PEG-*b*-PPA/DNA Micelles for Liver-Targeted Gene Delivery

Xuan Jiang[1], Hui Dai[2], Kam W. Leong[2], Hai-Quan Mao[1]
[1]Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21218
[2]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Introduction:

Liver is a critically important target for gene medicine applications because of the access of the transgene product to systemic circulation, and because it is the site of many metabolic genetic disorders, viral infection and malignancies. At present, the full potential of liver-targeted gene transfer is hindered by a lack of safe and efficient gene carriers. Recently, we have developed a new class of polyphosphoramidates (PPAs) as non-viral gene carriers. These PPA carriers exhibit favorable biocompatibility, DNA compaction and protection capacity, high transfection efficiency in several cell types in culture [1, 2]. However, PPA/DNA complexes, like many polycationic carriers, aggregate severely upon incubation in serum containing medium. This aggregation will lead to reduced cellular uptake by parenchymal cells and sequestration by reticuloendothelial system following intravenous injection, rendering the complexes inefficient for liver-targeted gene delivery. Here, we report a novel PEG-*b*-PPA block polymer carrier that can form nanoscopic micelles with plasmid DNA. The hydrophilic PEG shell layers can reduce micelle surface charge and serum protein adsorption under the physiological condition, which will lead to improved colloidal stability and enhanced gene expression in the liver.

Methods:

PEG-*b*-PPA copolymer was synthesized by initiating the cyclic monomer, 4-methyl-2-oxo-2-hydro-1,3,2-dioxaphospholane, with a macroinitiator, poly(ethylene glycol)-ate (PEGate) anion, followed by side chain conjugation before deprotection of the sidechain amine groups [1]. The molecular weight of PEG block is 2000, and M_W for PPA block was 22.5 kDa. PEG-*b*-PPA/DNA micelles were prepared by complexing the block copolymer and VR1255 plasmid DNA in 5% glucose solutions at various charge ratios. Tail vein injection of micelles in mice was performed at a DNA dose of 25 μg in 250 μl saline in 30 seconds. Intrabiliary infusion in rats was performed at a DNA dose of 20 μg in 4 ml micelle solution over 20 minutes using an infusion pump.

Results and Discussion:

The average diameter of PEG-*b*-PPA/DNA micelles remained nearly constant at an average diameter of 80 nm at all tested charge ratios (0.5 to 20). The size distribution of these micelles was unimodal with a low polydispersity. Transmission electron microscopic examination of PEG-*b*-PPA/DNA micelles revealed the spherical or rod like morphology of the micelles with diameter ranging from 70 to 80 nm (Fig. 1). Surprisingly, these micelles trandfect HepG2 cells at high efficiency, similar to that by PEI/DNA complexes and PPA/DNA complexes, without the aid chloroquine. In primary rat hepatocytes, the transfection efficiency of PEG-*b*-PPA/DNA micelles was about 15-fold lower than that of PPA/DNA or PEI/DNA complexes.

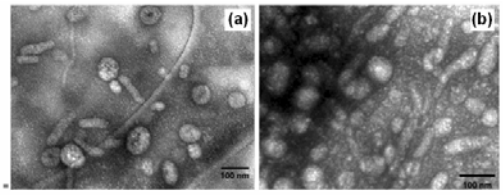

Figure 1. TEM images of PEG-*b*-PPA/DNA micelles (N/P=8).

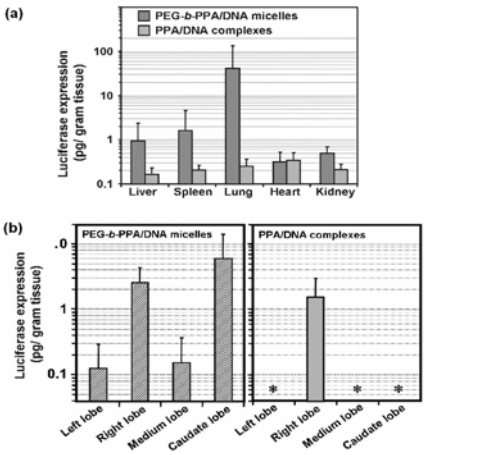

Figure 2. Luciferase expression in mice after tail vein injection (a), and in rat liver after intrabiliary infusion (b) of PPA/DNA complexes (N/P=10) and PEG-*b*-PPA/DNA micelles (N/P=8).

Following tail vein injection in Balb/c mice, PEG-*b*-PPA/DNA micelles mediated 5-, 8- and 150-fold higher luciferase expression than PPA/DNA complexes in the liver, spleen and lung (Fig. 2a). Although the highest transgene expression was found in the lung, this improved transfection efficiency, over PPA/DNA complexes, is likely the result of increased stability of PEG-*b*-PPA/DNA micelles in serum. After intrabiliary infusion in rats, PEG-*b*-PPA/DNA micelles also mediated 4-fold higher average luciferase expression in the liver than PPA/DNA complexes on day 3. No expression was detected in other major organs (lung, kidney, heart, spleen). Moreover, luciferase expression was only found in one of the liver lobes for PPA/DNA complexes, in contrast to a relatively widespread expression among different lobes of the liver in the micelle group (Fig.2b).

In summary, these results demonstrated unique features (size, stability, low cytotoxicity, high *in vitro* transfection efficiency) of the PEG-*b*-PPA/DNA micelles and the significantly improved transgene expression *in vivo*, suggesting the potential of this nanoscopic micellar carrier for liver-targeted gene delivery.

References:

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Acknowledgements:

The authors thank Xiao Mo and Decheng Wu for their technical assistance with TEM imaging and GPC analysis.