Cell targeting by a generic receptor-targeted polymer nanocontainer platform

Pavel Brož, Samantha M. Benito, CheeLoong Saw, Peter Burger, Harald Heider, Matthias Pfisterer, Stephan Marsch, Wolfgang Meier, Patrick Hunziker

Abstract

Nanotechnology promises new avenues to medical diagnosis and treatment. Of special interest are injectable nanovehicles that are programmable towards specific targets, are able to evade the immune defense, and are versatile enough to be suited as carriers of complex functionality. Biotin-functionalized (poly(2-methyloxazoline)–b-poly(dimethylsiloxane)–b-poly(2-methyloxazoline) triblock copolymers were self-assembled to form nanocontainers, and biotinylated targeting ligands were attached by using streptavidin as a coupling agent. Specifically, fluorescence-labeled nanocontainers were targeted against the scavenger receptor A1 from macrophages, an important cell in human disease. In human and transgenic cell lines and in mixed cultures, receptor-specific binding of these generic carriers was followed by vesicular uptake. Low nonspecific binding supported the stealth properties of the carrier while cytotoxicity was absent. This versatile carrier appears promising for diagnostic or therapeutic medical use.

Keywords: Polymer nanocontainer; Receptor targeting; Macrophage scavenger receptor A1; Drug delivery

1. Introduction

Generally, targeting specific cells or specific membrane proteins in the body is desirable in medicine for improved diagnosis and treatment [1]. The concept of nanoscale vehicles that carry complex functionality to such targets within the body while remaining intact and being able to escape the
innate defense system has stimulated broad interest, although the feasibility of combining these properties into a nanoscale structure remains far from proven.

For diagnostic use, current targeting strategies like radioisotope scintigraphy suffer from significant unspecific uptake of radioisotopes in multiple organs, or rapid clearing of the marker from the circulation by renal filtering. Problems arise also due to rapid removal from the circulation when unstable vehicles like liposomes are used, or when unspecific protein binding facilitates the uptake of the substances by the reticuloendothelial system [2].

In therapeutic applications, specific targeting of cells or receptors is desirable because many side effects of current drugs emerge from the undesired impact of a drug molecule on physiological pathways in a cell type or organ that is not involved in the disease process. For example, cytostatic drugs used in cancer therapy often also hit cell lines unrelated to the cancer (e.g., the fast-growing cells of the bone marrow, the immune system, the intestinal endothelium, and the hair follicles). This leads to well-known side effects, limits the therapeutic efficacy, and makes improved targeting strategies desirable. Even more important in our evolving era of somatic gene therapy may be the capability of carrying therapeutic genes to specific cellular targets within the body [3].

An optimal carrier should be versatile enough to target a cell of choice and be stable enough to avoid premature destruction. It should also show no unspecific adherence to proteins and cells, which could lead to its elimination from the circulation. In addition, it should be able to carry a load of molecules to its destination, where it would interact in a controlled way with the target receptor or target cell [4–13].

We explored the potential of synthetic, functional nanocontainers as generic, versatile carriers by examining their interaction with a clinically important, receptor-specific cell model. We thereby focused in particular on design requirements for receptor- and cell-specific binding, on specificity of binding, on the process of surface adherence, and the mode and time course of uptake, as well as on a first assessment of cytotoxicity. We chose macrophages and their scavenger receptor SRA1 (SRA1) as model target because macrophages play a major role in a wide range of disease states, including infection, autoimmune disease, cancer, and atherosclerosis [14–21].

As building blocks for the synthetic nanocontainers, the triblock ABA copolymer poly(2-methyl-oxazoline)–b-poly(dimethylsiloxane)–b-poly(2-methylloxazoline) (PMOXA–PDMS–PMOXA) was chosen (Fig. 1a). This amphiphilic block copolymer forms stable, closed vesicular structures in aqueous media. The wall thickness of these structures is about 10 nm (i.e., about three times thicker than biological lipid bilayer membranes). Containers with controlled diameters between 100 and 250 nm (mean diameter slightly less then 250 nm) [22], a volume of about $10^{-10}$ l, and a volume-to-shell ratio of approximately 4:1 (in the case of a 200-nm container) can be built. The chosen block copolymers have a proven record of good biocompatibility [23]. Moreover, the container walls are completely covered by PMOXA chains that show very low nonspecific protein binding [24]. The nanocontainers show high stability [25], low permeability [26], low plasma protein binding, stable encapsulation of hydrophilic contents [27]; and allow integration of whole, functional membrane proteins into their walls [28,29], the use of those containers as nanoscale bioreactors [27], and the exchange of genetic material between biologic structures and nanocontainers [22]. In the present study, the nanocontainers were loaded with fluorescent markers, allowing the microscopic observation of the binding and uptake of the nanocontainers by the cells.

As target receptor, we chose the macrophage scavenger receptor SRA1 [30], a pattern recognition receptor that is upregulated in activated tissue macrophages but not in monocytes and monocyte precursor cells [30]. It is associated with the endocytotic pathway and is responsible for binding and uptake of a number of polyanionic substances like oxidized, acetylated, or maleylated low-density lipoprotein (LDL), advanced glycation end-product (AGE) proteins, (lipo)polysaccharides, and polynucleotides like polyinosinic (poly-I) and polyguanylic acid quadruplexes (polyG) [31].

In the experiments, macrophages derived from the human monocytic THP-1 [32] cell line and the simian tumor cell line COS-7 were used as target cells expressing SRA1. When stimulated by phorbol esters, the THP-1 cell line differentiates into highly active macrophages with high expression rates of SRA-1, which closely mimic tissue-derived human macrophages [33]. In receptor binding and colocalization
experiments, COS-7 cells either expressing the transfected SRA1 receptor coupled to the fluorescent marker enhanced green fluorescent protein (EGFP) or lacking this target receptor were used. This cell line does not express SRA1 in its native state and is therefore particularly suited as its own negative control cell line.

As specific ligand for the SRA1 receptor, the oligonucleotide polyguanylic acid (polyG; 23 nucleotides per strand) that forms stable quadruple helices [34] was chosen for functionalization of the nanocontainers (Fig. 1b) after it proved resistant to degradation when exposed to human plasma (data not shown).

**Fig. 2a** shows the overall setup of the experiments comprising model cells, receptor-specific ligand bound via a biotin/streptavidin bound to the polymer molecules of the nanocontainers, and the loading of nanocontainers with a fluorescent marker molecule.

### 2. Materials and methods

#### 2.1. Nanocontainer synthesis

The polymer used in this work consists of a PDMS (poly(dimethylsiloxane)) middle block and two PMOXA (poly(2-methylloxazoline)) side chains. The
synthesis was carried out following a modification of the procedure already published [25]. The total number average molecular weight ($M_n$) of the polymer corresponds to 7090 g/mol. The number average molecular weight for the PDMS block is 4878 g/mol, whereas the number average molecular weight for each of the PMOXA chains corresponds to 1105 g/mol. This results in a hydrophilic weight ratio of 31%. Gel permeation chromatography in tetrahydropyran (THP) and with polystyrene standards revealed a weight average molecular weight...
molecular weight ($M_n$) of 9473 g/mol. In order to obtain the active drug delivery systems, we functionalized the ends of the triblock copolymer with biotin groups. For that purpose, a mixture of the polymer with excess of biotin in chloroform was stirred at room temperature in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). Elimination of unreacted biotin was achieved by ultrafiltration (Millipore Ultrafiltration Cell; membrane cutoff, 5000 Da) in a 1:5 ethanol/water solution. The preparation of the vesicles followed a slightly modified procedure described elsewhere[25,27]. Briefly, the polymer is dissolved in EtOH to give a 14.3% (wt/vol) solution; 250 µl of this ethanolic solution is subsequently added dropwise into 5 ml of PBS buffer containing 80 mM sulphorhodamine B (maximum exclusion 565 nm; maximum emission 586 nm) red fluorescent dye (Sigma-Aldrich, Buchs, CH, Switzerland). The diameters of the resulting vesicles could be controlled in a range from about 100 to 250 nm by repetitive filtration through filters of defined pore size (Milllex-GV, 0.45 µm and 0.22 µm; Millipore). For the preparation of biotinylated nanocontainers, we used mixtures of pure, nonmodified triblock copolymer and biotinylated polymer at a ratio 9:1 wt/wt.

2.2. Nanocontainer functionalization with polyG ligands

In order to obtain ligand-bearing NC, biotinylated nanocontainers were incubated with slight excess of streptavidin (with respect to the biotin groups at the surface of the NC). Frequently, we used a 1:1 ratio of biotin to streptavidin (i.e., 4 mol of binding sites) to avoid crosslinking via streptavidin bridges [35]. The excess of streptavidin was removed chromatographically (Sepharose 4B column; 37 cm length, ID 1 cm, Bio-Rad in PBS buffer). This procedure allowed the simultaneous elimination of nonencapsulated sulphorhodamine B from the samples. Subsequently, these nanocontainers were incubated with biotinylated polyG (Microsynth, Balgach, CH) in a 1:1 ratio of biotin sites to polyG, to produce active targeting drug delivery systems. Due to the high binding affinity between biotin and streptavidin [36] no further purification was necessary.

In order to evaluate the scavenger receptor A1 binding properties of the polyG ligand without possible nanocontainer-associated interferences, we tagged the biotinylated polyG ligand with a Cy3 (maximum exclusion 548 nm; maximum emission 562 nm)-labeled streptavidin (Sigma-Aldrich) to allow a direct imaging of the ligand (instead of imaging the NC content).

2.3. Cell cultures

COS-7 cells (ATCC no. CRL-1651) were cultured as described earlier [37]; 1 day prior to transfection, cells were seeded at about 40% confluence onto ethanol-washed cover slips into 12-well Falcon plates (Becton Dickinson, Basel, Switzerland). On the day of transfection, cell density was routinely $5 \times 10^4$ cells/cm². Lipid-mediated transfections with FuGENE™ (Roche Molecular Biochemicals, Rotkreuz, Switzerland) were carried out as described earlier [37]. Cells were transfected either with a plasmid carrying the open reading frame for EGFP (negative control), or with a plasmid containing the complete open reading frame of human SRA1 fused in frame to the C-terminal end of EGFP. All subsequent experiments were carried out between 24 and 48 h after transfection. THP-1 cells (ATCC no. TIB-202, Manassas, VA) were cultured as recommended by the manufacturer. Differentiation into functional and cover slip (also in 12-well Falcon plates) adherent macrophages was initiated with 100 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) [33], resulting in a mean cell confluence of approximately 30%. Human vascular smooth muscle cells (VSMC) were a gift from the group of Prof. M. Tamm (Basel) and were cultured according to their protocol [38,39]. Human umbilical vein endothelial cells (HUVEC; ATCC no. CRL-1730) were cultured according to the manufacturer’s instructions. In order to get mixed macrophage VSMC/HUVEC cultures, THP-1 cells were differentiated for 3 days on glass cover slips, and VSMC or HUVEC were added in slight cellular surplus and cocultured in mixed cell medium for 1 day. Surface adherence and viability were checked by means of light microscopy before performing experiments.

2.4. Experimental proceeding

Unless stated differently, the nanocontainer solutions (37 °C; with or without the tracking ligand...
polyG and controls; see Results section and figures for experimental setup) were then added dropwise to the prepared cell culture samples, resulting in a final nanocontainer concentration of 50 μg/ml in the cell medium. Standard incubation time was 2 h at 37 °C. Then the cells incubated with nanocontainers were fixed in 4% paraformaldehyde for 30 min at room temperature. Fixed cells were washed twice in PBS and then mounted in Mowiol (Hoechst, Frankfurt, Germany) containing 1% n-propyl gallate (Sigma-Aldrich). Using this fixation scheme, VSMC and HUVEC show faint blue/green autofluorescence that renders them detectable in fluorescence microscopy, while macrophages are distinguished by a strong blue autofluorescence signal, allowing clear distinction of macrophages from the other cell types. Cells were examined using a Nikon Diaphot 300 microscope (Nikon, Küssnacht, Switzerland), equipped with a SenSys Video imaging system. Images were assembled with the OpenLab software (Paul Bucher, Basel, Switzerland).

Quantitative image analysis (Fig. 3) was performed by digital fluorescence microscopy using the ImageJ software package (Version 1.28 u; NIH); for quantification, all cells in each of multiple views were localized in the green band (509 nm) for COS-7 cells and in the blue band (450 nm) for THP-1 (with the observer blinded for the red NC signal), and NC uptake for each cell was then quantified by quantifying fluorescence intensity in the red band (590 nm). After defining a region of interest (ROI) around an individual cell (with the observer blinded for the red NC signal), we first measured the mean green (for COS-7 cells) or blue (for THP-1 cells) light intensity, then the mean red light intensity in the same ROI in the red light picture. Cell numbers ranged from 78 to 92. As reference values for the calculations, we took the no-receptor/no-ligand sample for the COS-7 and the no-ligand sample for the THP-1 cells.

Uptake kinetic experiments were performed with the same setting and different incubation times reaching from 30 to 270 min; the increase of overall red light intensity was determined by using a Color Counter plug-in for ImageJ (same as above) on five different screenshots of the same cell population, representing approximately 200 cells in each setup.

Cytotoxicity of nanocontainers was studied in the THP-1 macrophages by [3H] adenine release [40]; cells labeled with [3H] adenine (1 μCi/ml) were incubated with nanocontainers for 4 and 24 h, and [3H] adenine release into the medium was determined using an automated scintillation counter; cells treated with 10% ethanol served as positive control whereas untreated cells served as a negative control.

Standard statistics were used, including calculation of means and standard deviations (S.D.), application of Student’s t test for group comparisons, and use of two-sided significance levels of 0.01.

For scanning electron microscopy (SEM), the cells were incubated in 4 or 37 °C before adding the nanocontainers; fixation was performed with 1% glutaraldehyde, then the samples were dehydrated with ethanol in increasing concentration, critical point-dried (CPD) and finally platinum-sputtered. Cell fixation for transmission electron microscopy (TEM) follows the standard methods [41].

3. Results

3.1. Nanocontainer binding to the cell surface

Nanocontainer binding experiments are schematically visualized in Fig. 2a.

In a first step, we performed studies on the ligand–receptor interaction. The chosen scavenger receptor A1 tracking ligand polyG labeled with the red fluorescent dye Cy3 showed a fast, signal-intensive, and receptor-specific binding in cell cultures of SRA-1-expressing COS-7 cells at 37 °C (Fig. 4a). No ligand binding was present in SRA-1 lacking COS-7 cells even when high concentrations of ligand were used. The biotin-bound Cy3 alone shows no binding to both receptor-expressing and receptor-lacking target cells.

In a second step, we introduced the fully functionalized ligand-labeled receptor-tracking nanocontainers as described in the Materials and Methods section. Binding of nanocontainers to the cell surface was analyzed in COS-7 cells with and without expression of the SRA1 receptors, and in THP-1 cells expressing SRA1. In both settings, we were able to repeat the results of the ligand–receptor studies by localizing the red fluorescent signal of the NC-encapsulated sulphorhodamine B on the cell surface. Further experiments were performed at 37 and 4 °C. At low temperature, receptor binding and uptake by
Fig. 3. Determination of specific versus unspecific nanocontainer binding by quantitative bispectral fluorescence microscopy. (a) Ligand functionalization and receptor expression are crucial points for selective and significant (>2 S.D. above mean red fluorescence intensity of negative control) nanocontainer (NC) uptake in transgenic SRA-1-expressing COS-7 cells (a) and THP-1 macrophages (b).
membrane fusion (like in liposomes) [42] can still take place, but cellular transport processes like endocytosis are inhibited [43]. No nanocontainers were seen by SEM in control experiments at both temperatures when cells devoid of receptor were used, no ligand was present, or nanocontainers lacking a biotin moiety were used. In contrast, after incubation of receptor-expressing cells with functionalized nano-
containers for 2 h at 4 °C, nanocontainers attached to the cell surface could be observed (Fig. 2b). When incubated at 37 °C, the nanocontainers did not remain at the cell surface but were taken up into the cell as documented in Section 3.2. This supports the importance of active transport processes for nanocontainer uptake (presumably by the endocytotic pathway known to be associated with the SRA1 receptor) [44], in contrast to mere membrane fusion. THP-1 cells and SRA1-expressing COS-7 cells showed similar nanocontainer binding, documenting the usefulness of both cell lines in our setup.

3.2. Cellular uptake of nanocontainers

To further explore the binding of functionalized nanocontainers to SRA-1 receptors and to observe their handling by the cells, we used fluorescence microscopy in conventional and confocal setups. In Fig. 3, quantitative uptake of nanocontainers in COS-7 and THP-1 cells is documented under various experimental conditions. The uptake of fluorescently labeled nanocontainers by COS-7 cells expressing SRA1/EGFP fusion receptors [45] or control cells lacking the receptor as well by differentiated THP-1 macrophages was visualized with selective excitation of the receptor-associated green fluorescence or the red nanocontainer fluorescence, together with comprehensive imaging of the cells using the specific autofluorescence activity (see Materials and Methods section). Uptake was quantified by measuring, for each cell, light intensity in the red channel (indicating nanocontainers), and the light intensity in the green or blue channel (indicating receptor/cell presence). These experiments showed a striking receptor specificity of nanocontainer uptake: absence of either the SRA1 receptor or the specific ligand on the nanocontainer completely abolished nanocontainer uptake in COS-7 cells as well as in THP-1-derived macrophages (Fig. 3). This important finding of strong specific uptake

Fig. 5. Localization of internalized nanocontainers. (a) In TEM, hollow vesicular structures can be found in THP-1 macrophages incubated with functionalized nanocontainers. (b) Functionalized nanocontainers seen by fluorescence microscopy are bound to the macrophage surface and inside intracellular vesicles.
supports the initial hypothesis that the tailored design of the nanocontainers renders them suitable as a receptor-specific carrier. In addition, the remarkable absence of unspecific binding shows that a key problem of conventional targeting approaches (i.e., the unspecific uptake of the carrier by phagocytic cells) can be circumvented using specific nanocontainer building blocks that exhibit very low polymer–protein interaction.

To further elucidate receptor-specific binding and uptake, colocalization of internalized nanocontainers and EGFP-tagged SRA-1 receptors was examined by confocal microscopy (Fig. 4b) in experiments performed at 37 °C. We found that both nanocontainer-associated and receptor-associated fluorescence were always colocalized in intracellular vesicles, further supporting the importance and specificity of the ligand–receptor interaction for nanocontainer uptake. Little fluorescence was localized at the cell membrane, pointing to the rapid uptake of membrane-bound nanocontainers. This supports the findings by SEM reported in Section 3.1.

Fig. 6. Cell type-selective targeting in human macrophage/human vascular smooth muscle cell and human macrophage/human endothelial cell cocultures. The upper panels show individual cell lines treated with red fluorescent nanocontainers without functionalization (left panel) and with functionalization by the scavenger receptor A1 (SRA-1)-specific ligand polyG (remaining panels). THP-1 macrophages (blue) exhibited strong uptake of polyG-functionalized nanocontainers, while no uptake of nonfunctionalized nanocontainers was discernible. Likewise, neither smooth muscle cells (green) nor endothelial cells (green) showed a discernible uptake of functionalized nanocontainers. The lower panels show mixed cultures containing activated THP-1 macrophages (blue) and smooth muscle cells (green) or endothelial cells (green). Treatment with functionalized nanocontainers led to strict colocalization of red nanocontainers with the macrophages only.
Competition experiments with fluorescence-tagged, oxidized LDL, an alternative ligand to the SRA1 receptor, strongly inhibited nanocontainer binding, thus demonstrating the receptor specificity of the nanocontainer–cell interaction (data not shown).

In uptake kinetics experiments shown in Fig. 4c, rapid uptake was observed within the first 30 min with saturation of uptake at 3 h.

In TEM (Fig. 5a), nanocontainer-treated macrophages showed characteristic intracellular accumulations of vesicular structures with sizes in the range of the nanocontainers. Moreover, these structures were not observed in control cells. Together with the confocal microscopy results (Fig. 5b), this finding is compatible with intracellular transport and accumulation of nanocontainers to certain locations in the cells, presumably linked to the endocytotic pathway.

3.3. Cell type specificity and cytotoxicity

Evidently, cell targeting makes sense only in an environment where more than one cell type coexists. We therefore expanded the targeting experiments to human cell lines known not to express SRA1 receptors, VSMC, and endothelial cells. No binding of SRA1-specific or nonfunctionalized nanocontainers was observed in cell cultures of VSMC and endothelial cells, in contrast to the human THP-1 macrophage culture, which showed strong uptake in fluorescence microscopy (Fig. 6). This was also even more evident in the mixed cultures of both macrophages–VSMC and macrophages–endothelial cells, where macrophages (bright blue dots) show strong red fluorescence activity indicating nanocontainer uptake, while both smooth muscle cells and human endothelial cells (darker, elongated green–blue structures after picture processing) show no signs of red fluorescence activity.

Despite the good record of biocompatibility of the polymers used [23], toxicity is an important consideration in the design of nanocontainers and other carrier systems. Although a cell culture model alone is not sufficient to exclude clinically relevant toxicity, we assessed cytotoxicity in an assay that measures release of radioactive [3H] adenine upon cell death, whereas viable cells with fully intact cell membranes retain their adenine content [40]. In untreated, differentiated THP-1 cells (negative control), mean adenine release over a series of experiments was 11.1% (S.D. 2.9%) of total cell-bound adenine at 4 h and 35.5% (S.D. 4.8%) at 24 h. In differentiated THP-1 cells treated with 10% EtOH (in order to promote cell death), adenine release was 51.6% at 4 h (S.D. 3.6%; \( P<0.001 \), always versus negative control) and 59.9% (S.D. 3.3%; \( P<0.001 \)) at 24 h. Treatment with native nanocontainers (i.e., without functionalization with ligand) led to an adenine release of 15.4% (S.D. 2.6%; \( P=0.4 \)) at 4 h and of 35.6% (S.D. 2.0%; \( P=0.59 \)) at 24 h. In functionalized nanocontainers, adenine release was 15.1% (S.D. 2.6%; \( P=0.68 \)) at 4 h and 36.7% (S.D. 1.4%; \( P=0.2 \)) at 24 h, thus indicating no significant cellular toxicity.

4. Discussion

Generic receptor-programmable nanocontainer carriers built by combining chemical design and synthesis, integration of biomolecules, and self-assembly are able to carry a load of molecules (in our case a fluorescent marker) to a target cell, where they show strong binding to the cell surface through receptor-specific interaction. In the case of the scavenger receptor targeted in our experiments, surface-bound nanocontainers are rapidly taken up by the cell through an active process, most probably the endocytotic pathway, without leading to discernible cytotoxicity. In strong contrast, no binding and uptake of the functionalized nanocontainers are observed in cells devoid of the target receptor. Macrophages are unable to bind and take up those nanocontainers not specifically targeted against them, thereby reducing the common problem of conventional targeting approaches, namely, unspecific elimination of carriers by the macrophage system.

The combination of chemical custom design and synthesis, incorporation of biomolecules, and self-assembly to form complex nanoscale structures is an illustration of the bottom–up manufacturing approach that has become a key paradigm of nanotechnology, and contrasts with the top–down approach characteristic of current microtechnology and chip technology, which is also under investigation for drug delivery applications [46]. As the sheer number of containers
needed for a biologic impact may grow very large with size reduction of the individual units, significant problems may be encountered with conventional top-down manufacturing approaches, and render self-assembly attractive as manufacturing technique. According to one’s need, building blocks can either be designed by chemical synthesis (as in our case, the polymer shell tuned for high stability, good biocompatibility, immunologic stealth properties, and propensity to self-assembly), while the synthetic polyG quadruplex ligand was chosen because of its desired targeting properties. Other building blocks can be chosen from biological systems (like in the present example, the streptavidin molecule used for linking), or a number of transmembrane proteins that we have successfully integrated into the polymer membrane of our nanocontainers and which retain their complex biological functions like switchable pores [28], exchange of genetic material with viruses [22], or chemoreactor functionality [27]. This large freedom in chemical design as well as the large spectrum of functionality offered by biomolecules available from living organisms, which are potential nanocontainer building blocks, render the described carrier system a truly generic approach for novel targeting strategies.

Polymer nanocontainers thus have a great potential as carriers for medical applications. Diagnostic applications and treatment approaches based on radioactive isotopes may form the simplest application of our platform, as no further design change is necessary except to load the nanocontainers with the desired isotope during self-assembly and to ‘reprogram’ the nanocontainer to its new target with a suitable ligand. For further therapeutic approaches, the release of nanocontainer contents within the cell, the intracellular pathways of the polymer materials, the behavior in a biologic environment of biomolecules built into the carrier membrane, and the distribution and kinetics of the nanocontainers in the intact organism, all topics of our ongoing research, need to be studied in more detail.

5. Conclusion

The concept of injectable nanovehicles that are programmable towards specific targets evades the immune defense, and is suited as carriers of complex functionality has caught the interest of a broad public. This interdisciplinary paper describes the design of such a generic, functionalizable platform by combining polymer chemistry, incorporation of biomolecules, and self-assembly; and studies the interaction of such carriers with human cell lines. Strong specificity, “stealth” properties, broad functionalizability, and absence of cytotoxicity of this synthetic vehicle in a biologic environment render this system promising for a broad spectrum of use, and may have a significant impact on medicine and biology.

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