Long-term overseas dispatch program in Friedrich Schiller University Jena

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1. MERIT Errantry as a chance for the long-term overseas dispatch

I joined a research group of Dr. Mosig at Friedrich Schiller University Jena in Germany for 2 months from September 2015. Dr. Mosig was a researcher I got to know during the MERIT errantry in March 2015. When I visited Jena to join a symposium during the errantry, I had a chance to listen to presentations from students in Jena. Fortunately, one student from Dr. Mosig's lab presented about cellular cultivation studies under flowing condition, which I was highly interested in. As I contacted Dr. Mosig through the student, he kindly took me to a short lab-tour and provided me an opportunity to take a look into their experimental setups. A long-term dispatch in this report was carried out by contacting Dr.Mosig again after the MERIT errantry.

2. Results from research activities during the dispatch

I have been working on development of gene delivery carrier for gene therapy. Gene therapy, which cures genetic disease by transfecting therapeutic proteins at targeted sites inside a body, has recently attracted many attentions. For systemic gene therapy, gene carriers to carry plasmid DNA (pDNA) to targeted sites are necessary. However, as the carriers are quickly eliminated from circulating blood by many biophylaxis systems, such as cleavage of pDNA by DNase, phagocytosis by macrophages, and so on, improvement of blood circulation property has been one of the biggest problems remain to be solved.

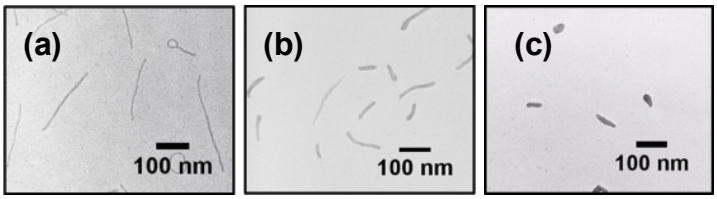

Figure 1. TEM images of PEG-PLys polyplex micelles with PEG -PLys. (a) Long rods (b) Short rods (c) Globules.

I have been working to develop polyion complex micelles (polyplex micelles, PMs) as a gene delivery carrier, which are formed between pDNA and block copolymer of poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-PLys), from a view point of control of micelle shape. As a result, a method to control micelle shape into Long-rods, Short-rods, and Globules was established (Figure 1). As a next step, it would be interesting to evaluate how pharmacokinetics of the PMs depends on shape of the PMs.

In Dr. Mosig's lab, they have been established methods to evaluate cell behaviors under *in vivo*-scaled flowing environment. They have reported cells behave more similar to *in vivo* when they are under flow compared to conventional static environment.

During the dispatch, PMs were prepared with PEG-PLys, which was brought from Japan, and cellular uptake by macrophage and HUVEC (Human Umbilical Vein Endothelial Cell) under *in vivo*-scaled flowing environment was evaluated. First, Monocytes purified from human blood and HUVEC from human umbilical vein were cultured together in Biochips for 5 days until the monocytes were differentiated to macrophages. Second, fluorescence labeled PMs were perfused to the Biochip under flow which gives 3 dyne/cm^2 of shear stress. Then, amounts of taken up PMs was evaluated

by measuring fluorescence intensity in each cell using flow cytometry. As a result, cellular uptake by macrophages for long-rods was significantly high compared to short-rods or globules. Note that uptake by HUVEC did not show significant difference depending on shape of PMs. Besides, cytokine release of IL-6 and TNF-α was also enhanced for long-rods compared to the other two shapes, which supported higher uptake of long-rods by macrophages. Therefore, it could be assumed that long-rod shaped PMs are more efficiently taken up by macrophages. In other words, it could be assumed that shape with short long-axis might be more favorable in aim to avoid unfavorable uptake by macrophages during blood circulation. Note that these tendencies were not observed significantly when the experiments were conducted with conventional well plates under static environment as a control. This suggested a possibility that it might be important to provide flow to cells for evaluation of PMs as a gene delivery carrier *in vitro*.

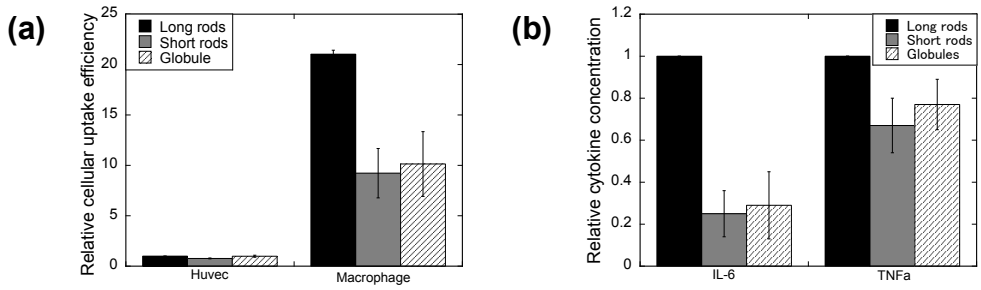

Figure 2. Evaluated cellular activity with PMs under flowing condition with 3 dyne/cm^2 of shear stress. (a) Cellular uptake efficiency of PMs for Huvec and Macrophage (b) Cytokine release (IL-6 and TNF-α) from co-cultured cells of Huvec and Macrophage.

3. Future plans

During this stay, I obtained interesting data also in addition to the ones shown in this report. With kind help from Marko Gröger, who helped me with my experiments during this stay, some additional experiments are planned as I heve left some polymer in the lab. I will proceed with other experiments in parallel in Japan and we plan to write a paper on this work together.

4. Acknowledgments

I would like to express my deepest gratitude to Marko Gröger for always being helpful and giving me a lot of constructive advice during my stay. I am also indebt to all the lab members for enormous helps, especially great supervising by Dr. Mosig. I appreciate MERIT program for providing such a chance to learn.

Figure 3. With the lab members.