Online Proceedings

POSTER SESSION 2

Commons East, Easel 77

12:45 PM to 2:15 PM

HPMA-oligolysine-oligohistidine Copolymers for Gene Delivery

Jennifer Lynn (Jen) Choi, Senior, Bioengineering
Amgen Scholar, Mary Gates Scholar
Mentor: Suzie Pun, Bioengineering
Mentor: Julie Shi, Bioengineering

One of the major intracellular barriers to non-viral gene delivery is degradation through the endosomal/lysosomal pathway. To overcome this barrier, pH-sensitive polymers that swell in acidic pH and ultimately disrupt the endosome can be designed to escape these compartments. Previously, the Pun lab developed HPMA-oligolysine copolymers that have transfection efficiencies approaching that of polyethylenimine (PEI), the gold standard for transfection. In order to increase the copolymers' transfection efficiency, oligohistidines were incorporated into the copolymer in both statistical and block architectures to impart pH-sensitivity. Transfection studies with these two copolymers showed that statistical incorporation of oligohistidine increased transfection efficiency, while block incorporation did not increase transfection efficiency. To determine if structure-function differences affected cellular uptake and endosomal buffering, we performed uptake inhibitor and endocytic buffering inhibitor studies. Through these studies, we found that both copolymer geometries preferred non-clathrin coated caveolae uptake pathways, and that they differed in endosomal buffering capabilities. The statistical copolymer showed increased endosomal buffering capacity, which was consistent with the overall higher transfection efficiency of the statistical copolymers over the block copolymers. From these two studies, we are better able to understand the relationship between structure and function in gene delivery using polymers.

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Commons East, Easel 78

12:45 PM to 2:15 PM

pH-Responsive Polymers for Improved Gene-Delivery Efficiency

Michael Joseph (Mike) Bocek, Senior, Biochemistry
Amgen Scholar, Goldwater Scholar, Mary Gates Scholar
Mentor: Suzie Pun, Bioengineering
Mentor: David Chu, Bioengineering

A major obstacle in the efficient delivery of genes using non-viral vectors is sequestration and subsequent degradation of the vector in the endosomal/lysosomal trafficking pathway. While incorporation of membrane-lytic peptides into gene delivery vectors has been shown to increase transgene expression by disrupting the endosomal vesicles, non-specific membrane-disruption caused by these modified vectors leads to significantly increased toxicity. One approach to mitigating this increased toxicity is to mask the membrane-lytic peptides inside a pH-responsive micellar core that destabilizes during vesicle acidification in the endosomal/lysosomal pathway, revealing the membrane-lytic segments only when internalized. Incorporating pH-responsive imidazole elements in the hydrophobic micelle core which become charged under acidifying conditions can disrupt the micellar structure by electrostatic repulsion, leading to display of the active peptide, membrane lysis, and increased DNA delivery to the cytoplasm. After synthesis of the pH-responsive motif a panel of representative DNA delivery polymers will be evaluated for pH-responsiveness, transfection efficiency, and cytotoxicity.

POSTER SESSION 2

Commons East, Easel 76

12:45 PM to 2:15 PM

Tet1 and Melittin-Grafted Copolymers for Neuronal Gene Delivery

Joshuel Arce (Josh) Pahang, Junior, Bioengineering
Mary Gates Scholar
Mentor: Suzie Pun, Bioengineering
Mentor: Joan Go Schellinger, Bioengineering

It has been hypothesized that effective treatment of neurodegenerative diseases, such as Huntington's Disease and Alzheimer's, may be possible through the manipulation of neural stem cells to divide and replenish afflicted neuronal cell populations. A crucial component of this treatment would be the creation of a vehicle for the efficient delivery

of stimulating genetic material into target cells. Prospective virus-based vectors accomplish this by making use of a recombinant virus and its natural ability for gene transfer. These viral vectors, while inherently effective, have significant safety issues with the possibility of eliciting harmful immune responses. Peptide-based copolymers are a promising non-viral alternative for delivery systems. While safer, these synthetic vectors have thus far proven less efficient. One problem is lack of consistent cell membrane penetration, preventing the therapeutics from reaching areas within the cell where they would come into effect. Our laboratory has recently incorporating the membrane-lytic peptide melittin (a molecule derived from bee venom) into our base DNA-binding peptide-based polymers. The melittin-functionalized materials were shown to increase gene delivery efficiency by at least 10-fold compared to control materials both in vitro and by injection into mouse brain. This current study seeks to develop a multifunctional delivery system by incorporating the neuronal-targeting peptide "Tet1," for specialized treatment of neurodegenerative diseases. Previous work showed that conjugation of Tet1 to control polymers results in increased transfer specificity to neuronal cells in vivo. Potentially, Tet1 and melittin can synergize to further improve gene delivery to the central nervous system.

POSTER SESSION 2

Commons East, Easel 66

12:45 PM to 2:15 PM

Development of an Ultrasound Mediated Neuronal Transfection Protocol Towards Treatment of Traumatic Brain Injury

Ashton Stuart (Ashton) Hemphill, Senior, Bioengineering
Mary Gates Scholar
Mentor: Pierre Mourad, Neurological Surgery
Mentor: Suzie Pun, Bioengineering

Traumatic brain injury (TBI) is injury to the brain caused by external trauma. Approximately 1.7 million cases of traumatic brain injury are reported every year in the US, and contributing to an estimated 30.5% of injury related deaths. TBI is currently treated by managing symptoms, present treatment methods are incapable of reversing the damage done by the initial trauma. Gene therapy is a promising method of treatment for TBI; by transfecting cells such as neural stem or progenitor cells (NSCs and NPCs respectively) in the brain with plasmids which induce differentiation and migration damage to neurons can be alleviated or even reversed. This projects aims to develop and optimize a protocol which utilizes non-viral transfection vectors in conjunction with ultrasound to efficaciously target and transfect neural progenitor cells (NPC), i.e. cells capable of differentiation into neurons. By transfecting NPCs with plasmids which cause differentiation and/ or migration NPCs can be induced to become new neurons and migrate to areas of neuronal injury caused by traumatic brain injury. In this manner some of the dead and dying neurons might be replaced and the initial trauma mitigated. This is a novel method of treatment both in its use of non-viral vectors capable of targeting NPCs in conjunction with ultrasound mediated increase in transfection efficiency and in its potential for reversing the initial damage seen in TBI; something that no current method of treatment is capable of.

POSTER SESSION 3

Commons East, Easel 52

2:30 PM to 4:00 PM

Application of Subcellular Fractionation Techniques to Investigate the Intracellular Trafficking of the Pro-apoptotic Peptide KLA for Tumor Targeting Applications

Ngoc Anh Luu (Anh Ta) Ta, Senior, Bioengineering
Mary Gates Scholar
Mentor: Suzie Pun, Bioengineering
Mentor: Julie Shi, Bioengineering

The pro-apoptotic (cell death inducing) peptide (KLAK-LAK)2 ("KLA") has been recently investigated for tumor killing applications. To increase the efficacy of the peptide, our group has added additional charged amino acids to increase cellular uptake (Gly-Lys-Arg-Lys-(KLAKLAK)2) ("GKRK-KLA") and synthesized a polymeric construct of this peptide and HPMA (N-(2-hydroxypropyl)methacrylamide) ("p(HPMA-co-GKRK/KLA"). Preliminary studies have shown an increase in cell death per KLA unit in the modified constructs over the unmodified KLA construct. However, the mechanisms of cellular internalization and subcellular distribution of the peptide is unknown. Recently, we showed using fluorescence microscopy that the KLA constructs do not colocalize with mitochondria, the organelle that was believed to be affected. In this work, we applied radiolabeling and organelle separation techniques to investigate the mechanisms behind cellular uptake and subcellular trafficking over time of both the KLA peptide and our modified designs. These insights will allow us to design better materials to increase the efficacy of KLA peptide delivery.