Hybrid Programmable Peptide Crosslinkers as Enzyme-Regulated Drug Delivery Vehicles

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ABSTRACT

Using a hybrid surfactant and peptide-based self assembly method, we have built a series of peptide crosslinked micelle systems that breakdown and release their internal and external cargo in response to the presence of specific enzyme targets. The nanocapsule displays highly specific release in the presence of closely related proteases, matrix metalloproteases (MMPs), and can be modified with electron dense tags such as gold nanoparticles for monitoring its assembly and disassembly using electron microscopy. We have shown through a combination of dynamic light scattering, fluorescence release assays, and cell viability studies that the location and enzyme expression levels in the vicinity of the peptide crosslinked nanocapsules can be used to specifically regulate the degradation of the nanocapsules shell for the controlled release of an internalized drug or externally linked oligonucleotide, which holds important implications for co-delivering small molecules and therapeutic oligonucleotides that result in synergistic effects.

Keywords: nanomedicine, peptides, nucleic acids, drug delivery, self-assembly

1 INTROUDUCTION

To date, many nanomaterial-based forumlations have focused on the effective synthesis and assembly of materials that can encapsulate small molecule drugs and trigger their release in a stimuli-responsive fashion. Beyond using light and pH as location specific triggers, many nanocapsule-based systems are moving their focus to release mechanisms that utilize enzymes as triggers as they are highly sequence-specific in relationship to their cleavage targets. Their specificity in controlling the realase of a nanomaterial's cargo comes from their selectivity for their peptide substrates, and to date, many nanoparticle systems have shown control over the depolyment of drugs due to this aspect. [1] In the work presented here, we merge the enzyme specific release mechanism for deploying a small molecule drug with the ability to co-deliver a DNA molecule into a cell simultaneously. These materials, which we refer to as nucleci acid nanocapsules (NANs) were developed in part to deliver oligonucleotides into cells, with the added capability of triggering the co-delivery of a small molecule in their hydrophoic center. [2] The outward facing nucleic acid structure takes inspiration from the spherical nucleic acid (SNA) structures developed by Mirkin and coworkers. [3] By displaying nucleic acids at their surface, the NAN structure has a greater likilhood of undergoing scavenger receptor mediated endocytosis, the preferred mechanism of uptake due to the design princples behind its enzyme-specific degradation.

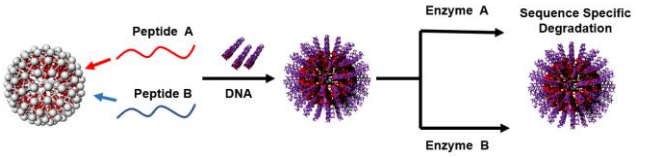

Figure 1. Crosslinkages within the nanocapsule provide enzyme specific release capabilities. The external layer of outward facing oligonucleotides aid the particles entry into cells, and when the particle breaks down due to enzyme specific recognition, nucleic acids can be relased into the cell as active therapeutics (i.e. antisense oligonucleotides, siRNA, etc.) alongside the interior small molecule cargo.

2 SYNTHESIS DETAILS

The NANs are formed using a stepwise assembly of a novel surfactant molecule which lends itself well to covalently linking to both an enzyme-cleavable crosslinker, and a thiolated DNA molecule. This two-step synthesis is achieved using back to back photoinitiated thiol-yne reactions, wherein the first reaction is between the thiol of a cysteine terminated peptide sequence and surfactant alkynes, and the second is between a thiolated DNA molecule and the crosslinked particles surface (Figure 2). The results of these reactions is a nanocapsule which presents an enzyme cleavable exterior, and a dense shell of oligonucleotides that can be depolyed into a cell upon degradation of the nanocapsule. The third and final component of the particle is the utility of the hydrophobic pocket in the center of the particle, which is useful for encapsulation of a small molecule drug or dye during the initial self assembly step. The contents of this hydrophobic region are shown to also be simultaneously released upon breakdown of the particle, thereby consitituting a co-delivery system. [4]

The synthesis and design of these materials is motivated by the idea that the inhibition of a biochemical pathway by a small molecule in conjungtion with the knocking out of a

gene in a related pathway has promise as a way to enhance the therapeutic efficacy of certain drugs and siRNA combinations. These combinations of materials are commonly explored through step wise administration, limiting their precision and interpretation of their effects.

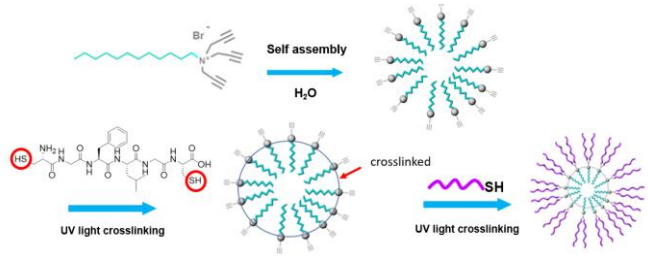

Figure 2. Schematic detailing the step wise self assembly of the nucleic acid peptide-crosslinked nanocaspules (Peptide-NANs). The peptide-NANs result in a highly charged DNA coated nanocapsule with the option of encapsulation of small molecule cargo in the hydrophobic center of the particle.

Therefore, to address the need for a materials platform that can efficienctly co-deliver both a small molecule drug and a therapeutic oligonucleotide - with specificity over where and when it would be deployed - the nucleic acid nanocapsule was developed. Paying particular attention to the biochemical environment that the nanocapsule would experience has guided the design of the particle.

3 RESULTS

To determine the control that these particles provided through their embedded enzyme-triggerable crosslinkers, the particles were subjected to a number of different biochemical conditions, including various combintations of enzymes under varying pHs.

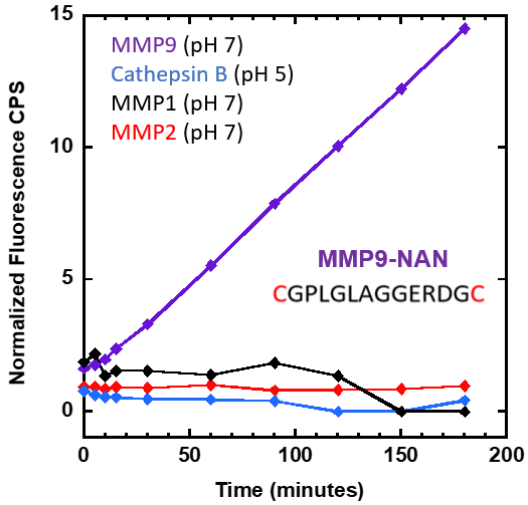

Figure 3. Enzyme specific recognition of the peptide crosslinked nanocapsule (synthesized with an MMP9 specific substrate) by matrix metallaprotease (MMP9). MMP9 NANs were subjected to various enzymes at their optimal pHs and it was shown that only the right combination of peptide and enzyme could release the dye encapsulated within the nanocapsules. [4]

The results consitently indicated that the crosslinkers were specific for their targeted enzyme as shown through the release (or lack of release) of dyes embedded within the nanocapsules (Figure 3). Using a combination of self-assembly and enzymatic reactions we show that a enzyme-specific, DNA functionalized nanocapsule, can be controllably assembled and dissasembled in vitro and in cell culture (Figure 4).

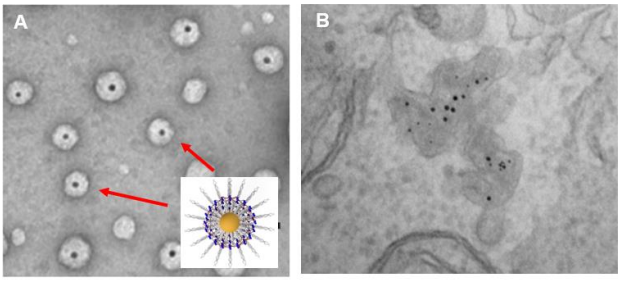

Figure 4. TEM images of a DNA functionalized peptide crosslinked nanocapsule. A) A gold nanoparticle was encapsulated into the peptide-NAN in order to be able to track it in cells. B) A cell sectioning TEM image showing the uptake of the peptide NANs into the endosomes of HeLa cells. (Cells were incubated with 1uM peptide-NANs for 16 hours and then fixed, sectioned, stained and imaged.) [4].

The results of this work show the promise that these structures hold in the area of co-delivery of therapeutics into cells, and in particular, the extent of the specificity that can be imparted into these materials through the incorporation of peptides and other synthetic crosslinkers. Future studeies will pair theraeptuically active nucleic acids such as siRNA with small molecule drugs which can help to augment the gene silencing capabilities of the siRNA. Future work is also focused on understanding if we can control the stability of the nanocapsules by mixing and matching peptides with synthetic crosslinkers as a way to tune the degration rates of these materials. The ability to tune the opening of these materials over a greater range of times will enable more precise control over the rate of delivery from a vehicle that is tailored both to its cargo and its intended cellular location.

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