Antisenseoligo nucleotide delivery using polymer vesicles

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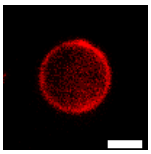
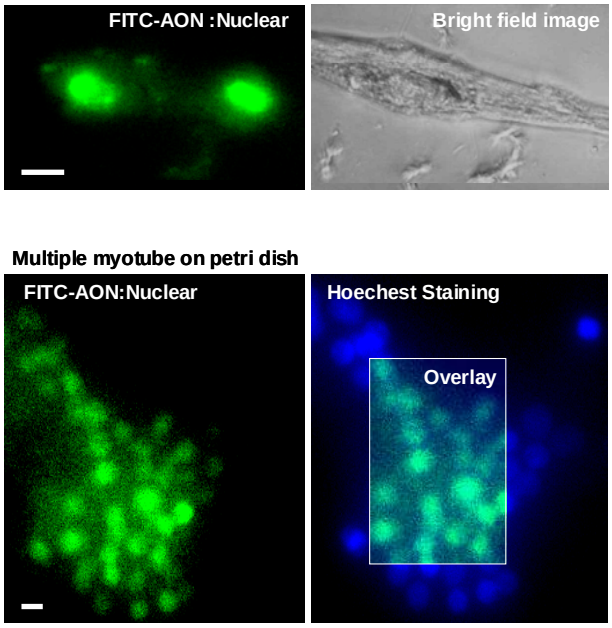
Statement of Purpose: Synthetic vesicles by PEO-PBD or PEO-PCL have advantages 1) carriers are so inert that it does not cause inflammation 2) they are so stealthy that they can circulate in vivo for long time. [1,2,3,4,5]. Programmable release of encapsulants by controlling the ratio of PEO-PBD/PEO-polyesters would be another advantage [6,7]. Here we detail the gene delivery by controlled-release polymersomes. Antisenseoligo nucleotide (AON) was encapsulated within PBO-PBD/PEO-PCL blend vesicles for programmable release. The AON delivery into muscle cells for corrective therapy depends much upon the delivery system that provides advantages of site-specific delivery, protection from nucleases and tailored release.

Methods: Vesicles form spontaneously upon mixing of aqueous solution and DMSO stock solution at room temperature. Vesicles formed from blends of PEO-PCL and PEO-PBD and were used immediately after 2days dialysis at cold room to remove organic solvents. The excitation/emission maxima of fem-AON are 492/520 nm. TMRCA and fluorescein-5-carbonyl azide were conjugated to copolymer as described elsewhere [8]. Hydrophobic fluorescent dyes (PKH26 or PKH67) were added directly to a vesicle suspension [5].

The fluorescent intensity of AON and TMRCA-tagged vesicles were measured with spectrofluorimetry. UV scattering was measured using spectrophotometer, and the base line correction for DMSO adsorption was considered. Surface tension measurement was done using normal glass slide glass.

Results / Discussion: The vesicle forming process was visualized with copolymers that have chemically conjugated fluorophore on them (left, scale bar is 2 micro meter). The surface tension was a key control factor for the size of vesicles. So, the vesicle size and numbers were highly reproducible. The encapsulation efficiency of OB2 vesicles was more than 21%, which was usually much higher than typical vesicle forming methods. Degradable and biocompatible PEO-PCL or PEO-PLA blending with stable PEO-PBD copolymers generates the poration of vesicles. Self-porating vesicles and their endosomal release in vitro were thoroughly investigated by our lab (2005) [9]; blending vesicles with two copolymers were also able to give a programmable release profile over time at 37C. In-vitro application of self-porating vesicles gave promising results. C2C12 cells were prepared in petri dish and patterned single cell strips to see delivery. The endocytosis rate was measured with well-defined patterned C2C12 cell strips and was able to exclude non-specific binding. OB2 vesicles gave a saturation profile; the influx of vesicles into cells has a plateau at almost 6hr time-point. Copolymer structures were able to travel to near nuclei after being pinocytosed to cells. Also they were not able to enter nuclei, because of the selectivity of





nuclear membrane. Perinucleus localization in day two pictures was obvious. Finally, encapsulated AON was delivered into the nuclei of C2C12 cells. The optimization of delivery can be tuned with the ratio of OB2 and OCL4 for controlling release-half time. These deliveries were clearly shown in the above pictures. Green labeled AON image was overlaid to blue stained nucleus to show nucleus delivery.

Conclusions: A process of forming polymer vesicles by cosolvent extraction was developed to maximize encapsulation efficiency of highly charged antisense-oligonucleotides (AON). Along with stable encapsulation of fluroescent-AON at 4 °C, programmable release was made possible by blending hydrolysable PEO-polyester with PEO-poly butadiene. Passive uptake by cells was studied with C2C12 muscle cells grown on patterned micro-strips of collagen. Labeled vesicle carriers show perinuclear localization within several hours, and self-porating vesicles lead to nuclear localization of AONs. More broadly, the results demonstrate that polymer vesicles can load and release nucleus-targeted drugs.

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