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Polymersomes: A new multi-functional tool for cancer diagnosis and therapy

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ABSTRACT

Nanoparticles are being developed as delivery vehicles for therapeutic pharmaceuticals and contrast imaging agents. Polymersomes (mesoscopic polymer vesicles) possess a number of attractive biomaterial properties that make them ideal for these applications. Synthetic control over block copolymer chemistry enables tunable design of polymersome material properties. The polymersome architecture, with its large hydrophilic reservoir and its thick hydrophobic lamellar membrane, provides significant storage capacity for both water soluble and insoluble substances (such as drugs and imaging probes). Further, the brush-like architecture of the polymersome outer shell can potentially increase biocompatibility and blood circulation times. A further recent advance is the development of multi-functional polymersomes that carry pharmaceuticals and imaging agents simultaneously. The ability to conjugate biologically active ligands to the brush surface provides a further means for targeted therapy and imaging. Hence, polymersomes hold enormous potential as nanostructured biomaterials for future *in vivo* drug delivery and diagnostic imaging applications.

1. Background

Nanosized carriers are prime candidates for the delivery of highly toxic and/or hydrophobic therapeutic agents. These delivery vehicles have the potential to augment the pharmacodynamic and pharmacokinetic profiles of drug molecules, thereby enhancing the therapeutic efficacy of the pharmaceutical agents [1]. Further, encapsulating the drug molecule in a delivery system can increase *in vivo* stability, extend its blood circulation time, and further provide a means for controlling the release of the agent [1]. Moreover, the delivery system can alter the biodistribution of the drug molecule by allowing the agent to accumulate at the tumor site, either passively or actively with targeting [1]. In addition to therapeutic drug delivery, serving as diagnostics tools, nanosized carriers can deliver imaging agents to detect and non-invasively diagnose disease.

Polymersomes, polymer vesicles self-assembled from a diverse array of synthetic amphiphilic block copolymers containing hydrophilic and hydrophobic blocks [2–4], have been shown to possess superior biomaterial properties, including greater stability and storage capabilities [5–7], as well as prolonged circulation time, as compared to liposomes (vesicles derived from phospholipids) [8]. A particularly attractive storage feature, highlighted in Fig. 1, is the large hydrophobic core of the polymersome membrane, which follows from the membrane-forming amphiphilic polymers being larger than conventional phospholipids [9]. Further, block copolymer chemistries can be tuned through polymer synthesis to yield polymersomes with diverse functionality [10]. A vast majority of vesicles made of synthetic copolymers have dense polyethylene oxide (PEO) outer shells, which affords them "stealth" like character that may lead to increased circulation times and *in vivo* biocompatibility [5]. Thus, although liposomes are presently used in various biotechnological and pharmaceutical applications to improve therapeutic indices and enhance cellular uptake [4], it appears that polymersomes can offer superior advantages for future clinical therapeutic and diagnostic imaging applications.

In aqueous solutions, amphiphilic block copolymers can self-assemble into mesoscopic structures ($\leqslant$200 nm–50 μm in diameter) [3]. The ratio of hydrophilic to hydrophobic block volume fraction determines whether micelles (spherical, prolate, or oblate), or vesicles (polymersomes) will form [2,11–13]. As a general rule, however, a ratio of hydrophilic block to total polymer mass of approximately $\leqslant$35% ± 10% yields membrane structures, while copolymers with ratios greater than 45% generally form micelles; those with ratios less than 25% form inverted microstructures [14].

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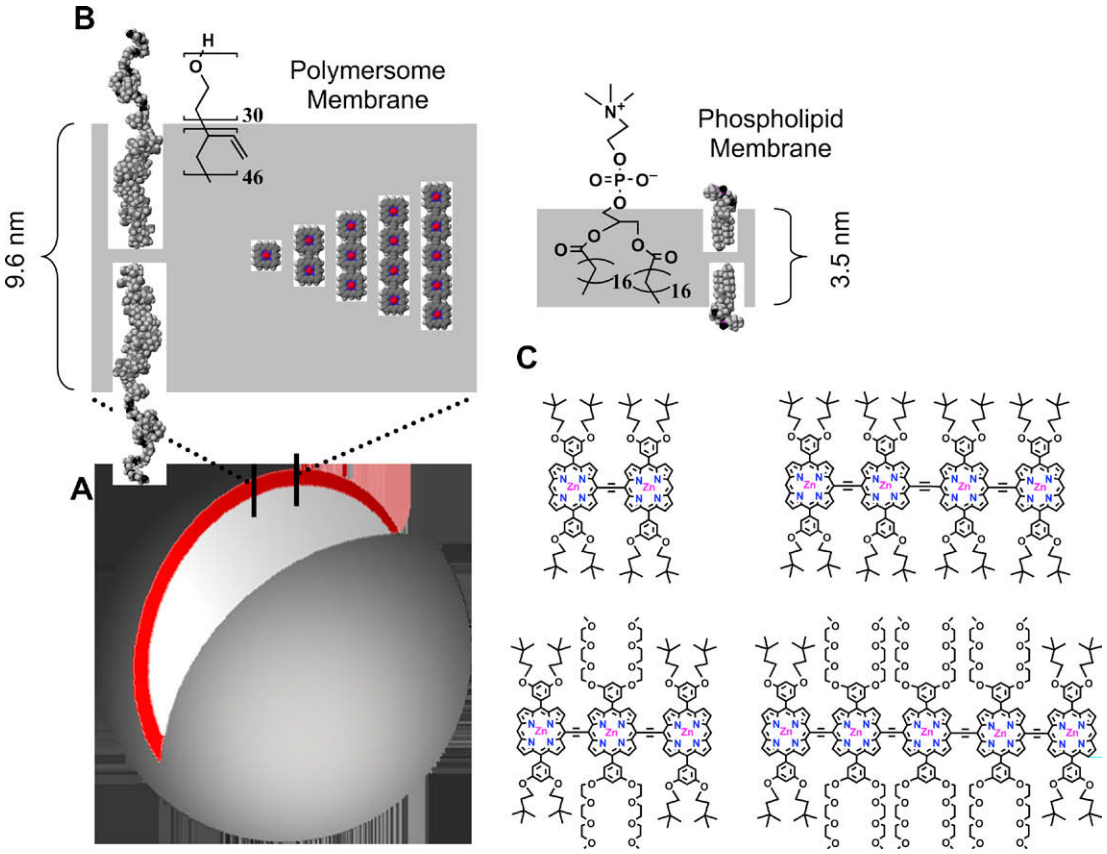

Fig. 1. Schematic representations of NIR-emissive polymersomes. (A) In aqueous solution, amphiphilic diblock copolymers of polyethyleneoxide-1,2 polybutadiene (PEO_{30}–PBD_{46}) self-assemble into polymer vesicles (polymersomes) with the hydrophobic PBD tails orienting end-to-end to form bilayer membranes. The depicted unilamellar polymersome displays an excised cross-sectional slice illustrating the bilayer PBD membrane (gray) containing the hydrophobic (porphinato)zinc(II) (PZn)-based near-IR fluorophores (NIRFs, red). (B) CAChe-generated sectional schematic of the NIR-emissive polymersome membrane indicating the molecular dimensions of: (i) the PBD component of the bilayer (9.6 nm); (ii) the large, dispersed PZn-based NIRFs (2.1–5.4 nm); and, (iii) a typical liposome membrane (3–4 nm) comprised of phospholipids (1-stearoyl-2-oleoyl-sn-Glycero-3-Phosopho-choline—SOPC). (C) Chemical structures of NIR fluorophores PZn_2–PZn_5. [This image was reproduced from Ghoroghchian et al. [9] with permission from Copyright (2005) National Academy of Sciences, USA.]

Micellar structures have been used as intracellular and systemic delivery systems [15–18] but present significant limitations when compared to polymersomes. In aqueous solutions, they can only encapsulate hydrophobic molecules unless strong binding or covalent linking strategies are incorporated for sequestering aqueous-soluble components.

In contrast, polymersomes can *simultaneously* encapsulate hydrophilic components in their aqueous interior and hydrophobic molecules within their thick lamellar membranes [10]. In addition, biologically active ligands, such as antibodies, can be readily conjugated to the exterior brush surface to target the vesicles or to provide a therapeutic response [19–22]. These properties of the vesicle architecture effectively create a multimodal platform, which can be used for therapeutic (drug delivery) and/or diagnostic (imaging) applications (Fig. 2).

Although vesicles can be targeted to specific sites using biologically active ligands, the anatomical and pathophysiological abnormalities of the tumor tissue alone can be utilized to aid in the localized delivery of macromolecules [23]. The tumor vasculature, characterized by irregularly shaped, dilated, defective, and/or leaky blood vessels, disorganized endothelial cells with fenestrations, as well as other abnormalities, allows for the passive accumulation of macromolecules at the tumor site [24]. Further, due to the poor lymphatic drainage, nanoparticles can accumulate and remain at the tumor site even in the absence of a targeting moiety [25]. This phenomenon is known as the enhanced permeability and retention (EPR) effect and makes it possible to achieve high local concentrations of macromolecules at the tumor site without specific targeting [24]. However, a question that has yet to be addressed with polymersomes is how much additional accumulation is possible with targeting.

2. Diblock copolymers forming vesicles and release mechanisms

In this section, we highlight some of the polymer formulations, which have led to the formation of polymersomes, that have demonstrated promise for controlled release of pharmaceuticals.

Initial polymersome research by Hammer and Discher used poly(ethylene oxide)-block-poly(ethylethylene) (PEO-b-PEE) diblock copolymers to demonstrate the formation of polymersomes in aqueous solution, as well as to characterize the vesicles material and physical properties [3]. Additional work in the field has led to the synthesis of a number of biocompatible PEO-based amphiphilic block copolymers that form aqueous vesicles dispersions, including poly(ethylene oxide)-block-poly(butadiene) (PEO-b-PBD) [7].

A significant limitation of these polymers for *in vivo* therapeutics is that they are not biodegradable and likely not fully biocompatible. In an effort to create vesicles that degrade and release their contents *in vivo*, PEO-b-PBD polymers have been blended with hydrolysable block copolymers, such as poly(ethylene oxide)-block-poly(lactic acid) (PEO-b-PLA) or poly(ethylene oxide)-block-poly(caprolactone) (PEO-b-PCL); these vesicles have been shown