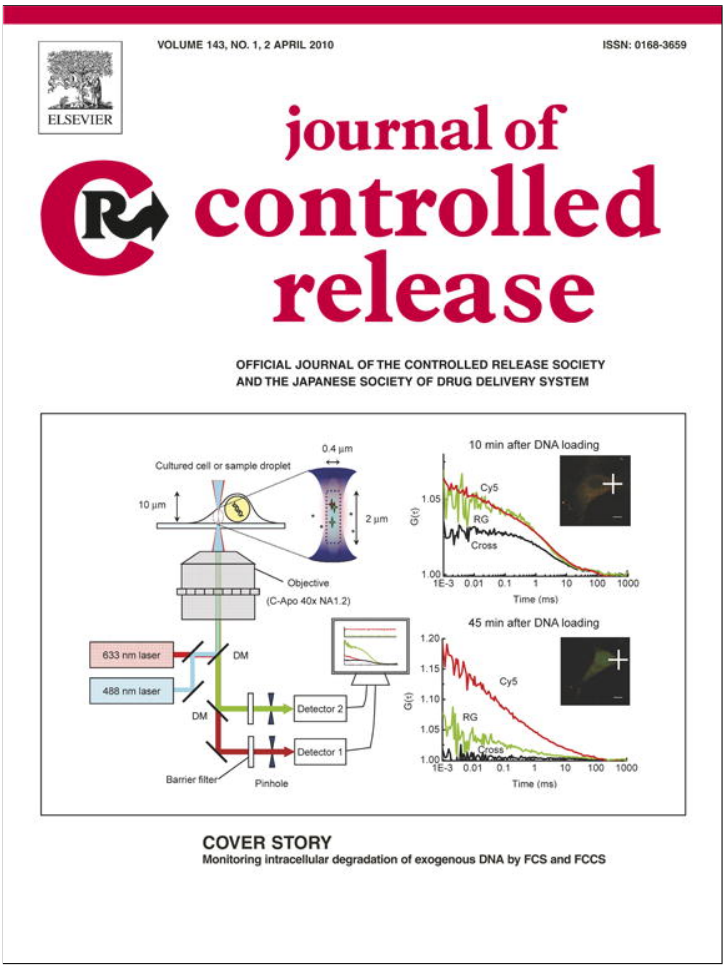

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Cover Story

Monitoring intracellular degradation of exogenous DNA by FCS and FCCS

Cationic polymers and cationic lipids have been widely used in the gene delivery research. While they have been effective, the non-viral vectors need to overcome the main disadvantage of low gene expression efficiency as compared with viral vectors. The non-viral gene delivery systems have a few distinct limitations. First, non-viral vectors are not as effective as viruses in delivery of genes to the target cells. After reaching the target, the non-viral vector needs to enter the target cell and release the DNA into the cell cytoplasm. For gene expression, the delivered DNAs are required to reach the nucleus in the intact forms. The presence of cytoplasmic nucleases presents another hurdle in the ultimate gene expression. Protecting the exogenous DNA from the nuclease attack in the cytoplasm is expected to enhance the transfection efficiency significantly. Nevertheless, this particular step in gene delivery has not been studied carefully, simply because there have been no reliable methods for continuous monitoring of the nuclease activity in a quantitative manner *in vivo* and *in situ*.

An article by Sasaki and Kinjo in this issue employed fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) for *in situ* monitoring of the nuclease activity [1]. This method is based on recent advances in fluorescence microscopic techniques. Since it does not involve any separation procedure for nuclease products, the method can be applied for quantitative measurements in living cells. They used FCCS for continuous, quantitative monitoring of the time-dependent changes in the relative abundance of degraded DNAs in living cells. Furthermore, the distribution analysis of the sizes of degraded DNA fragments indicated that the main mechanism of exogenous DNA degradation in cytoplasm was 5′ to 3′ exonuclease activity rather than endonuclease. They also confirmed that the exonuclease degradation can be inhibited by end-capping and end-elongation. The result showed that exonuclease activity affected the transfection efficiency, although the level of exonuclease activity in cytoplasm might be different depending on the cell line. The study by Sasaki and Kinjo presents a new approach of evaluating the nuclease barrier in different cell lines. It also offers a theoretical background for examining exogenous gene expression of various cell lines. This approach may also be important for enhancing the production of induced pluripotent stem cells using non-viral strategies, because it has a potential to evaluate the stability of each plasmid factor in a target cell. The work by Sasaki and Kinjo is highly useful in understanding the fate of exogenous DNA and the efficiency of DNA integration in living cells, both of which are critical in development and evaluation of clinically useful non-viral vectors.

Reference

[1] A. Sasaki, M. Kinjo, Monitoring intracellular degradation of exogenous DNA using diffusion properties, J. Control. Release 143 (2010), 104–111.

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