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Polymersomes as a potential platform for cancer immunotherapy

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ABSTRACT

With the focus in the field of cancer nanomedicine shifting from direct tumor targeting to modulation of the immune system, new opportunities arise for the employment of nanocarrier systems. Polymeric nanovesicles, or polymersomes, have been under investigation as a potential nanocarrier platform for the past decades. These investigations have enhanced our fundamental knowledge on how to tailor physicochemical properties, such as size and shape, surface chemistry and functionalization, and membrane characteristics. The versatile nature and high structural stability of polymersomes makes them suitable for cancer treatment that goes beyond drug delivery. Rational nanocarrier design allows for the spatiotemporal control over the function of specific immune cell targets to enhance cancer immunotherapy. This review provides a perspective view on the potential of polymersomes as a multifunctional platform for *in vivo* cancer immunotherapy. We discuss opportunities to implement polymersomes in the field, elaborate on their design considerations for immunotherapeutic applications and compare polymersomes with lipid nanoparticles and other relevant systems. Current challenges and future perspectives are addressed to underline what is needed to employ polymersomes as a platform for cancer immunotherapy.

1. Introduction

Immunotherapy has become a powerful treatment modality that has changed the way cancer is treated [1]. Research into immune checkpoint blockade (ICB) and chimeric antigen receptor (CAR) T cells has pioneered the field and exemplifies the rapid developments of the last decade [2,3]. Nevertheless, the clinical translation of new cancer immunotherapies is losing its momentum, because the current strategies demonstrate limited efficacy and can cause systemic autoimmunity [4,5]. Recently, nanomedicine has been proposed to enhance cancer immunotherapy by providing control over the timing and localization of immune stimulatory cues, which current approaches do not offer [6–9]. By capitalizing on nanocarrier technology, the therapeutic window of cancer immunotherapy can be widened through spatiotemporal regulation of antitumor immunity.

Traditional nanomedicine was originally envisioned to improve the pharmacokinetics of chemotherapeutic agents and was especially aimed at prolonged circulation time and high tumor accumulation through the enhanced permeability and retention (EPR) effect [10]. The importance of particle design to achieve therapeutic goals is evident from the first clinically approved nanomedicine, Doxil®, a liposomal formulation of doxorubicin. Functionalization of the liposomal surface with neutrally charged and hydrophilic poly(ethylene glycol) (PEG) reduced particle clearance from the blood and promoted accumulation in the tumor [11]. Since then, a vast amount of research has been dedicated to the optimization of nanomedicine design to attain superior tumor targeting and avoid clearance by the immune system [12–15].

Although traditionally the field of nanomedicine has tried to minimize interactions with the immune system, its application in cancer immunotherapy requires an opposite approach [16]. The convergence between cancer immunotherapy and nanotechnology aims at specifically targeting and modulating immune cells in a spatiotemporally controlled manner, which inherently implies a desire for high nanocarrier accumulation in lymphoid organs. Interestingly, this paradigm would thus deem the clearance of nanoparticles by phagocytic immune cells as a favorable trait rather

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than a clinical hurdle to overcome. Control over this intrinsic avidity towards immune cells, as well as gaining immunomodulating capacity, may be achieved by tuning the physicochemical properties of the nanoparticle system.

Polymersomes are nanoscale vesicles formed via the self-assembly of amphiphilic block copolymers into stable membranes [17,18]. Due to their physicochemical versatility, polymersomes are of substantial interest to the field of cancer immunotherapy. The past decades of polymersome research have yielded a thorough understanding on how to tailor their physicochemical characteristics [19]. However, pre-clinical studies exploring materials for cancer immunotherapy have mainly focused on liposomes or other systems (e.g. inorganic or solid polymeric nanoparticles) [20]. Importantly, polymersomes and liposomes are structurally similar vesicles that originate from either amphiphilic block copolymers or phospholipids, respectively. Polymersomes have several advantages over liposomes due to their thicker membrane and entanglement of their hydrophobic chains, which provide them with higher stability and better cargo retention [21]. In addition, synthetic block copolymers display a greater chemical versatility in contrast to the limited number of naturally occurring phospholipids [21]. The benefits of polymersomes over their lipid counterparts led to the belief that polymersomes would become the next generation drug delivery system. Nonetheless, lipid nanoparticles (LNPs), including liposomes and solid LNPs, dominate the clinical landscape whereas polymersomes are conspicuous by their absence in clinical trials [22,23].

Several factors hamper clinical translation of polymersomes. First of all, only a handful of polymers, including PEG and poly(lactic-co-glycolic acid) (PLGA), have been approved by the US Food and Drug Administration (FDA), whereas most lipids are certified for clinical use due to their intrinsic biocompatibility. Moreover, methods for large-scale and reproducible production of polymersomes are lacking, while this has already been established for LNPs. In addition, the chemical versatility of polymersomes increases the design possibilities, but it complicates interstudy comparisons between structurally and chemically different systems. Consequently, clinical translation of polymersomes has proven difficult in spite of their potential as a versatile and designable platform, especially in the field of cancer immunotherapy.

In this review, we aim to appraise the potential of polymersomes as a platform for cancer immunotherapy. In spite of the advances of nanomedicine in immunotherapy for other diseases, including cardiovascular disease [24] or autoimmune disorders [25], we narrow our scope by only highlighting opportunities to implement polymersomes in the field of cancer immunotherapy, although the discussed principles would also hold for other classes of immunotherapy. Emphasis will be placed upon the optimal design features for biodistribution to lymphoid organs and therapeutic targeting of immune cells. The advantages and disadvantages of polymersomes over LNPs and other relevant systems will be argued in the context of applications for cancer immunotherapy. Finally, we will present future objectives that should be pursued and discuss how current challenges can be overcome in order to exploit polymersomes as a nanomedicine platform.

2. Opportunities for polymersomes in cancer immunotherapy

To appreciate the potential of polymersomes in cancer immunotherapy, it is important to understand the rationale behind the current treatment strategies and identify the gaps and limitations in the field. Conventional cancer immunotherapies have been based on the intrinsic ability of the immune system to inhibit carcinogenesis and seek to enhance a naturally occurring cascade of events known as the cancer-immunity cycle [26]. Typically, dying tumor cells release tumor antigens and create an immunogenic milieu through so-called immunogenic cell death (ICD), which stimulates antigen-presenting cells (APCs), such as dendritic cells (DCs), to internalize, process and present tumor antigens. The DCs migrate to lymphoid tissues to activate and expand tumor-specific T cells, which home to the tumor site and extravasate into the tumor. Cytotoxic T cells recognize and kill tumor cells presenting their cognate antigen, which causes the release of additional tumor antigens and reinvigorates the cancer-immunity cycle. This paradigm allocates a central role to T cells, which logically have become the focus of current cancer immunotherapies, including immune checkpoint blockade (ICB), adoptive cell transfer (ACT) and cancer vaccines [27]. However, cancer immunity acts as a double-edged sword as the tumor develops an immunosuppressive or non-immunogenic tumor microenvironment (TME), consisting of tumor-promoting stromal cells and anti-inflammatory immune cells [28,29]. T cell-based approaches often succumb to this TME for different reasons depending on the tumor type, such as impaired T cell functionality or exclusion of T cells from the tumor [30].

Interestingly, cancer immunotherapy has shifted its focus from augmenting T cell responses to normalizing T cell function by turning immunosuppressive “cold” tumors into immunogenic “hot” tumors [31]. Likewise, the field recognizes the systemic nature of cancer immunity and the interplay of distinct immune cells from multiple lymphoid organs that dictate the immunogenicity of the local TME [32]. For instance, tumor presence induces alterations in spleen and bone marrow myelopoiesis to ensure that short-lived myeloid cells in the TME, like tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), are replenished [33]. These interacting immunological processes offer a targeting framework for nanomedicine in cancer immunotherapy. Hence, numerous reviews have discussed the use of nanoparticle systems for immunotherapeutic applications [34–37]. The development of nanovaccines, artificial APCs and the modulation of innate immune cells represent major research lines that have been pursued to enhance the efficacy and safety of cancer vaccines, ACT and ICB respectively (Fig. 1).

Conventional cancer vaccines deliver personalized neoantigens to tissue-resident DCs to induce antitumor T cell responses. However, the formation of robust T cell responses remains problematic, because of tolerance induction or insufficient antigen cross-presentation to cytotoxic T cells [38]. Nanovaccines may overcome these drawbacks by co-encapsulating antigen and adjuvant, targeting cross-presenting DC populations or delivering antigens to intracellular compartments involved in cross-presentation. Furthermore, antigen encapsulation into nanovaccines protects the cargo from degradation, which is particularly relevant for fragile mRNA-based antigens.

The need for antigen delivery to DCs and subsequent presentation to T cells is circumvented by ACT, which involves the isolation, *ex vivo* expansion and reinfusion of highly specific antitumor T cells. Clinical grade ACT protocols apply micron-sized artificial APCs (i.e. microbeads) with strictly defined stimulatory molecules for the expansion of T cells, but these cells often suffer from poor *in vivo* survival due to the long and cumbersome *ex vivo* culture protocols [39]. Furthermore, the potential of microbeads is limited to *ex vivo* use as intravenous administration poses the size-related risk of pulmonary embolism formation. The development of nano-sized artificial APCs has made the system suitable for safe and direct *in vivo* T cell activation, which increases the practicality and efficacy of T cell therapies.

Intrinsic T cell responses are further augmented by ICB, which effectively relieves inhibitory T cell signaling [2]. Nevertheless, the

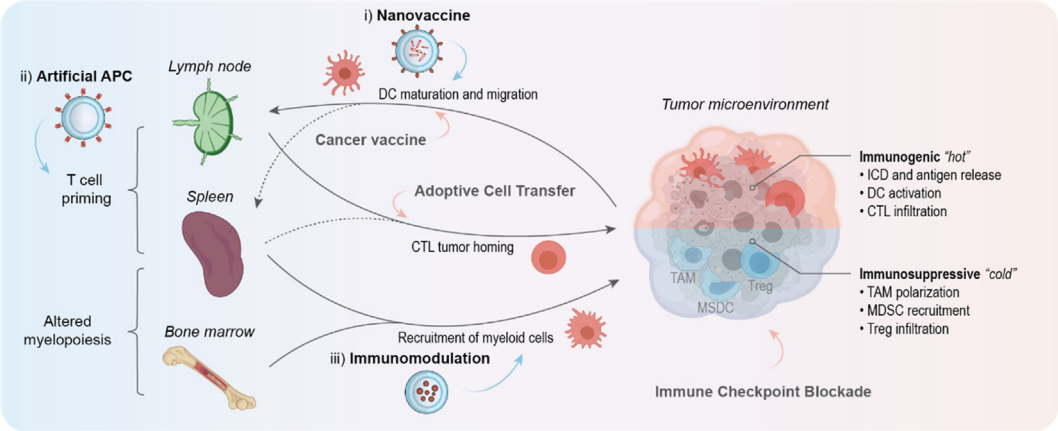

Fig. 1. | Opportunities to implement polymersomes as a nanomedicine platform for cancer immunotherapy. Cancer immunity is orchestrated systemically and involves immune cells in multiple lymphoid organs as well as the tumor microenvironment (TME). Cancer immunotherapy can utilize polymersomes as *i)* nanovaccines for antigen and adjuvant co-delivery to improve cancer vaccine immunogenicity, *ii)* artificial APCs for *in vivo* T cell activation to substitute for adoptive cell transfer (ACT) and *iii)* drug carriers for the modulation of immunosuppressive myeloid cells to enhance immune checkpoint blockade (ICB). APC = antigen-presenting cell, CTL = cytotoxic T lymphocyte, DC = dendritic cell, ICD = immunogenic cell death, MSDC = myeloid-derived suppressor cell, TAM = tumor-associated macrophage, Treg = regulatory T cell.

lack of tumor specificity often results in systemic autoimmunity, while the cytotoxic effect towards the tumor is limited by the immunosuppressive TME [30,40]. Nanomedicine has the capacity to improve ICB by specifically delivering it at the tumor site, although modulation of innate immune cells to alleviate immunosuppression in the TME represents a more interesting approach [41]. The pivotal role of myeloid cells in tumor immune escape and the intrinsic avidity of nanoparticles for these phagocytic cells makes them ideal targets for nanomedicine-based cancer immunotherapy. Moreover, the discovery of innate immunological memory, designated as trained immunity, has provided an easily druggable targeting framework to modulate myeloid cells in lymphoid organs even before their recruitment by the tumor [42].

These new insights into tumor immunology and the shortcomings of traditional cancer immunotherapy provide opportunities for the implementation of polymersomes in the applications described above (Fig. 1). Rational design is key to enhance cancer immunotherapy with nanomedicine, and polymersome research has deepened our fundamental understanding of how to tailor their topological design features, including morphology, surface chemistry and membrane properties [43–48]. For *in vivo* cancer immunotherapy, efficient biodistribution to lymphoid organs is the first priority when considering polymersome design, while the spatiotemporal control over immune cells within these tissues is of second importance. In the following sections, we will elaborate on optimal polymersome size and shape, surface chemistry and functionalization, and membrane properties in the context of targeting lymphoid organs and modulating specific immune cells.

3. Polymersome morphology

Polymersomes and LNPs share a similar degree of size control within the nanometer range. In terms of shape however, polymersomes have the advantage over LNPs as they can be kinetically trapped in a variety of shapes, such as discs, ellipsoids and tubes, while LNPs cannot maintain a shape other than spherical [43].

Polymersome size and shape can be tuned during or after the self-assembly process, which has been extensively reviewed elsewhere [43–46]. Generally, the dispersity of the size distribution depends on the preparation method and some approaches are more scalable and reproducible than others. For example, thin film rehydration and direct injection yield polydisperse polymersomes, while alternative formation methods, such as electroformation and emulsion phase transfer (i.e. solvent switch method), allow for better size control [44]. Additionally, extrusion, which is well-established for LNPs, has been employed as a size control measure for polymersomes [49,50]. More recent advancements, including microfluidics, polymerization-induced self-assembly (PISA), and flash nanoprecipitation, represent more practical, scalable, and reproducible options, potentially suitable for clinical use [44,45,51]. Non-spherical polymersomes are either prepared by shape transformation of spherical polymersomes, via osmotic pressure or chemical modification (e.g. cross-linking), or spontaneous self-assembly of modified block copolymers into non-spherical morphologies, known as the liquid-crystalline lattice confinement strategy [43,46].

Polymersome morphology can thus be simply adjusted and we envision a key role of these design parameters in biodistribution to lymphoid organs and towards the development of polymersome-based artificial APCs.

3.1. Biodistribution to lymphoid organs

Polymersome size and shape largely direct biodistribution, but the route of administration is also important. *In vivo* administered polymersomes need to efficiently reach specific immune cells in lymphoid organs, such as the spleen, bone marrow and lymph nodes, to effectively enhance cancer immunotherapy. Nanovaccines should target DCs in peripheral or lymphoid tissues, while artificial APCs ought to penetrate deep into the lymph node paracortex where T cells reside. Nanocarriers for the delivery of immunomodulators have to engage with myeloid cells or their progenitors in the bone marrow or spleen. Several polymersome examples illustrate the role of morphology and administration route in biodistribution to the spleen, bone marrow and lymph nodes (Fig. 2).

3.1.1. Distribution to the spleen

The spleen is effectively targeted via intravenous administration as circulating particles are naturally taken up by the mononuclear phagocyte system (MPS), which includes monocytes and macrophages. Particles generally distribute most efficiently to this organ when their size ranges between 100 and 200 nm[13–15]. In line with these observations, our group has demonstrated that increasing the size of PEG-polybutadiene (PEG-PBd) based polymersomes from 90

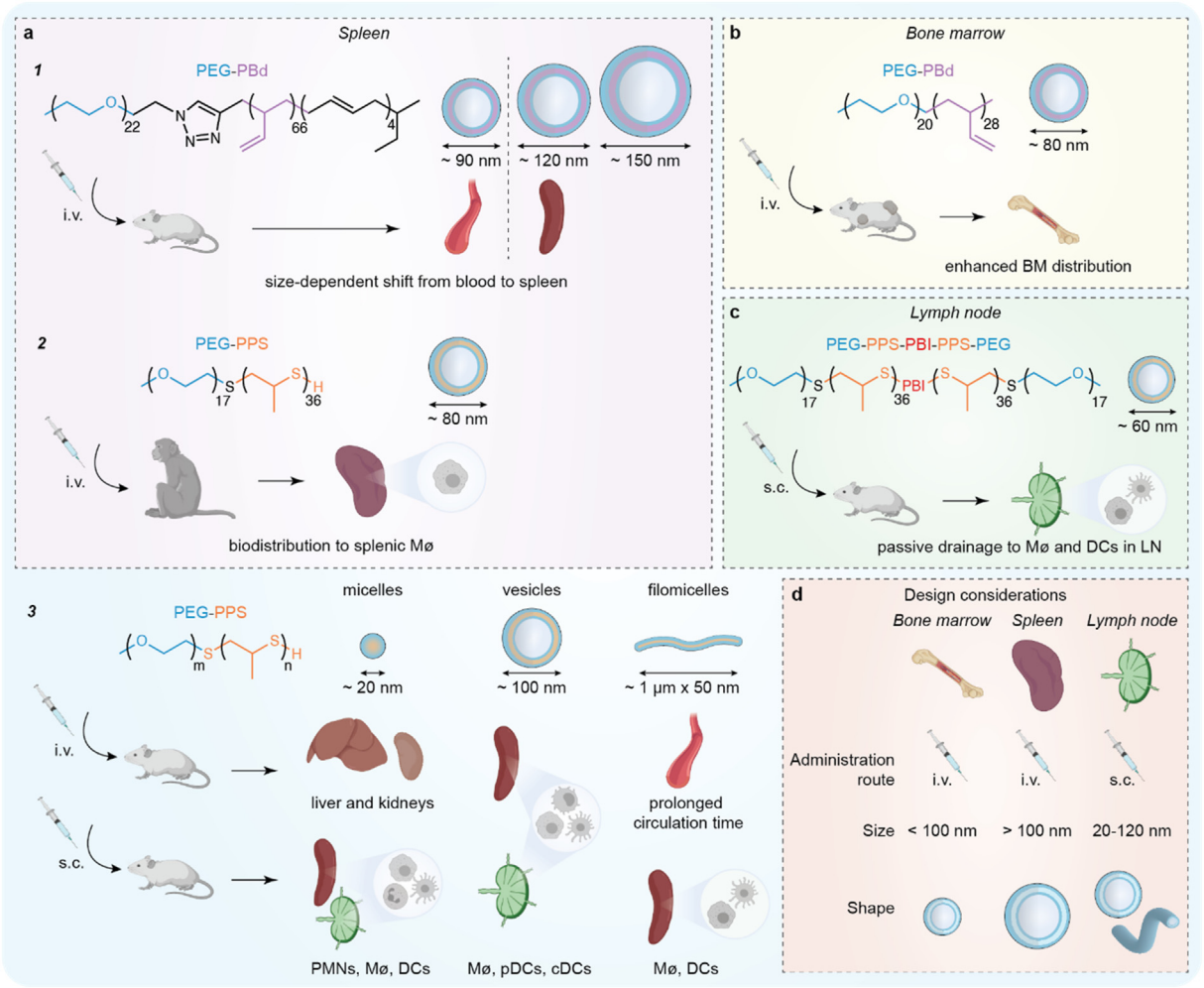

Fig. 2. | Overview of polymersome morphologies used for lymphoid organ targeting. Polymersomes with various morphologies have been used to investigate biodistribution to the spleen, bone marrow or lymph nodes upon i.v. or s.c. administration. Direct comparative studies in representative *in vivo* models have the most value in determining the optimal design considerations for lymphoid organ targeting. **a/1** was adapted from Ref. [52]. **a/2,3** was adapted from Refs. [54–56]. **b** was adapted from Ref. [57]. **c** was adapted from Ref. [65]. BM = bone marrow, LN = lymph node, s.c. = subcutaneous, i.v. = intravenous, PMN = polymorphonuclear leukocyte, Mφ = macrophage, pDC = plasmacytoid dendritic cell, cDC = conventional dendritic cell.

to circa 120 nm induced a sharp transition from long circulation times to accumulation in the spleen and liver of mice [52] (Fig. 2a/1). This change in biodistribution was more abrupt for rigid polymersomes than normally observed for flexible liposomes of similar size [52,53]. Polymersomes might thus have an advantage over LNPs in defining stricter size considerations for applications in cancer immunotherapy.

The biodistribution of polymersomes to the spleen has been further elucidated by Scott et al., who have developed PEG-poly(propylene sulphide) (PEG-PPS)-based nanoparticles of three different structures: micelles (20–30 nm), polymersomes (100–120 nm) and filomicelles (~50 nm in diameter and several microns in length) [54–56] (Fig. 2a/2,3). They observed that polymersomes most effectively and specifically targeted macrophages, plasmacytoid DCs (pDCs) and conventional DCs (cDCs) in the spleen upon both intravenous and subcutaneous administration (Fig. 2a/3). In contrast, micelles and filomicelles associated less efficiently with splenic macrophages and DCs, and distributed either to the liver and kidneys or spent more time in circulation, respectively [54,55]. Despite their difference in structure, the surface properties of these polymersomes, micelles and filomicelles are comparable and these results thus indicate a role for size and shape in polymersome biodistribution to the spleen. These studies were extended in an immunologically more relevant non-human primate model, in which they accumulated more in splenic macrophages than DCs in contrast to the observations in mice [56] (Fig. 2a/2). Given the higher suitability of non-human primates as a model for the human immune system, optimizing polymersome morphology to target the spleen intravenously may be more appropriate for the delivery of immunomodulatory drugs to macrophages than for directing nanovaccines to DCs.

3.1.2. Distribution to the bone marrow

Similar to the spleen, systemically injected particles accumulate within the MPS cells of the bone marrow [13–15]. Optimal morphological design of polymersomes to enhance bone marrow distribution has not been defined in detail yet, but PEG-PBd polymersomes of 80 nm in size readily distributed to the bone marrow, especially in tumor-bearing mice [57] (Fig. 2b). The altered bone marrow myelopoiesis induced by the tumor might have contributed to this change in bone marrow biodistribution. It is notable however, that the polymersomes showed relatively high splenic accumulation compared to the bone marrow.

Other important lessons can be learned from the nanobiologic

systems developed by the group of Mulder [58–61]. This apolipoprotein A-1 (apoA1)-based nanobiologic has demonstrated excellent bone marrow avidity and myeloid cell uptake, partly due to interactions of apoA1 with receptors commonly and abundantly found on these innate immune cells [58–60]. In a recent study, a screening for bone marrow affinity among 20 nm discoidal, and 35, 65 and 120 nm spherical nanobiologics, revealed optimal uptake by myeloid cells of 35 nm spheres compared to the smaller discs and larger spheres, which suggests superior biodistribution to the bone marrow of very small and spherical particles [61]. When translated to polymersomes, preparing vesicles within this size range may be troublesome due to the formation of micelles instead of polymersomes.

3.1.3. Distribution to the lymph nodes

The lymph nodes can be accessed via three distinct routes: 1) through the blood vasculature, 2) via afferent lymphatics, or 3) by intranodal injection. The latter approach is the most direct, although the procedure is generally more invasive and may disrupt the structural and functional integrity of the lymph node [62,63].

Entry into the lymph nodes via the vasculature is promising for T cell-targeting strategies, such as artificial APCs, as T cells reside near the blood capillaries in the paracortex. However, systemically injected polymersomes have shown minimal engagement with lymph node-resident T cells in mice and negligible lymph node accumulation in non-human primates [54,56].

Lymph node access through subcutaneous injection occurs either via passive drainage of relatively smaller particles within the 10–100 nm size range or through active DC-mediated trafficking of particles larger than 100–200 nm [62–64]. With oxidation-sensitive PEG-PPS-perylene bisimide(PBI)-PPS-PEG polymersomes that switch their fluorescent emission upon intracellular processing in reactive oxygen species (ROS)-rich compartments, Scott and colleagues demonstrated that polymersomes with a size of 20–50 nm associated with subcapsular sinus macrophages and accumulated inside mature DCs that locate close to the afferent lymph vessels [65] (Fig. 2c). Entry into the lymph nodes via the afferent lymphatics thus favors DC-targeting approaches, including nanovaccines, although passively draining polymersomes might penetrate deep enough into the lymph node to reach T cells as well.

Additionally, the Scott group also screened their PEG-PPS micelles, polymersomes and filomicelles for their distribution to lymph node-resident immune cells [54,55] (Fig. 2b/2). Compared to micelles and filomicelles, polymersomes targeted macrophages, pDCs and cDCs in the lymph nodes best after both systemic and subcutaneous injection with minimal accumulation in migratory DCs or polymorphonuclear leukocytes (PMNs) [54,55]. Similar to the effect of size and shape on spleen targeting, this data indicates a role for polymersome morphology in lymph node accumulation.

In summary, the route of administration and optimal polymersome size mainly determine biodistribution to lymphoid organs without the need to change the default spherical shape (Fig. 2d). Polymersomes smaller than 100 nm most likely achieve optimal bone marrow distribution, while a size above 100 nm favors spleen targeting. Polymersomes of 20–120 nm effectively drain to lymph nodes, although active DC-mediated trafficking should be explored in the context of nanovaccines, given the importance of DC maturation during lymph node migration.

Remarkably, indirect comparisons between the biodistribution of polymersomes and LNPs suggest similar morphological effects. The advantage of polymersomes might thus not directly be clear and warrants further investigation. Direct comparative studies in appropriate *in vivo* models are essential to elucidate the role of polymersome physicochemical properties on biodistribution and contribute to the clinical translation of polymersomes for cancer immunotherapy.

3.2. Towards shaping artificial APCs

Apart from their role in biodistribution, nanoparticle size and shape also affects the interaction of artificial APCs with T cells. Ideally, artificial APCs mimic the interface between natural APCs and T cells, known as the immune synapse [66]. They should provide a large surface area and stimulate ligand aggregation at the pre-organized T cell receptor (TCR) nanoclusters on the T cell surface [67]. Both size and shape play an important part in the formation of a strong and persistent IS. However, polymersomes have not yet been explored as artificial APCs, so the current knowledge on the effect of particle size and shape mainly comes from superparamagnetic nanoparticles, solid polymeric nanoparticles or polyisocyanopeptide (PIC) nanoworms, and has been thoroughly reviewed elsewhere [68–71].

Spherical microbeads have been extensively used as clinical grade artificial APCs in *ex vivo* expansion protocols due to their large surface contact area with the T cell. Non-spherical elongated artificial APCs have recently gained interest for *in vivo* application, because they can make up for the required decrease in particle size and surface area. Moreover, substrate flexibility enables multivalent binding and dynamic receptor clustering [72,73]. Therefore, the importance of size and shape is evident [68–71], although direct extrapolation of these findings to polymersomes should be avoided given the vast differences in physicochemical properties between polymersomes and the systems currently under investigation. However, our group has recently achieved control over the morphology of biodegradable poly(ethylene glycol)-*block*-poly($_{D,L}$-lactide) (PEG-PDLLA) polymersomes by optimizing the manufacturing procedure [74,75]. Through incorporation of a size extrusion methodology and an osmotically-induced shape transformation process, polymersomes with well-defined morphologies were prepared, including uniquely shaped nanotubes [74,75]. Interestingly, the different polymersome morphologies retained their surface functionalization [74] and drug loading [75] capacity, which readily allows for the conversion of these polymersomes into artificial APCs to systematically study the role of polymersome morphology on T cell activation.

Overall, each nanoparticle platform now pursued in the development of artificial APCs possesses promising features, e.g. large surface area [76] or flexible morphology [72,73]. It is therefore expected that the potential of shaping polymersomes into artificial APCs lies in the incorporation of both flexibility as well as an elongated surface area, which would allow for the systematic evaluation of nanoparticle morphological features for artificial APC development.

4. Polymersome surface chemistry and functionalization

Polymersomes and LNPs can employ similar surface functionalization strategies, but the chemical versatility of synthetic polymers, in contrast to natural phospholipids, can benefit the control over ligand conjugation through multiple coupling chemistries. Hence, polymersomes allow for facile surface functionalization of a wide variety of molecules and the most common methods, including non-covalent (e.g. streptavidin-biotin interaction) and covalent conjugation (e.g. azido-alkyne or maleimide-thiol click chemistry), have been comprehensively reviewed elsewhere [77]. Notably, polymersome surface chemistry, which is partially

determined by the degree and type of functionalization, can affect protein corona composition and ultimately their biological identity upon *in vivo* administration. Control over surface functionalization is thus an important design feature of polymersomes for cancer immunotherapy and we foresee particular significance with respect to biological identity as well as immunomodulatory features, such as active targeting, antigen display and the development of artificial APCs (Fig. 3).

4.1. Biological identity

The protein corona acquired by nanoparticles within the first few hours of injection largely dictates their biological identity and affects their performance [78]. Control over protein corona formation and composition is therefore therapeutically relevant and has historically been reduced through PEGylation to prevent nanoparticle clearance. Interestingly, PEG often comprises the entire surface of polymersomes due to its incorporation into the block copolymer, while the degree of PEGylation for liposomes is limited to low molecular ratios [79]. Consequently, the polymersome surface most likely constitutes of dense PEG chains in 'brush' conformation, which may result in a distinct biological identity compared to that of liposomes with PEG chains in a collapsed 'mushroom' state. Nevertheless, the biological identity of PEG-based polymersomes has not been fully elucidated yet and may also depend on interactions between physicochemical properties, including surface chemistry and functionalization.

Recently, Scott and colleagues have made initial steps towards controlling the biological identity of PEG-based polymersomes. They screened a small library composed of PEG-PPS micelles, polymersomes and filomicelles functionalized with methoxy, hydroxy or phosphate end groups to study the effect of surface chemistry and morphology on protein corona formation and composition [80]. Protein corona quantification and identification via proteomics revealed that human plasma proteins most abundantly adsorb to negatively charged phosphate surfaces, whereas the relatively inert methoxy end group displayed very low protein adsorption. A more detailed investigation into the protein corona composition disclosed a complex interplay between surface chemistry and morphology, of which the former largely dictated nanoparticle immunogenicity (i.e. complement activation) and the latter determined uptake by cells of the MPS. This suggests that the morphology-dependent biodistribution described in the previous sections is at least partially explained by a difference in biological identity [80]. Remarkably, regardless of surface chemistry, polymersomes adsorbed the most proteins associated with clearance by the MPS.

These findings suggest that PEG-based polymersomes intrinsically show enhanced internalization by immune cells because of a certain protein corona composition. So, their biological identity

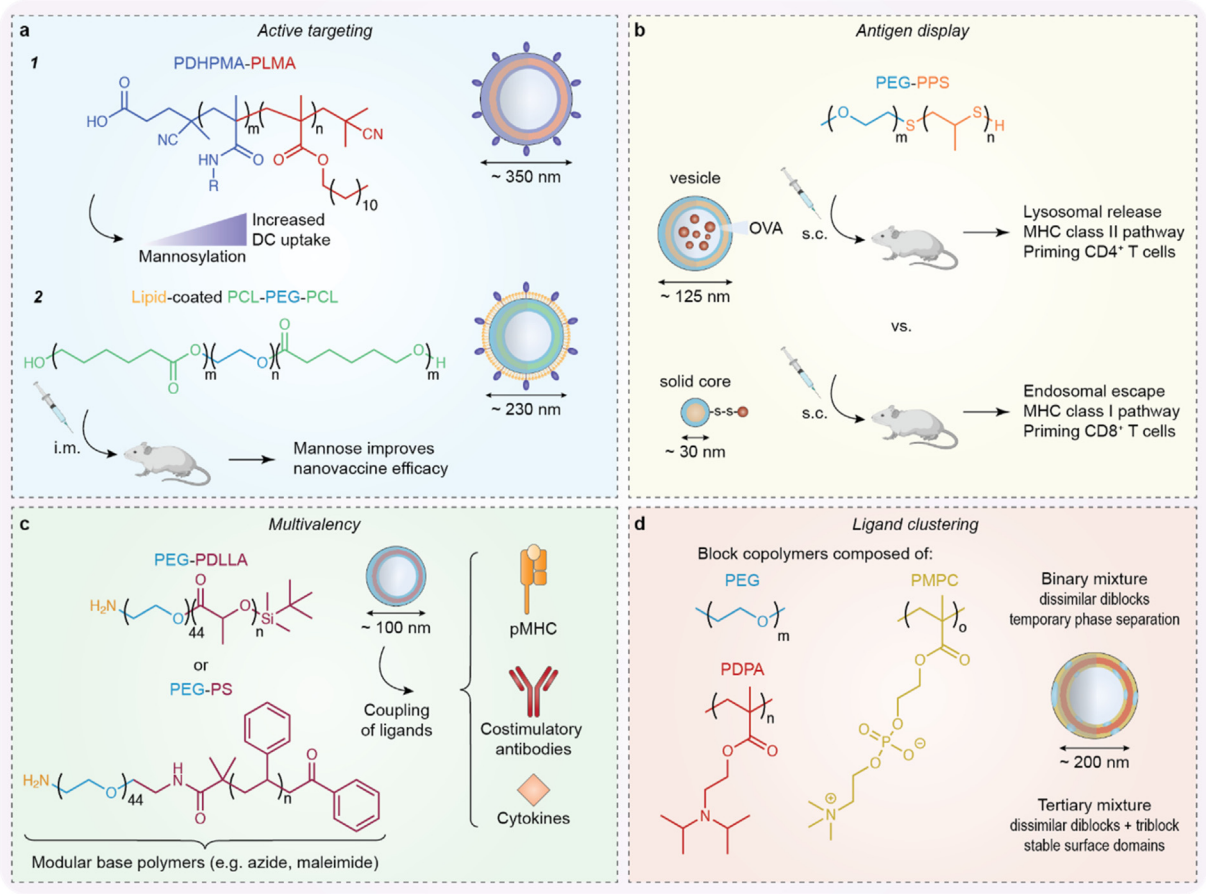

Fig. 3. | Overview of polymersomes with surface functionalization applicable in cancer immunotherapy. Functionalization of the polymersome surface may contribute to the targeting of immune cells, the processing of cargo and the presentation of stimulatory signals to immune cells. **a**, Active targeting of DCs with mannose moieties increases polymersome uptake and enhances nanovaccine efficacy. **b**, Surface display of antigens may increase the priming of cytotoxic $CD8^+$ T cells by altering the intracellular fate of the antigen. **c**, Block copolymer versatility allows for surface functionalization with multiple ligands via distinct coupling chemistries or **d**, the formation of functionalized surface domains through blending dissimilar polymers at varying ratios. i.m. = intramuscular, pMHC = peptide-major histocompatibility complex. **a/1** is adapted from Ref. [81] and **a/2** from Ref. [82]. **b** is adapted from Ref. [88]. **c** is adapted from Ref. [93]. **d** is adapted from Refs. [99,100].

could have vast implications for their use in cancer immunotherapy, although the protein corona composition of polymersomes functionalized with large immunomodulatory or targeting ligands remains unknown. Nonetheless, polymersome surface functionalization likely influences their performance in cancer immunotherapy through biological identity and warrants further investigation.

4.2. Active targeting moieties

In cancer immunotherapy, the utility of active targeting largely depends on the target cells, although reaching the desired lymphoid organ remains the first step. The improved efficacy of active targeting may not outweigh the trouble of target identification, ligand selection, and conjugation. In other words, phagocytic cells naturally internalize nanoparticles and may not require active targeting; rather, optimized particle morphology might sufficiently favor biodistribution to these cells. Alternatively, targeting of non-phagocytic cells, like T cells, or delivery to specific DC or other myeloid cell subsets, might require targeting moieties to improve spatiotemporal control. The potential of targeting does not end at the cellular level, because control over intracellular localization of cargo might ultimately translate into a higher therapeutic efficacy. Surface functionalization of polymersomes with targeting moieties can thus improve association with a particular cell type or direct transport to distinct intracellular compartments.

4.2.1. Actively targeting cell types

Active targeting of polymersomes to cells should exploit the expression of specific surface markers present on therapeutically relevant immune cell subsets, which are suitable for high affinity antibody or ligand-mediated targeting. Polymersome systems have only been used to explore targeting of the mannose receptor, a common C-type lectin receptor expressed by multiple innate immune cells including DCs [81,82] (Fig. 3a). Scherer and colleagues functionalized poly(2,3-dihydroxypropyl methacrylamide)-poly(lauryl methacrylate) (PDHPMA-PLMA) polymersomes with mannose to investigate the uptake by bone marrow-derived DCs in relation to the degree of mannosylation and found that only display of multiple mannose derivatives per polymer chain (4.3% mannosylation) increased internalization by DCs compared to less mannosylation (2.2%) and unmannosylated controls [81] (Fig. 3a/1). The group of Zhang enhanced antitumor immunity *in vivo* via the co-delivery of a model antigen, ovalbumin, and multiple Toll-like receptor (TLR) agonists to DCs in mannosylated dioleoyl-3-trimethylammonium propane (DOTAP) lipid-hybrid poly(-caprolactone)-PEG-poly(caprolactone) (PCL-PEG-PCL) polymersomes [82] (Fig. 3a/2). Mannosylation thus represents a valid strategy for active targeting of nanovaccines to DCs, although the use of more subset-specific markers should be investigated to enhance spatiotemporal control in cancer immunotherapy.

4.2.2. Actively targeting intracellular compartments

As different immunomodulatory drugs act at distinct intracellular locations, cancer immunotherapy may profit from the potential of polymersomes to direct their cargo to specific subcellular locations, including the cytosol, endosomal or lysosomal compartments, nucleus or mitochondria. Different research groups have equipped polymersomes with organelle-targeting moieties specific for transport to either the mitochondria or the nucleus [83–85]. Polymersomes functionalized with cationic triphenylphosphonium (TPP) ions targeted and traversed the highly negatively charged inner mitochondria membrane and delivered their cargo inside the mitochondria [83]. Similarly, conjugation of a nuclear localizing sequence (NLS), which naturally tags cellular proteins for transport to the nucleus, resulted in more efficient nuclear entry of polymersomes, although only particles smaller than 50 nm may be able to transport through the nuclear pores [84,85]. Thus, organelle-targeting moieties show promise in directing subcellular drug delivery, yet TPP and NLS are unspecific moieties and should be combined with active targeting of specific immune cell receptors to obtain a higher specificity.

In brief, active targeting is a promising strategy to achieve greater specificity due to the relatively distinctive expression patterns of certain cell surface markers. However, polymersomes may still predominantly reach phagocytic myeloid cells and active targeting might skew the biodistribution only slightly towards the desired target cell type. Alternatively, active cell targeting may have functional implications in terms of immune cell activation and intracellular processing [86]. Engaging immune stimulatory receptors with agonistic ligands may aid in DC maturation or immune cell activation in general, whereas binding to either recycling receptors or receptors tagged for degradation results in enhanced endosomal escape or preferred lysosomal degradation of the polymersome, respectively. Consequently, identification of ideal receptors for targeting, with high immune cell specificity, desired function and preferred intracellular dynamics, is of great importance.

4.3. Mode of antigen display

Polymersomes offer the possibility to encapsulate antigens in their vesicular lumen and conjugate them on their surface via covalent bonds or non-covalent adsorption. Antigen display through degradable or responsive-linkers, such as disulphide bridges, allows for the processing and presentation of antigens by DCs in nanovaccine applications. Co-delivery of both encapsulated and conjugated antigens can improve the antigen-presentation process and induce more robust antitumor immune responses. Furthermore, the antigen delivery method can affect the intracellular release and processing of antigen and thereby modulate the formation of either cellular or humoral immune responses [87].

Within a nanovaccine context, the group of Hubbell has directly compared PEG-PPS polymersomes encapsulating ovalbumin with PEG-stabilized PPS solid nanoparticles conjugated to ovalbumin via a reduction-responsive disulphide linker [88,89] (Fig. 3b). In one study, subcutaneous immunization of mice with either encapsulated or conjugated ovalbumin revealed that antigen encapsulation favored the activation of polyfunctional $CD4^+$ T cells and conjugated ovalbumin induced activation of effector $CD8^+$ T cells [88]. A follow-up study clarified the underlying mechanisms of different intracellular antigen processing within DCs and altered antigen distribution to specific lymph node-resident DC subsets [89]. Upon internalization by DCs, conjugated antigen was cleaved off inside the endosome and escaped into the cytosol for further processing and presentation to $CD8^+$ T cells on MHC class I molecules. Encapsulation in the polymersome protected the antigen until it reached the lysosomal compartment where the antigen entered pathways dedicated to presentation on MHC class II molecules to prime $CD4^+$ T cells. In addition, encapsulated antigen was retained in the lymphatic sinuses of lymph nodes and associated with DCs that preferably prime $CD4^+$ T cells. Conjugated antigen, however, penetrated deeper into the lymph nodes and gained access to $CD8^+$ DCs that activate $CD8^+$ T cells.

These studies excellently exemplify the importance of rationally considering the antigen delivery method. With regard to cancer immunotherapy, antigen delivery through conjugation can be preferable given the preference for antigen cross-presentation and priming of cytotoxic $CD8^+$ T cells. However, other well-established nanovaccine platforms, like PLGA nanoparticles, show opposing

effects [90]. Moreover, discrepancies in size and rigidity between the polymersomes and solid nanoparticles more likely explain the altered particle distribution, antigen processing, and ultimately priming of distinct T cell populations.

4.4. Towards functionalizing artificial APCs

Surface functionalization plays a vital part in artificial APC function by providing the necessary immune stimulatory cues to activate T cells. Optimal T cell activation by natural APCs requires the presentation of three functional signals: 1) an antigen-specific signal in the context of MHC molecules that engages TCRs, 2) costimulatory cues, like CD80 or CD86, which bind to the costimulatory molecule CD28 on the T cell and 3) signaling of cytokines, such as interleukin (IL)-12 or interferon (IFN)-α, secreted by the APC to shape the induced T cell response. Artificial APCs should at least mimic the first TCR signal and second costimulatory signal to induce T cell activation, although the third cytokine signal should ideally be included to direct T cell differentiation. However, effective mimicking of the complex organization and dynamics of receptors and ligands within the synapse is equally important. Namely, newly triggered TCR nanoclusters and CD28 costimulatory molecules constantly re-organize into larger microclusters in the peripheral synapse and translocate to its center to sustain prolonged TCR signaling and thereby induce strong T cell activation [91]. Therefore, the use of polymersomes as artificial APCs demands multivalency, i.e. the ability to present multiple signals, and control over the density or clustering of surface-conjugated ligands.

4.4.1. Multivalent signaling

The facile surface functionalization of polymersomes allows for the relatively easy fabrication of multivalent polymersomes, although it remains challenging to achieve control over ligand ratio and spatial arrangement. Conjugation of multiple ligands along the same coupling methods, either simultaneously or stepwise, uncontrollably skews the ligand ratio due to competition for the same binding sites or because of bias towards more reactive or sterically exposed functional groups [92]. The versatility of polymersomes allows for the incorporation of multiple conjugation strategies specific for each ligand. Synthesis and subsequent blending of different pre-functionalized block copolymers would improve control over ligand ratio and density, although this brings about the problem of having to repeat polymer synthesis for each specific ligand and associated reactive group.

Rijpkema et al. have found a solution to this issue by constructing amine-functionalized base block copolymers made of PEG-PS or PEG-PDLLA that can accommodate virtually any reactive moiety prior to self-assembly and conjugate a desired ligand specifically to this reactive group after polymersome formation. They created a library of 32 differently functionalized polymers, including amino-, azido-, and maleimide-moieties, which readily self-assembled into polymersomes [93] (Fig. 3c).

Nonetheless, these ligation strategies conjugate at random sites and cause suboptimal spatial orientation, which can hamper ligand binding capacity and functionality. Polymersome surface functionalization may benefit from the conjugation of site-specifically engineered proteins that direct spatial arrangement to enhance the control over multivalent ligand presentation and improve the reproducibility of functionalized polymersomes [69,94].

4.4.2. Controlling ligand density and clustering

Apart from multivalency, ligand density and clustering have great implications for the initiation and sustenance of T cell signaling and robust activation by increasing TCR binding avidity and mimicking TCR dynamics. The ability of ligands to cluster depends on the membrane fluidity of the applied platform or the ability to form pre-organized surface domains. Liposomes seem the ideal artificial APC given their biocompatibility and high membrane fluidity, which permits liposomes to dynamically rearrange and organize conjugated ligands. Several liposomal artificial APC formulations have demonstrated potential in T cell activation, although these favorable dynamic properties could also render them unstable and thus less suitable for therapeutic use [95–97]. Polymersomes are composed of building blocks with higher molecular weight and increased chain entanglement compared to liposomes, which improves their stability but also reduces motility of conjugated molecules. Nonetheless, the chemical versatility of block copolymers has proven an opportunity to tune membrane fluidity by choosing optimal polymer molecular weight, although the maximal lateral ligand diffusion did not match that of liposomes [98].

Alternative to enhancing polymer membrane fluidity, the Battaglia group has developed a method, based on phase-separation of dissimilar diblock polymers, to form stable surface domains that could potentially be used to pre-cluster ligands on a polymersome surface [99,100] (Fig. 3d). A binary mixture of PEG-poly(2-(diisopropylamino)ethyl methacrylate) (PDPA) (PEG-PDPA) and poly((2-methacryloyloxy)ethyl phosphorylcholine)-PDPA (PMPC-PDPA) polymers caused a temporary phase separation, whereas a PEG-PDPA-PMPC triblock stabilized the surface domains when added to the mixture. Variations in molar ratio between the polymers could tune the size and shape of these domains to some extent [99,100].

On the whole, several strategies exist to find the highest ability of ligand clustering possible. However, this degree of control could render the polymersome system also very complex and one could argue if it is necessary to incorporate this amount of complexity into the system in terms of polymersome functionality. Moreover, less complex methods exist that facilitate TCR dynamics, such as shape transformation, which are unavailable to LNPs.

5. Polymersome membrane properties

Similar to surface functionalization, the chemical versatility of polymersomes also outcompetes that of LNPs with respect to designing membrane properties. The use of biodegradable or stimuli-responsive (e.g. pH, redox, temperature or light) block copolymers to tune membrane properties has been comprehensively reviewed elsewhere [47,48]. Typically, polymersomes provide the ability to create multi-responsive nanocarriers to precisely direct both the encapsulation of hydrophilic and hydrophobic drugs as well as the spatiotemporally controlled cargo release (Fig. 4).

5.1. Encapsulation efficiency

Moderate to high encapsulation efficiencies of hydrophilic or hydrophobic small molecule drugs into polymersomes are quite easily achieved by adding the compound either to the organic solvent or the aqueous phase during self-assembly. Polymersomes have displayed similar encapsulation efficiencies for both drug types compared to liposomes [101]. However, the encapsulation of therapeutically relevant hydrophilic cargo, such as proteins (e.g. antigens or cytokines) or nucleic acids (e.g. mRNA or siRNA), remains difficult and LNPs have already proven to be a superior platform for mRNA delivery [102]. Adjustments to the polymersome membrane chemistry may assist in achieving high quantities of encapsulated cargo (Fig. 4a).

The group of Meng and Zhong has demonstrated that electrostatic interactions between cationic polymers and anionic cargo increase the encapsulation efficiency of proteins and nucleic acids

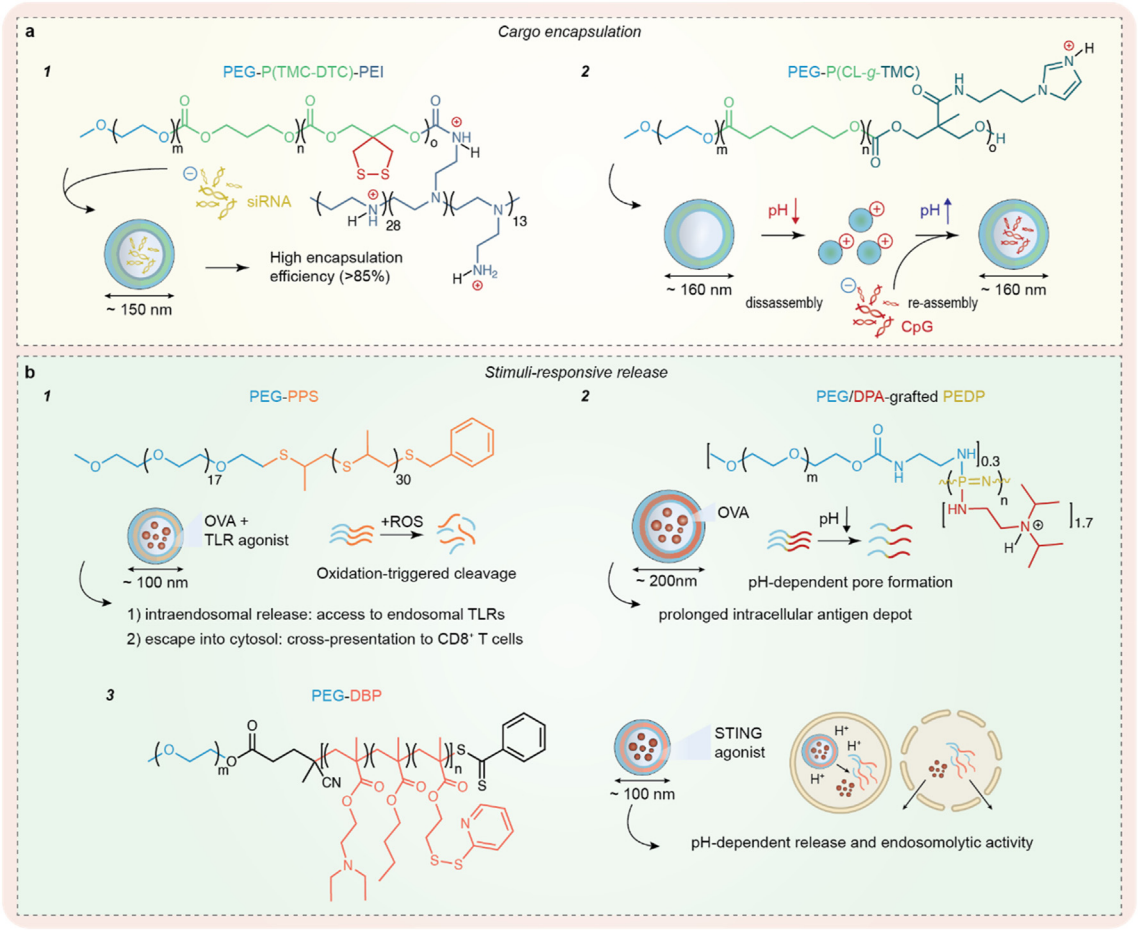

Fig. 4. | Overview of polymersome membrane properties applicable in cancer immunotherapy. Polymersome membrane properties facilitate high contents of encapsulated cargo or spatiotemporal control over cargo release. **a**, Incorporation of cationic polymers promotes the encapsulation efficiency of anionic and therapeutically relevant proteins and nucleic acids. Encapsulation techniques that utilize stimuli-responsive membranes enable high drug content loading. **b**, Stimuli-responsive (e.g. oxidation or pH) membranes control intracellular localization and release of cargo through polymer cleavage, membrane pore formation or disassembly. siRNA = small interfering RNA, OVA = ovalbumin, ROS = reactive oxygen species, STING = stimulator of interferon genes, TLR = toll-like receptor. **a/1** is adapted from Refs. [103,104] and **a/2** from Ref. [106]. **b/1** is adapted from Ref. [112], **b/2** from Ref. [113], and **b/3** from Ref. [114].

[103–105] (Fig. 4a/1). Asymmetric polymersomes composed of triblocks with PEG as the outer hydrophilic block, poly(trimethylene carbonate-co-dithiolane trimethylene carbonate) (P(TMC-DTC) as the hydrophobic block and either cationic poly(2-(diethylamino) ethyl methacrylate) (PDEA), poly(ethylenimine) (PEI) polymers or spermine, a polyamine, as the inner hydrophilic blocks have shown 85–90% encapsulation efficiency for proteins and almost 100% for siRNA and CpG, respectively [103–105]. The asymmetry of the triblock caused the longer PEG chains to constitute the outer membrane layer and the shorter PDEA, PEI or spermine chains to preferably orientate towards the vesicle lumen, which would potentially reduce the toxicity of the cationic polymer.

Building on the principle of electrostatic interactions, our group utilized pH-responsive imidazole-functionalized PEG-P(CL-g-TMC) polymers to augment the encapsulation of ovalbumin and CpG, a nucleic acid TLR agonist often used as an adjuvant in cancer vaccines [106] (Fig. 4a/2). The positive charge of protonated imidazole at acidic pH enhanced the electrostatic interaction between the polymer and ovalbumin and improved its encapsulation into polymersomes via a conventional encapsulation method in comparison with non-responsive polymers [106]. The pH-responsiveness also allowed for the full disassembly and subsequent reassembly into equally sized vesicles by merely tuning pH, and offered the possibility to load hydrophilic drugs upon reassembly. Encapsulation through reassembly increased the loading of CpG almost five-fold as compared to the traditional encapsulation method and also made the system more biocompatible because it eliminated the need of potentially harmful organic solvent [106].

In general, increasing the association between the polymersome and the cargo will increase its encapsulation, although adjustments in polymersome structure or chemistry may have consequences for other aspects, including self-assembly and toxicity profile. On the other hand, too strong polymer-cargo interactions may hamper drug release or function at the target site.

5.2. Controlled release

Control over the timing and localization of drug release by the polymersome is of major concern in cancer immunotherapy. Temporal control implies either the rapid burst release or the sustained release over prolonged periods of time, while spatial regulation means that drug release should only occur near or inside the target cell and sometimes even at specific intracellular locations. Biodegradable or stimuli-responsive polymersome membranes fulfil the needs to obtain high control over drug release in

immunotherapeutic applications, although the latter provides the most influence over spatiotemporal drug release and has been most explored in polymersome systems for cancer immunotherapy.

5.2.1. Biodegradable membranes

Several biodegradable polyesters and polycarbonates have been used for polymersome assembly, including poly(lactide) (PLA), PCL and PTMC [74,107,108]. Additionally, polypeptides and polysaccharides, such as poly(L-glutamic acid) (PGA) and dextran, have been utilized [109,110]. PEG, although non-biodegradable, is considered non-toxic and biocompatible. Polymersomes made from biodegradable polymers are susceptible to hydrolysis, which causes progressive pore formation in the membrane and ultimately results in the disintegration of the vesicle. Encapsulated cargo is completely released from the polymersome within a certain time frame, which varies from hours to days, and the hydrolysis rate can be modulated by increasing the hydrophilic to hydrophobic ratio of the block copolymer or by blending biodegradable polymers with non-biodegradable ones [111]. Sustained release creates a cargo depot for the prolonged delivery of immunomodulatory drugs or exposure to antigen and may be especially useful in the stimulation of T cells with cytokines to maintain activation and induce memory formation.

5.2.2. Stimuli-responsive membranes

Stimuli-responsive polymersomes either non-reversibly disassemble or undergo a reversible increase in membrane permeability upon receiving an environmental trigger. The stimulus is often chosen to be specific for a certain subcellular compartment, for instance the acidic and oxidative endosomal milieu may trigger pH or oxidation-sensitive polymersomes (Fig. 4b).

Scott et al. have developed an oxidation-susceptible nanovaccine formulation based on PEG-PPS polymersomes for the delivery of ovalbumin and a TLR ligand to DCs, which improved DC maturation and the proliferation of effector $CD8^+$ T cells compared to soluble antigen and adjuvant [112] (Fig. 4b/1). The cargo release occurred in two stages: 1) intraendosomal release, which promotes binding of adjuvant to endosomal TLRs, and 2) endosomal escape into the cytosol, which facilitates antigen cross-presentation to $CD8^+$ T cells [112].

Similarly, Gao and co-workers established a pH-responsive nanovaccine based on polymersomes self-assembled from PEG and DPA grafted polyphosphazene (PEDP) polymers [113] (Fig. 4b/2). A drop in pH caused an almost complete release within one week and the vesicles surprisingly did not fall apart immediately upon pH change, but their structure gradually became perforated and less compact. So, these stimuli-responsive polymersomes displayed a sustained antigen release profile and created an intracellular antigen depot that enhanced the following T cell response [113].

Apart from nanovaccine applications, stimuli-responsive polymersomes are also useful in the delivery of immunomodulatory drugs to innate immune cells. The group of Wilson has produced a pH-responsive polymersome platform, based on PEG-poly(2-(dimethylamino)ethyl methyacrylate-co-butyl methacrylate-co-pyridyl disulfide ethyl methacrylate) (PEG-DBP), with pH-triggered cargo release and endosomal membrane destabilizing activity through interpolymer crosslinking, for the delivery of stimulator of interferon genes (STING) agonists to the cytosol of innate immune cells [114–116] (Fig. 4b/3). STING agonists have proven a promising therapeutic target in cancer immunotherapy and several nanomedicine systems have been used for their delivery [117,118]. STING polymersomes, either with or without co-encapsulation of antigen, provoked antitumor immunity in synergy with ICB upon subcutaneous or intravenous injection respectively [114,115]. In a recent biodistribution study, high accumulation in splenic macrophages and DCs was deemed as a clinical problem due to the significant splenic toxicity observed, which the authors attributed to the high STING expression in this lymphoid organ [116]. However, the high sensitivity of the spleen for STING agonists does not necessarily have to be a hurdle to clinical translation; rather, the spleen could be an interesting target given its role in tumor-induced altered myelopoiesis and the fact that lower drug concentrations might be sufficient to reach a desired therapeutic effect with minimal toxicity and safety issues.

In short, polymersome membrane properties play an essential part in achieving spatiotemporal control over both drug encapsulation and delivery. The chemical versatility of block copolymers permits the design of polymersomes with multiple distinct responsive elements, specific for either cargo loading or triggered release [119]. Ultimately, this may lift polymersomes to a new level as a drug delivery system, but also add to the complexity of the system.

6. Conclusions

Taken together, the examples described in this review illustrate the potential of polymersomes as a platform for cancer immunotherapy. The versatility of polymersomes justifies their use as nanovaccines, artificial APCs or drug carriers to modulate myeloid cells. All these applications require certain specific physicochemical properties to optimize biodistribution and maximize therapeutic efficacy (Fig. 5). Polymersome morphology generally directs a favorable biodistribution to lymphoid organs, which is the most critical parameter for *in vivo* cancer immunotherapy. Polymersome nanovaccines ideally prevent tolerance induction and induce cross-presentation of the delivered antigen by co-encapsulation of antigen and adjuvant or through active targeting of specific DC subsets and the triggered intracellular cargo release, respectively. Effective polymersome-based artificial APCs closely resemble the immune synapse in terms of both cell-cell and receptor-ligand interactions via shape transformation and design of ligand density. Optimal polymersome drug carriers promote receptor-mediated internalization and regulate the subcellular localization of a variety of immunomodulatory drugs through the conjugation of surface moieties and the incorporation of stimuli-responsive cargo release.

In spite of the preclinical progress so far, the emergence of polymersomes as a platform for cancer immunotherapy has been hampered and several challenges to improve clinical translation currently remain. The *in vivo* behavior and biodistribution of polymersomes, and the role of design characteristics herein, stay largely unknown, despite the importance of these parameters in cancer immunotherapy. Systematic *in vivo* studies to investigate biodistribution in relation to polymersome design should receive more priority alongside *in vitro* studies to explore the interactions between polymersomes and immune cells in more detail. Moreover, these *in vivo* studies should not be limited to the effect of polymersome morphology as other properties, like rigidity and chemical nature may also affect pharmacokinetics. Similarly, understanding the interplay between polymersomes and immune cells, and the role of surface functionalization and membrane properties, requires close collaborations within the field. Rapidly advancing methods, such as PET/CT or flow cytometry, as well as newly emerging techniques, like high-resolution microscopy are exceptionally suitable to investigate the biodistribution and cellular interactions of polymersomes in great detail.

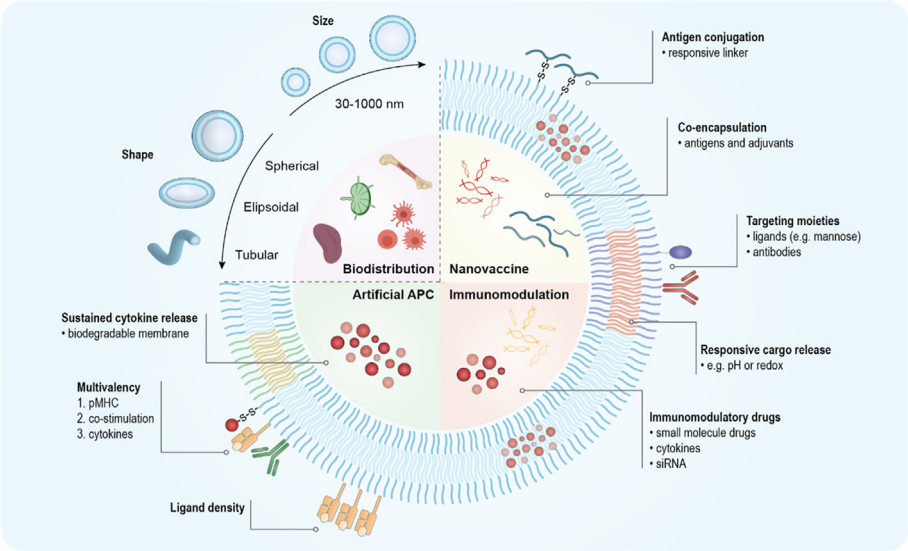

Fig. 5. | Physicochemical properties essential to polymersome applications in cancer immunotherapy. The different polymersome applications in cancer immunotherapy each require specific physicochemical properties to optimize biodistribution and maximize therapeutic efficacy. Optimized size and shape generally result in favorable biodistribution to lymphoid organs, while ideal surface and membrane characteristics play important parts in interactions at the polymersome-cell interface and inside the target cell. Artificial APC = artificial antigen-presenting cell, pMHC = peptide-major histocompatibility complex, siRNA = small interfering RNA.

Establishing optimal polymersome design considerations for applications in cancer immunotherapy requires the thorough investigation of all kinds of different characteristics. A way to systemically screen for these optimal parameters is by the creation of block copolymer or polymersome libraries. Methods to easily fabricate many physicochemically slightly different polymersomes have already been established to create a variety of polymer compositions or to control surface functionalization, membrane permeability or morphology [75,80,93,120,121]. These techniques can be utilized as a facile way to produce large libraries that differ only in the property under investigation. It is important that the polymersomes are compared with physicochemical identical counterparts and other clinically relevant systems, like LNPs, to promote intra and inter study comparisons and clinical translation. Finally, polymersomes' translation to the clinic is highly dependent on the development of scalable, reproducible and safe preparation methods, such as the rapid fabrication of polymersomes through rehydration of lyophilized polymers [122].

The designability of polymersomes may also add to the complexity of the system. The effect and interplay between the different aspects of a complex multivalent polymersome system on its functionality and clinical efficacy is unknown. An overly designed polymersome may not only hamper clinical translation by creating manufacturing problems or introducing new safety issues, but may also be unnecessary to achieve the desired therapeutic effect. For example, a spherical shape seems to result in optimal biodistribution to lymphoid organs compared to non-spherical shape, which suggests that it is not necessary to incorporate a shape transformation, although direct comparative evidence is lacking. Nevertheless, to enhance the progress in polymersome research and improve clinical translation, simpler is often better. Apart from the unnecessity of certain physicochemical properties, some may also have opposing effects and cannot be incorporated in the system simultaneously. For instance, a non-spherical shape might improve T cell activation, but may reduce the accumulation in T cell-rich areas, such as the lymph node. Ultimately, rational trade-offs have to be made to decide which polymersome properties to include in the design and which not.

In conclusion, this review highlights the potential of polymersomes as a platform for cancer immunotherapy. Polymersomes possess several advantages over other conventional systems and have the prospect of becoming an integrated immunotherapeutic platform, that is especially appropriate for, but not limited to, immunotherapy of cancer.

Author contributions

Jari F. Scheerstra: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. Annelies C. Wauters: Conceptualization, Writing – review & editing, Visualization, Supervision. Jurjen Tel: Writing – review & editing. Loai K. E. A. Abdelmohsen: Conceptualization, Writing – review & editing, Supervision. Jan C. M. van Hest: Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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