A Novel Family of Polymeric Carriers for Intracellular Drug Delivery

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Statement of Purpose: Drug delivery to the intracellular space remains a challenge for safe and effective administration of plasmid DNA, silencing RNA (siRNA), antisense oligonucleotides (AS-ODNs), peptide, and protein therapeutics. Drug carriers must overcome multiple obstacles, including targeting to and uptake by the appropriate cell, endosomal escape, and release of drug molecules from the carrier once in the cytosol. Here we highlight recent advances in the development of polymeric intracellular drug delivery carriers.

Methods: This abstract represents a review of recent research; please see referenced publications for more detailed methods.

Results/Discussion: Endosomal escape poses a significant challenge for intracellular drug delivery. Molecules internalized by a cell would eventually be degraded by enzymes in the low pH environment of the endosomal-lysosomal pathway unless they are able to escape the endosome. Poly(propylacrylic acid) (PPAA) is a pH-sensitive polymer that is hydrophilic at pH 7.4 but becomes hydrophobic and membrane-disruptive at low pH (as measured by red blood cell hemolysis), with maximum hemolytic activity below pH 6.3.[1] Incorporation of a pH-sensitive molecule such as PPAA can significantly improve endosomal escape and cytosolic delivery.

Fusion of streptavidin to the cell-penetrating peptide, TAT, allows targeted delivery of a wide array of biotinylated molecules.[2] By conjugating biotinylated PPAA to TAT-streptavidin, it is possible to direct both cell internalization and endosomal escape, significantly improving cytoplasmic drug delivery. Furthermore, conjugation of PPAA to an antigenic peptide increases major histocompatibility complex 1 (MHC 1) presentation and subsequent cytotoxic T cell activation in an *in vitro* vaccination model.[3]

Nucleic acid delivery can be significantly enhanced with complexation to pH-sensitive polymers. Complexation of plasmid DNA to the cationic lipid DNA vector, DOTAP, along with PPAA stabilizes the polyplex in blood, and also enhances delivery of plasmid DNA to the nucleus.[4] In addition, ternary complexes of a diblock co-polymer poly[dimethylaminoethylmethacrylate-b-(dimethylaminoethylmethacrylate-co-butyl methacrylate-co-methyl methacrylate)] (p[DMAEMA-b-(DMAEMA-co-BMA-co-MMA)]) and PPAA have been used to successfully deliver siRNA against GAPDH to a macrophage-like cell model. The addition of PPAA to the complex results in enhanced delivery and decreased cytotoxicity.[5]

Once the carrier and drug are released from the endosome, the challenge remains of "unpackaging" the drug molecule from the drug carrier. Conjugation of peptides or proteins to the glutathione-reactive polymer, pyridyl disulfide acrylate (PDSA) can improve intracellular delivery by facilitating release via glutathione from the polymeric carrier once inside the reducing environment of the cytoplasm.

PDSA has been used to successfully deliver peptides and antisense nucleotides.[6] Complexation of PDSA to the polymeric DNA carrier, poly-L-lysine, by ionic complexation can improve intracellular delivery of nucleic acid therapeutics including AS-ODNs.[7] Furthermore, copolymers of butyl acrylate, PPAA, and PDSA can improve intracellular delivery of proteins by first enhancing endosomal escape then providing release of drug from the polymer. This might have significance, for example, in the development of protein vaccines.[3]

Alternative pH-sensitive molecules also have significant potential for delivery of therapeutic molecules to cells. Poly(N-isopropylacrylamide-co-propylacrylic acid) (p[NIPAAm-co-PAA]) is a temperature- and pH-sensitive polymer that exhibits a cloud point in a physiologically-relevant range that is highly tunable by altering the relative ratios of PNIPAAm to PPAA.[8] In addition, poly(styrene-alt-maleic anhydride) (PSMA) and its derivatives is another family of pH-sensitive polymers that shows significant potential for use in intracellular drug delivery applications.[9]

Conclusions: Polymers that respond to special conditions within the intracellular environment have wide-ranging applicability in the development of novel drug delivery carriers. Research in our lab demonstrates that pH- and glutathione-sensitive polymeric systems can provide effective intracellular delivery of biotherapeutic macromolecules.

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References:

[1]Murthy N. J Control Release. 1999;61:137-143.
[2]Albarran B. Protein Eng Des Selection. 2005;18:147-152.
[3]Flanary S. Unpublished data, University of Washington, October, 2006.
[4]Cheung C. J Biomat Sci Polym Ed. 2005;16:163-179.
[5]Johns R. Unpublished data, University of Washington, October, 2006.
[6]Bulmus V. J Control Release. 2003;93:105-120.
[7]El-Sayed M. Unpublished data, University of Washington, October, 2006.
[8]Yin X. Biomacromol. 2006;7:1381-1385.
[9]Henry SM. Biomacromol. 2006;7:2407-2414.