Polymersome-templated porous and hollow nanogels for dual intracellular delivery

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Intracellular delivery has attracted substantial attention due to applications in medicine and investigations into cellular function. Among the current nanocarrier systems, nanogels are very promising vehicles due to their high stability, tunable bioactivity, and minimized protein adsorption. In particular, the ability to specify hydrogel porosity and chemistry permits size- and charge-dependent control over the influx or efflux of molecules. Furthermore, vesicular nanostructures are capable of encapsulating hydrophilic molecules without requiring chemical modification, allowing the transport of protein therapeutics in their native conformation. A nanogel system with a porous and hollow structure may therefore serve as a platform for the delivery of multiple diverse therapeutic payloads via distinct mechanisms of release.

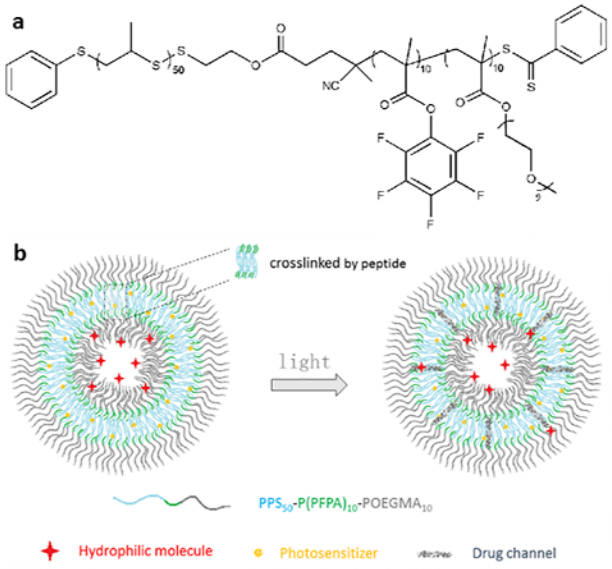

Figure 1. Chemical structure of PPS_{50}-$P(PFPA)_{10}$-$POEGMA_{10}$ (a) and schematic of photo-oxidation triggered release of payloads from nanogel pores (b).

Here we present a novel porous and hollow nanogel system for dual intracellular delivery of protein and small molecule therapeutics via redox-responsive triggers. The loading and assembly of our nanogel vesicles are guided by a self-assembled polymersome template, which is subsequently crosslinked into a stable hydrogel membrane via PEG or enzyme-degradable peptides. Release of encapsulated molecules is specified by a dynamic membrane porosity, which increases with oxidation and has an upper limit set by the length of the crosslinker.

In a stepwise synthesis, well-defined amphiphilic triblock copolymers PPS-P(PFPA)-POEGMA (Figure 1a) were obtained by the living polymerization of propylene sulfide (PS) initiated with benzyl mercaptan and subsequent RAFT polymerizations of pentafluorophenyl methacrylate (PFPA) and oligo(ethylene glycol) methacrylate (OEGMA). All the intermediate and final products were characterized by ^{1}H NMR and gel permeation chromatograph (GPC). Polymersomes of PPS_{50}-$P(PFPA)_{10}$-$POEGMA_{10}$ were formed by thin film rehydration. Urokinase plasminogen activator (uPA)-degradable peptides (GSGRSAGK) or PEG-diamine of various chain lengths was used to crosslink the shells by the aminolysis of the pentafluorophenyl esters in P(PFPA) blocks, resulting in a stable hollow nanogel structure. The nanogels were characterized by Nano Sight and cryogenic electron microscopy (cryo-TEM).

To verify the oxidation-dependent release dynamics of encapsulated payloads *in vitro*, the nanogels were photosensitized through the incorporation of ethyl eosin within the hydrophobic PPS layer. We have previously demonstrated the on-demand spatiotemporally controlled degradation of PPS vesicles via the photo-oxidation of PPS to poly(propylene sulfone) by ethyl eosin.[1] The relative hydrophilicity of the sulfones increases the accessibility of the disulfide bonds linking the PPS and P(PFPA) blocks to cleavage in reductive environments, resulting in pore formation (Figure 1b). We hypothesized that the pore size could be controlled via the extent of oxidation to specify the influx/efflux of encapsulated payloads. Nanogels were thus simultaneously loaded with rhodamine B (RhB) and green fluorescent protein (GFP) and the selective release of these differently sized molecules was monitored with fluorescence microscopy following illumination with a 488 nm light source at various time points. The release rates were found to be dependent on the optical excitation time, with short times of exposure triggering the release of RhB, while additional light duration was needed for the unloading of GFP. The balance between the oxidative and reductive potential within the endosomes of phagocytic cells is highly dependent on the specific cell subset and pathway of endocytosis.[2] We additionally observed time-dependent selective release of payloads following phagocytosis and macropinocytosis by murine bone marrow-derived macrophages and dendritic cells.

In summary, we have synthesized a novel nanogel vesicle with oxidation-dependent porosity. Release rates of payloads was size dependent and could be controlled by photo-oxidation, enzymatic degradation, or targeted cell type. These nanogels have immense potential for selective therapeutic imaging and/or modulation of different phagocytic cell subsets.

References:

[1] Vasdekis & Scott et al., ACS Nano 2012, 7850-7857.

[2] Savina et al., Immunol. Rev. 2007, 143-556.