancer using mice.[36] Prostate stem cell antigen (PSCA) is a specific glycosyl phosphatidylinositol-anchored glycoprotein used for prostate cancer targeted imaging and therapy. It is reported that surface treated radioactive polymersomes are an exceptional diagnostic

tool for imaging in the fields of cardiology, neurology and oncology to provide three-dimensional images of radiotracer distribution.[36] Prolonged circulation time and distribution to the reticuloendothelial system and cancer cells exhibited by radiolabelled ^{25}I polymersomes, ensure effective SPECT/CT imaging and inhibition of tumor growth in mice with significant survival benefits.

Internal and External Stimuli Responsiveness of Polymersomes

On reaching the target site, polymersomes have to release their entrapped molecules successfully. The hydrolytic degradation of the hydrophilic portion helps the polymersomes to release its content at the target site. Drug delivery from polymersomes generally follows passive diffusion, impelled by concentration gradients. This mechanism alone won't be assuring the expected release of the payloads at the target site. To overcome this problem, sensitizing the polymersomes to various stimuli (both internal and external) like pH, redox reaction inducing molecules, specific enzymes, ultra sound, light, temperature, and magnetic field can be adopted at a greater extent.[11] pH responsive polymers like poly(2-(methacryloyloxy)ethylphosphorylcholine-*b*-poly(2-(diisopropylamino)ethyl methacrylate) (PMPB-*b*-PDPA) and poly(D,L- lactide)-*b*-poly(2-methacryloyloxyethyl phosphorylcholine) (PLA-*b*-PMPC) are the most sort after pH sensitive block polymers. Substitution with esters, acetates in the hydrophobic diblock of polymer can facilitate the response to the acidic environment. Incorporation of ionizable amine group is also one of the acceptable methods. Polymersomes can be sensitised by the presence of oxidizing and reducing agent in the biological systems. Glutathione, a biological reducing agent can act as an excellent stimulus for cellular targeting of bio molecules. In tumor cells, level of glutathione is found to be high than the normal concentration. Incorporating covalent bonds in the polymersomes that can split or dissociate by the change in concentration of glutathione is an excellent approach for tumor targeting. Similarly, Introduction of a cleavable disulphide reduction sensitive link between the hydrophobic and hydrophilic parts is also beneficial. Such polymersomes will remain intact in oxidizing biological circumstances (i.e. blood plasma). Another approach is the development of a sugar responsive polymersome using poly(styrene boroxole) and poly ethylene boroxole. The release of the encapsulated insulin is triggered by the high concentration of monosaccharides in the blood.[37-38] Battalia G *et al.* reported the use of polymer vesicles for plasmid DNA specially designed for gene delivery and therapy. This method uses Encapsulation of DNA in pH sensitive PMPC-PDPA polymer vesicles. The release of the genetic material is assured by the exposure of pH 5.5 condition once it entered into the lysosomes and endosomes.[39] A detailed information on different polymers prepared with the corresponding entrapped molecules and the release mechanism is shown in Table 1. Vesicles fabricated with copolymers of PEG and the temperature sensitive Poly(N-isopropylacrylamide) (PNIPAM) dissimilate with change in temperature. PNIPAM demonstrates reversible coil-to-aggregate transition below and above the lower critical solution temperature (LCST). Thus the entrapped molecules are released from the polymersomes when the temperature lowers below the LCST.[40] Triggering the release of a payload in the presence of certain enzymes is also an alternative approach followed for polymersomes. The destabilization of the vesicles is induced by proteinase K-modulated hydrolysis. Engineered peptide sequences are easily incorporated between poly(D,L – lactide) and poly(ethylene glycol) that are programmed to destabilized

Table 1: Polymersomes and its release mechanisms.[18,27]

Co polymer	Drug/Macromolecule	Release Mechanism
PMOXA-*b*-PDMS-*b*-PMOXA	Nucleoside hydrolase, Trypsin	pH responsive
PEG-*b*-PPS	Ovalbumin, Bovine -globulin, Bovine serum albumin	Reduction
PEO-*b*-PCL-*b*-PLA	Serum albumin	pH and temperature
PEO-*b*-PLA; PEO-*b*-PCL	Hemoglobulin	pH and temperature
PEO-*b*- PNIPAm	Doxorubicin	pH
PEG-*b*-SS-*b*-PPS	Calcein	Reduction sensitive
PMPC-*b*-PDPA	Doxorubicin	pH sensitive
PEG-*b*-PLGA	Bovin serum albumin -Gd	MRI
PEG-*b*-PBOx	Insulin	Sugar responsive
PAA-ONB-PMCL	eGFP	Photo sensitive
PEG-*b*-PAA(SH)-*b*-PDEA	FITC-BSA, FITC-CC, CC	pH and reduction
(PEG-*b*-PLA	Arsenic trioxide	pH
(PPS-*b*-PEG)	Doxorubicin	X ray
PEG-*b*- PLA	Docetaxel	Reduction-responsive
PLL-*b*-PBLG-*b*-(PEO)	Paclitaxel and	Temperature and pH
PEG-*b*- P(TMC-DTC)- *b*-PEI	Pemetrexed	Reduction responsive

by lysosome to release the content. Polysaccharide when introduced to the polymer structure can attract enzymatic degradation. *Clostridium* and *Staphylococcus aureus* use hyaluronan as a carbon resource. Hyaluronic acid substituted bacteria sensing polymersomes can be used for lysis of bacteria.[18]

Polymersomes that respond to external stimuli have a wide range of practical applications. The penetration of biological macromolecules to solid tumors which are located away from the vasculature can be enhanced by assigning monocytes as a the cellular carrier typically enclosed with polymersomes and polymer bubbles.[41] Theses assembly ensures the penetration deeper up to >150 μm into the tumor cells. The polymer bubbles can be allowed to collapse by external application of ultrasound waves. Polymersomes can be disrupted by the shear force of the bubble breakdown and in turn allows the entrapped biomolecule to release at the targeted site. Huang W *et al.* reported the successful use of this approach for the precise and specific delivery of doxorubicin to the tumor cell in rats.[42] Light sensitive polymersomes can also be prepared by incorporating photosensitizing materials in the bilayer.[43] The external application of suitable radiations can induct physical changes and prompt deformation of the structure of the polymersomes and release of the therapeutic moiety to the site of delivery.

Huang W *et al.* reported the use of magnetic field as an external stimulus for the delivery of high concentration of oxygen to the tumor cells. Iron oxide nano particles and chlorin e6 photosensitizer are also encapsulated in to the polymersomes.[43] By applying laser radiations the chlorin e6 can transform oxygen to super reactive oxygen groups. The heat produced by the application of high frequency of magnetic field induces the release of oxygen to the tumor cells. According to him, the combined use of magnetic and photodynamic approach drastically improved the therapeutic effectiveness of monocytes to impede tumor growth by 75% in mice.

Apart from the drug targeting and imaging, the emergence of intriguing studies focused on gene delivery, nano reactors, and nuclear medicine using polymersomes from various corners of the globe, is clearly indicating the application of polymersomes for broader medical applications in future.

CONCLUSION

The acceptance of polymersomes as a successful drug delivery modem and diagnostic tool is governed by its perfect resemblances to cell membrane and organelle. Unparalleled self-assembling with tunable physical and morphological characteristics added charm to this systems. Nonetheless, the pharmaceutical market currently only has liposomal formulations, demonstrating the need for rapid development of synthetically highly regulated and sensitive polymersome formulations. A revolutionary future for polymersome in biomedical perceptions and drug delivery arena can only be assured when the researchers concentrate on the engineering aspects for its overall functionality. If customized and engineered thoughtfully, theses supramolecular polymersomes can be a wonder device for a wide range of biomedical applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV: Ultra violet; **BBB:** Blood brain barrier**; mV:** – Millivolts; **KDa:** Kilo Dalton**; IR:** Infra-red; **SPECT/CT:** Single-photon emission computed tomography; **mM:** Millimolar; **A549:** A human caucasian lung carcinoma- cell line; **SK-MES-1:** Human lung cancer cell line; **PMDA:** Poly(methyloxazoline); **PDMS:** Poly(methyloxazoline); **PMOXA:** Poly-(2-methyl-2-oxazoline); **PEG:** Polyethylene Glycol; **PPS:** Poly(propylene sulphate); **PCL:** Poly(caprolactone); **PLA:** Poly(lactic acid); **PEO:** Polyethylene Oxide; **PPS:** Poly(propylene sulphide); **PNIPAm**; Poly(N-isopropyl)- acrylamide); **PMPC:** Poly (2-(methacryloyloxy)ethyl-phosphorylcholine; **PDPA:** Poly(2-(diisopropylamino)- ethyl methacrylate); **PLGA:** Poly(lactic-co-glycolic acid); **PBOx:** Poly(phenylene benzobisoxazole); **PAA:**- Poly acrylic acid; **ONB:** Ortho nitro benzyl alcohol; **PMCL:** Poly(γ-methyl-ε-caprolactone); **PDEA:** Poly(2-(diethylamino)ethyl methacrylate); **PLL:** Poly(L-lysine hydrochloride); **PBLG:** Poly(g-benzyl- L-glutamate); **PTMC:** Poly(trimethylene carbonate); **DTC:** Dithiolane trimethylene carbonate; **PEI:** Polyethylenimine; **SS:** Reduction sensitive disulphide linkage; **eGFP:** enhanced green fluorescent protein; **FITC-BSA:** Fluorescein isothiocyanate labelled bovine serum albumin; **FITC:** Fluorescein isothiocyanate labelled cytochrome C; **CC:** Cytochrome C; **MRI:** Magnetic resonance imaging.

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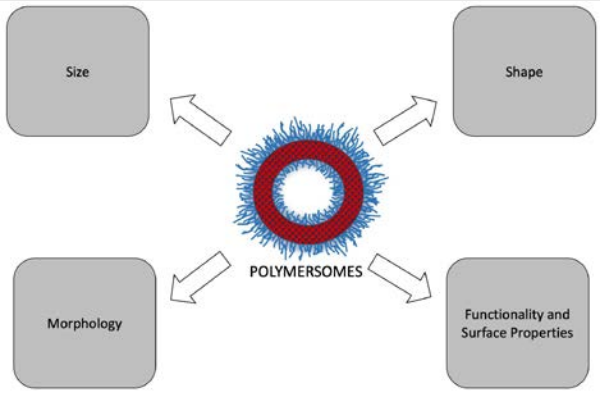
PICTORIAL ABSTRACT



SUMMARY

The development of drugs that target specific locations within the human body remains one of the greatest challenges in biomedicine today. Advances in nanomedicine have led to the development of nano-sized polymersomes as vehicles for different medical applications. Polymersomes or polymeric vesicles are structurally like liposome and acclaimed stable than liposome. Theses vesicles are made by block co-polymer through various methods. Hydrophilic block in amphiphilic block copolymer stabilize the hydrophobic bilayer in aqueous solution and form core-shell structure. Therefore, polymersomes have been used for delivery of both hydrophobic/ hydrophilic drugs. Versatility in polymer chemistry led polymersome as an ideal drug carrier for drugs having non-favourable physicochemical parameters. Various novel functionalized, stimuli-responsive and self-targetable polymersomes has been investigated and are being continuously evaluated for biomedical application.

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