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Abstract: Background: The worldwide medicine research is dedicated towards improvement of patients’ health and diseased state. The use of nanotechnology is an upcoming area which is at present, is highly focused in the filed of medicine. Continuous studies in the area have given rise to the development of a novel field called “nanomedicine” which mainly aims to open new perspective of treatments and increasing therapeutic efficacy of existing therapies.

Methods: We searched Google, PubMed portals for the literature survey of the following subjects so as to get latest updated information pertaining to latest developments in the field of polymerosomes in nanomedicine.

Results: In most of the recent past studies, nanomedicine has gain attention all over the world and has lead to development of new approaches for medical treatments. Some of these approaches involved solubilization, targeting of cancer medicine or application as a diagnostic tool. Polymerosomes, which are artificial amphiphilic vesicles, made up of different chemical polymers, are currently being investigated for delivering various probes for imaging target tissues/ organs and cytotoxic drugs to tumor cells and also for gene therapy. In the present review, we discuss the current views on polymerosomes and their medical applications, a prominent emerging area in the field of nanomedicine.

Conclusions: Advances in nanomedicine have led to the development of nano-sized polymerosomes as vehicles for different medical applications.

Keywords: Nanomedicine, nanotechnol, poymerosomes, therapy.

INTRODUCTION

Polymersomes are artificial vesicles enclosing an aqueous cavity, resulting from self-assembly of amphiphilic copolymers [1, 2]. Additionally, optimization on the vesicular membrane can help to adjust the polymerosomes for different applications, e.g. as drug delivery vehicles or artificial organelle [3-7]. Polymers are chemical compounds consisting of many repeating subunits called monomers, and they can exist as chains or in branched form. Besides synthetic polymers, natural polymers are essential in nature, as they are involved in different aspects of life on the molecular level. Deoxyribonucleic acid (DNA) and proteins for examples are two essential biopolymers and without them life would not exist in the form we know it. DNA and proteins consist of different subunits (monomers), while PET or PEG are buildup of only one kind of monomer. Polymers consisting only of one type of repeating unit are called homopolymers, while polymers made from different building blocks are called copolymers. As polymers (homo and copolymer) are made up of typically more than ten repeating units and have a high molecular weight, they are also classified as macromolecules. Polymerosomes are highly versatile and biologically stable systems and their overall properties and drug encapsulation and release capabilities can be easily tuned by applying various block copolymers that are biodegradable and/or stimuli-responsive. These advantages make polymerosomes one of the most interesting supramolecular structures for potential applications in delivery of drugs, genes and proteins in the newly emerging area of nanomedicine and nanobiology.

BLOCK COPOLYMERS

Polymers that are used to form polymerosomes are called block copolymers and have an amphiphilic nature [8]. Block copolymers are macromolecules that contain different adjacent blocks of chemically distinct monomers, different composition or different sequence distribution [9]. A block copolymer that consists of two types of monomers is called a
was used as hydrogel for drug delivery for controlled release even at high concentration of up to 1000 μg/mL [21]. PNVP, another hydrophilic polymer, shows low toxicity be introduced on the surface of the nanoreactors [20]. Functionalization additional properties e.g. cell targeting, can for medical applications [18,19] and by end-tentions. Additionally, it was shown that PMOXA can be used response and therefore be less tolerable for medical applica-

PMOXA (the hydrophilic block) has been observed to be biocompatible and is mostly cleared from the blood stream after 24 h14. As degradation of PDMS in the body is slow, the clearance from the blood stream is linked to renal clearing. PDMS with lower molecular weight (around 5 kDa) can be removed from the body easily, while higher molecular weight PDMS has a tendency to accumulate in tissue [14]. The molecular weight cut-off in kidney is approximately 30-50 kDa [15], which explains that smaller the polymers are, the better they get cleared from the body, even if they are not biodegradable. Additionally, an advantage of PMOXA is that it is protein repellent [16], which could be helpful for medical application to avoid an immune response. Being protein repellent refers to the stealth properties of pegylated liposomes, which show better blood circulation properties as non-pegylated [17]. The hydrophilic block of the polymer is in contact with the body fluids, PMOXA is preferred because of its non-ionic nature. In case of charged polymers the self-assembled polymersome could induce stronger immune response and therefore be less tolerable for medical applications. Additionally, it was shown that PMOXA can be used for medical applications [18,19] and by end-functionalization additional properties e.g. cell targeting, can be introduced on the surface of the nanoreactors [20].

PNVP, another hydrophilic polymer, shows low toxicity even at high concentration of up to 1000 μg/mL [21]. PNVP was used as hydrogel for drug delivery for controlled release of different drug substances [21,22]. PNVP can also be used as solubilization agent, due to its high water solubility, which then can be used to conjugate for example to tumor necrosis factor-α(TNF-α) and then apply it intravenous to mice [23]. The PNVP conjugated TNF-α had extended blood circulation time compared to PEG-TNF-α and a 90-fold higher plasma-life than native TNF-α, which proves the bio-compatibility of PNVP in animals.

PDMS, the hydrophobic block, which is used as constituent material for contact lenses or breast implants, is known to be biocompatible for a long time [24]. Due to the good ability to form PDMS with accuracy of few nanometers [25], it has also been used for life-saving devices like pacemakers and is well-known in the medical field. PDMS is used in food industry as anti-foaming agent as additive E900 in concentrations up to 10 mg/L, and shows good tolerability in humans. Additional silicon based polymers also have a good bio durability helping to improve stability of self-assembled structures made of PDMS. PDMS has a low glass transition temperature (Tg= 146 K), which makes the PDMS chain flexible at room temperature or higher [26]. Otherwise it is also stable under oxidative conditions and at higher temperatures, which partially explains the good chemical and biological stability [27-29]. The combination of one hydrophilic polymer (PMOXA and PNVP) with the hydrophobic PDMS allows to build an amphiphilic polymer, which can then be used for self-assembling nanomedical structures, since all of the polymers are known to be tolerable for medical applications.

**SELF-ASSEMBLY OF AMPHIPHILIC BLOCK COPOLYMER**

Amphiphilic block copolymers can, as already mentioned, self-assemble in aqueous solution into different structures. The obtained architecture depends on several parameters such as concentration, molecular weight, geometry of the amphiphilic polymer or the ratio of the different blocks. The preferred structures are polymersomes and therefore the chosen triblock copolymers need to be optimized to form vesicular structures with an aqueous core. A similar behavior can be observed if lipids – naturally amphiphilic molecules – are dissolved in aqueous solution. Liposomes (from lipids

| Polymers | Formation method | Drug/stimulus for release | Degradability |
|----------|------------------|--------------------------|--------------|
| PEG-PBE  | Film rehydration | None reported             | No           |
| PEG-PMBD | Film rehydration | Paclitaxel, doxorubicin   | No           |
| PMOXA-PDMS-PMOXA | Phase inversion, UV crosslinking | Calcein | No |
| PEG-PLA | Phase inversion | Carboxyl fluorescein      | Yes          |
| PEG-PCL | Phase inversion | Carboxyl fluorescein      | Yes          |
| PEG-PTMC | Phase inversion | none reported             | Yes          |
| PEG-PTMBPEC | Phase inversion | Paclitaxel, doxorubicin/ pH-triggered hydrolysis | Yes |

PEG-PBE: poly(ethylene glycol)-b-poly(ethyl ene); PEG-PMBD: poly(ethylene glycol)-b-poly(butadiene); PMOXA-PDMS-PMOXA: poly(2-methyl-2-oxazoline)-b-poly(dimethylsiloxane)-b-poly(2-methyl-2-oxazoline); PEG-PLA: poly(ethylene glycol)-b-poly(lactide); PEG-PCL: poly(ethylene glycol)-b-poly(e-caprolactone), PEG-PTMC: poly(ethylene glycol)-b-poly(trimethylene carbonate); PEG-PTMBPEC: poly(ethylene glycol)-b-poly(2,4,6-trimethoxybenzylidenepentaoxyrithiol carbonate).
and the Greek word soma (body)) are normally formed in aqueous solution, which is also the case for their synthetic analogue: the block copolymers [30,31]. The self-assembly is mainly driven by non-covalent interaction (van der Waals forces) of the hydrophilic block. The aqueous phase favors the hydrophilic blocks and this triggers the self-assembly process to avoid water contact with the hydrophobic part of the block copolymer [32].

Polymersomes can be generated in different sizes, from tens of nm up to μm - so called giant polymersomes [33,34]. The size of polymersomes is influenced by different parameters from the amphiphilicity of the polymers themselves, up to the preparation methods used to self-assemble the polymersomes. After the formation of the polymersomes their structure can be additionally influenced by external factors such as extrusion, sonication or freeze/thaw cycles [35,36]. The thickness and fluidity of the membrane depends on the character of the block copolymer. The thickness of the polymersome membrane is influenced by the molecular weight and the block number (tri- or diblock) [37]. Triblock copolymers, with the same molecular weight as diblock copolymers, form thinner membranes and higher the molecular weight of a block copolymer is, the thicker is the polymersome membrane.

Polymersomes are mainly utilized for the purpose of encapsulation of the molecules. Further developments of polymersomes as nanoreactors, include the in situ production of active molecules. Nanoreactors combine the possibility to shield active molecules such as enzymes or proteins in nanometer size compartments, while preserving their functionality in situ [38].

MEDICAL APPLICATIONS OF SELF-ASSEMBLED STRUCTURES

Polymersomes

Lipid based drug carriers are already approved for nanomedical purposes and in clinical trials [39-42]. Nowadays drug delivery systems are created by encapsulation of the drug substance in the aqueous core of a self-assembling structure [38]. The drug can be then released from the delivery system can be triggered by, pH, redox potential, light, magnetic field, differences in ionic strength or by instability of the system [12]. The advantages of higher stability of polymersomes over liposomes can be used to obtain more controlled release kinetics. Release that will start only after the delivery to the specific site would be an additional improvement of nanomedical formulations. For example, poly (butadiene-ethylene oxide) PB-PEO polymersomes were loaded with paclitaxel and they showed a steady release over 5 weeks at 37°C, correlated with reduced cytotoxicity [43]. Therefore, a long term drug releasing system can be achieved by choosing the appropriate polymer system. This is helpful to maintain a constant concentration of drug in the blood or target tissue for a long time, without the requirement of administration of further doses.

Fluorescently labeled poly(2-methacryloyloxyethyl phosphocholine)-poly(2- (diisopropylamino)ethyl methacrylate) (PMPC-PDPA) polymers were used for example for in vivo studies, because they preferentially accumulate in tumor tissue, which may enhance polymersome based cancer therapy [44]. The accumulation of a delivery cargo in tumor tissue is beneficial due to the reduction of the initial doses, related with lower side effects, as the drug is only released at the targeted site. Proper targeting and release of the active molecules in the desired location still needs to be improved for in vitro applications. Nevertheless, polymersomes have a broad range of biomedical applications in cancer therapy, diagnostics and vaccination [38]. Various types of commonly used polymers for the preparation of polymersomes is given in Table 1.

Polymersomes are usually employed for targeted delivery of loaded active biomolecules. Further, developments of polymersomes as nanoreactors, included the in situ production of the desired active molecules. The greater stability of the nanoreactors compared to liposomes, is related to the polymer membrane and helps to maintain the encapsulated molecule within the cavity and let the active molecule react in the presence of the substrate or when activated by an external trigger. To supply the bio-active molecule with starting materials for the reaction and to guarantee the release of the newly formed molecules from the nanoreactor, a selective permeability of the nanoreactor membrane is required. Approaches to bring the bio-active molecule together with its substrate are i) using a substrate-permeable polymeric membrane or ii) inserting membrane proteins into the polymeric membrane to allow efficient exchange of molecule across the polymeric membrane [45]. PNVP-PDMS-PNVP block copolymers as well as PMOXA-PDMS-PMOXA are permeable for reactive oxygen species [46,47], while PMOXA-PDMS-PMOXA is also able to reconstitute channel proteins as the outer membrane protein F (OmpF), the ferric hydroxamate uptake protein component A (FhuA), the receptor protein for the phage T6 and colicin K (Tsx) or the Aquaporin Z (AqpZ) to increase the permeability [48]. In all cases, small molecules can be exchanged from the aqueous cavity of the nanoreactor to the nanoreactor’s environment and vice versa, while the bio-active molecule possesses a much higher molecular weight than the cut off of the channel protein cannot escape. The function of the nanoreactor depends on the nature of encapsulated bio-active molecule. For example, by choosing the encapsulated enzymes, the nanoreactors can be used either to produce antibiotics in situ [49] or to act as an artificial organelle [50]. Encapsulated photosensitizer can be used to generate a Trojan horse like nanoreactor for application in photodynamic therapy (PDT) [51]. Trojan horse nanoreactors generating ROS (reactive oxygen species) are also being tried to target cancer cells as specific tumor targeting approaches (Fig. 1).

POSSIBLE APPLICATIONS

Polymersomes are better suited for in vivo medical use as compared to many other similar types of vesicle structures. This is because the capability of polymersomes to encapsulate hydrophilic, hydrophobic and amphiphilic molecules like other vesicular structures, in combination with their thick and tough membrane, provides them with better stability in vitro as well as in vivo. Besides, the presence of a dense PEG brush with relatively long PEG polymers on the
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Fig. (1). Trojan horse like nanoreactor targeted into a cancer cell. Depiction of A ROS (reactive oxygen species) generating nanoreactor targeting a cancer cell to induce specific cytotoxicity to the cancer cell.

surface of polymersomes likely increases their biological stability and decreases immune reactivity (stealthiness), which helps prolong the circulation times in blood.

Photodynamic therapy is a new approach to treat the leading cause of death in developed societies - cancer. The current most widespread clinical strategies to treat cancer patient are: i) surgery, ii) radiation therapy and iii) chemotherapy [52-54]. Surgery is the fastest method but it has its drawbacks if the tumor is located close to a sensitive area or if the tumor has already formed metastasis no complete treatment can be achieved. Radiotherapy on the other hand, damages DNA, but lacks selectivity and therefore damages healthy neighboring cells or tissue.

Chemotherapy suppresses cell growth or kills quickly dividing cells within the body. As chemotherapeutic agents are distributed normally throughout the whole body undesired toxic side effects appear with this therapy, although the approach is advantageous in case of metastasized tumors. In the past few years, the efficacy of chemotherapeutic agents has been improved by formulating them within nanocarriers, which improve the circulation time in the blood, by preventing renal clearance and non-specific uptake [54]. Additionally, an increased and targeted uptake into tumor tissue can be achieved by the enhanced permeability and retention effect (EPR).

EPR relies on the ability of tumor cells to grow faster and therefore on the fact that these cells require more nutrients and oxygen. Consequently, the new formed blood vessels in tumor tissues show defects and larger openings that can allow the passage of structures with size around 200 nm compared to healthy blood vessel [7]. Therefore, nano-sized structures such as liposomes, polymersomes or micelles are favored in drug delivery by taking advantages of the EPR effect. Due to their size, these nanoparticles accumulate preferentially in the tumor tissue, while smaller molecules and larger assemblies do not profit from this size selection. The selectivity is only generated by the size and does not need an additional active targeting to the tissue. The selectivity is a crucial point of the treatment, as the more selective a treatment is, the less side effects can occur. Therefore, novel methods for more selective treatment are being developed to fight cancer and improve patient’s conditions.

Because of the better stability and versatility of polymersomes than liposomes [55], they found many applications in nanomedicine. Hydrophilic, hydrophobic or amphiphilic compounds can be loaded in polymersomes, which makes them very attractive vesicles for various applications in drug delivery, biomedical imaging and diagnostics [56]. Thus, highly lipophilic anticancer drugs [57], dyes [55] and quantum dots [58] as well as amphiphilic dyes [59], transgenes [45] and membrane proteins (i.e. OmpF, LamB and FhuA [60] could be integrated within the polymersome membrane without compromising their functionality. Cationic polymersomes have been found to be useful in the longitudinal monitoring of transplanted stem cells by using dual-modal MRI and optical imaging, for tracking their migration [61]. It has also been demonstrated that cationic, SPION-loaded polymersomes can be effectively and safely used for labeling stem cells for cellular MRI [62]. These properties make them more applicable than liposomes and other vesicle structures.

CONCLUSION

Advances in nanomedicine have led to the development of nano-sized polymersomes as vehicles for different medical applications such as prodrug delivery, gene therapy and for the delivery of imaging and other therapeutic probes to precise targets in the body. Utilization of polymersomes is appearing to be a promising strategy. However, more clinical studies are essential for its establishment as gold standard avenues.

CONFLICT OF INTEREST

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

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REFERENCES

[1] So, S.; Lodge, T.P. Size control and fractionation of ionic liquid filled polymersomes with glassy and rubbery bilayer membranes. Langmuir, 2016, 32(19), 4959-4968.
[2] Ruiz-Pérez, L.; Messager, L.; Gaitzsch, J.; Joseph, A.; Sutto, L.; Gervasio F.L.; Battaglia, G. Molecular engineering of polymer-some surface topology. Sci. Adv., 2016, 2(4), e1500948.
[3] Chen, P.; Deng, C.; Meng F.; Zhang J.; Cheng R.; Zhong Z. Cha-miacer polymersomes based on poly(ethylene glycol)-b-poly(l-leucine)-b-poly(l-glutamic acid) for efficient delivery of doxorubi-cin hydrochloride into drug-resistant cancer cells. J. Control. Release, 2015, 213, e87-8.
[4] Alibolandi, M.; Alabdollah, F.; Sadeghi, F.; Mohammadi, M.; Abnous, K.; Ramezani, M.; Hadizadeh, F. Dextran-b-poly(lactide-co-glycolide) polymersome for oral delivery of insulin. In vitro and in vivo evaluation. J. Control. Release, 2016, 227, 58-70.
[5] Pippa, N.; Pispas, S.; Demetzos, C. Polymer self-assembled nanostructures as innovative drug nanocarrier platforms. Curr. Pharm. Des., 2016, 22(19), 2788-2795.
[6] Tuguntuev, R.G.; Okeke, C.I.; Xu, J.; Li, C. Wang, P.C.; Liang, X.J. Nanoscale polymersomes as anti-cancer drug carriers applied
for pharmaceutical delivery. *Curr. Pharm. Des.*, 2016, 22(19), 2857-2865.

[7] Simón-Gracia, L.; Hunt, H.; Scodeller, PD.; Gaitjzsch, J.; Braun, G.B.; Willmore, A.M.; Ruoslahi, E.; Battaglia, G.; Teesalu, T. Paclitaxel-loaded polymersomes for enhanced intraperitoneal chemotherapy. *Mol. Cancer Ther.*, 2016, 15(6), 670-679.

[8] Anajafi, T.; Scott, M.D.; You, S.; Yang, X.; Choi, Y.; Qian, S.Y.; Mallick, S. Acridine orange conjugated polymersomes for simultaneous near-IR selective delivery of gemcitabine and doxorubicin to pancreatic cancer cells. *Bioconjug. Chem.*, 2016, 27, 762-771.

[9] Hu, M.; Shen, Y.; Zhang, L.; Qin, L. Polymersomes via self-assembly of amphiphilic β-cyclodextrin-centered triarm star polymers for enhanced oral bioavailability of water-soluble chemotherapeutics. *Biomacromolecules*, 2016, 17(3), 1026-1039.

[10] Mai, Y.; Eisenberg, A. Self-assembly of block copolymers. *Chem. Soc. Rev.*, 2012, 41, 5969.

[11] Letchford, K.; Burt, H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanoparticles, nanocapsules and polymersomes. *Eur. J. Pharm. Biopharm.*, 2007, 65, 259.

[12] Lee, J.S.; Feijen, J. Polymersomes for drug delivery: design, formation and characterization. *J. Control. Release*, 2012, 161, 473.

[13] Rodriguez-García, R.; Mell, M.; Lopez-Montero, I.; Netzel, J.; Hellwig, T.; Monroy, F. Polymersomes: smart vesicles of tunable rigidity and permeability. *Soft Matter*, 2011, 7, 1532.

[14] Goddard, P.; Hutchinson, L.E.; Brown, J.; Brookman, L.J. Polymers in aqueous media: performance through association. *J. Control. Release*, 1989, 16, 5.

[15] Ruggiero, A.; Villa, C.H.; Bande, E.; Rey, D.A.; Bergkvist, M.; Batt, C.A.; Manova-Todorova, K.; Deen, W.M.; Scheinberg, D.A.; Meier, W. Molecular organization and dynamics in polymersome membranes: A lateral diffusion study. *Macromolecules*, 2014, 47, 5788.

[16] Stump, E.; Ghadiri, M.R. Protein delivery: from conventional drug delivery carriers to artificial organelles: artificial peroxisomes play their role. *Int. J. Nanomed.*, 2012, 7, 49.

[17] Allen, T.M.; Cullis, P.R. Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.*, 2013, 65, 36.

[18] Li, W. Biopharmaceutical benchmarks 2006. *Trends In BioPharmaceutical Industry*, 2006, 5.

[19] Fenske, D.B.; Cullis, P.R. Liposomal nanomedicines. *Expert Opin. Drug Deliv.*, 2008, 5, 25.

[20] Li, S.; Byrne, B.; Welsh, J.; Palmer, A.F. Self-assembled poly(butadiene)-b-poly(ethylene oxide) polymersomes as paclitaxel carriers. *Biotecnol. Progr.*, 2007, 23, 278.

[21] Murdoch, C.; Reeves, K.J.; Hearnend, V.; Colley, H.; Massignani, M.; Canton, I.; Madsen, J.; Blanazs, A.; Armes, S.P.; Lewis, A.L.; MacNeil, S.; Brown, N.J.; Thorndill, M.H.; Battaglia, G. Internalization and biodistribution of polymersomes into oral squamous cell carcinoma cells in vitro and in vivo. *Nanomedicine*, 2010, 5, 1025.

[22] Balasubramanian, V.; Onaca, O.; Enea, R.; Hughes, D.W.; Palivan, C.G. Protein delivery: from conventional drug delivery carriers to artificial organelles: artificial peroxisomes play their role. *Expert Opin. Drug Deliv.*, 2010, 7, 63.

[23] Axthelm, F.; Casse, O.; Koppenol, W.H.; Nauser, T.; Meier, W.; Palivan, C.G. Antioxidant nanoreactor based on superoxide dismutase encapsulated in superoxide-permeable vesicles. *J. Phys. Chem. B*, 2008, 112, 8211.

[24] Spulber, M.; Baumann, P.; Saxer, S.S.; Pielue, M.; Meier, W.; Bruns, N. Poly(N-vinylpyrrolidone)-poly(dimethylsiloxane)-based polymersome nanoractors for laccase-catalyzed biotransformations. *Biomacromolecules*, 2014, 15, 1469.

[25] Marais, J.; Baumann, P.; Langowska, K.; Onaca, O.; Bruns, N.; Meier, W. Selective and responsive nanoractors. *Adv. Funct. Mater.*, 2011, 21, 1241.

[26] Langowska, K.; Palivan, C.G.; Meier, W. Polymer nanoractors shown to produce and release antibiotics locally. *Chem. Commun.*, 2013, 49, 128.

[27] Tanner, P.; Balasubramanian, V.; Palivan, C.G. Aiding nature’s organelles: artificial peroxisomes play their role. *Nano Lett.*, 2013, 13, 2875.
Polymersomes in Nanomedicine - A Review

[54] Master, A.; Livingston, M.; Sen Gupta, A. Photodynamic nanomedicine in the treatment of solid tumors: perspectives and challenges. *J. Control. Release, 2013, 168, 88.*

[55] Photos, P.J.; Bacakova, L.; Discher, B.; Bates, F.S.; Discher, D.E. Polymer vesicles in vivo: correlations with PEG molecular weight. *J. Control. Release, 2003, 90, 323-334.*

[56] Massignani, M.; Lomas, H.; Battaglia, G. Polymersomes: A synthetic biological approach to encapsulation and delivery. *Adv. Polym. Sci., 2010, 229, 115-154.*

[57] Ahmed, F.; Pakunlu, R.J.; Brannan, A.; Bates, F.; Minko, T.; Discher, D.E. Shrinkage of a rapidly growing tumor by drug-loaded polymersomes: pH-triggered release through copolymer degradation. *J. Control. Release, 2006, 116, 150-158.*

[58] Schelly, Z.A. Subnanometer size uncapped quantum dots via electroporation of synthetic vesicles. *Colloid Surf. B-Biointerfaces, 2007, 56, 281-284.*

[59] Battaglia, G.; Ryan, A.J.J. Bilayers and interdigitation in block copolymer vesicles. *Am. Chem. Soc., 2005, 127, 8757-8764.*

[60] Stoenescu, R.; Graff, A.; Meier, W. Asymmetric ABC-triblock copolymer membranes induce a directed insertion of membrane proteins. *Macromol. Biosci., 2004, 4, 930-935.*

[61] Wen, X.; Wang, Y.; Zhang, F.; Zhang, X.; Lu, L.; Shuai, X.; Shen, J. In vivo monitoring of neural stem cells after transplantation in acute cerebral infarction with dual-modal MR imaging and optical imaging. *Biomaterials, 2014, 35, 4627-4635.*

[62] Guo, R.M.; Cao, N.; Zhang, F.; Wang, Y.R.; Wen, X.H.; Shen, J.; Shuai, X.T. Controllable labelling of stem cells with a novel superparamagnetic iron oxide-loaded cationic nanovesicle for MR imaging. *Eur. Radiol., 2012, 22, 2328-2337.*