Synthesis and Applications of New Contrast Agents for *In Vivo* Cell Tracking

Yoichi, Tachibana[1]; Jyunichiro, Ennmi[2]; Hidehiro, Iida[2] and Tetsuji, Yamaoka[1].
1. Department of Biomedical Engineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute
2. Department of Investigative Radiology, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute

Statement of Purpose: Recently, there has been an increasing interest in developing various *in vivo* imaging techniques. Among them, magnetic resonance imaging (MRI) has proven to be a particularly powerful tool because of its ability to noninvasively attain the visualization of the internal structure in a high resolution. The noninvasive assessment is one of the most important factors for tracking the targeted cells .in the field of cell transplantation therapy. To detect the targeted cells in distinction from the other cells, contrast agents by enhancing the signal intensity are needed. The contrast agents based on chelates of gadolinium (Gd) are the most widely applied contrast agents for general clinical MR imaging. Mainly, low molecular weight agents, such as Gd-DTPA (diethylenetriaminepentaacetate), are routinely used. However, when these agents were injected or somehow delivered into the cell for labeling them, they rapidly diffused into the extracellular space space with a short retention time due to their small sizes. In order to prolong the retention of contrast agents, a novel technique should be developed made. We then tried to increase the molecular size of the contrast agents using polymer which does not interact with cell membrane.

Polyvinyl alcohol (PVA) and its hydrogels have been studied extensively to produce new materials for various applications. PVA has advantages that make itself excellent candidates for biomaterials in the biomedical and pharmaceutical field. Some of these advantages include their water soluble, non-toxic and non-carcinogenic characteristics. In addition, the body distribution of PVA with different molecular weights was investigated in developing the drug delivery[1]. The half-life of PVA with high molecular weights was much longer than that of the other polymers because of an insignificant interaction of PVA with cells, such as macrophages and blood cells. This weak interaction with various cells is important feature for cell tracking.

In this study, we have synthesized new contrast agents for cell tracking based on PVA and studied their behavior in the cells.

Methods: Contrast agents based on PVA were prepared by the reaction of the hydroxyl group on the PVA side chain and Gd chelates.

NIH/3T3 cells were cultured in DMEM-LG medium and labeled with the novel MRI-imaging agent by the method of electroporation. The number of surviving cells was assessed by WST-1 cell proliferation assay.

Relaxivity and MR cell studies were also performed using Tecmag Apollo NMR spectrometer equipped with a 20 mT/m max gradient set and 47 mm ID coil operating at 200 MHz, at ambient temperature (25 ^{o}C). Imaging was performed with a T1-weighted saturation recovery spin echo sequence with differing repetition times and an echo delay time of 16 ms.

Results/Discussion: Novel contrast agents were synthesized by using PVA (Mw:74800, DS:98%) and Gd. The degree of Gd introduction was 9.2, 5.3, and 2.5 mol %. The relaxivity of these PVA-Gd conjugates was slightly higher but on the same order of magnitude as Magnebist, which is typically using. This result demonstrates that these PVA-Gd conjugates can be used as effective contrast agents.

To determine the toxicity of these PVA-Gd conjugates, the synthesized PVA-Gds were added to the culture medium for NIH-3T3 cell at various concentrations, and the WST-1 cell proliferation assay was performed. All PVA-Gd conjugates did not affect the cell proliferation or viability of cells even at high concentration.

The intracellular behaviors of these PVA-Gd conjugates were examined using FITC-labeled PVA-Gd. In this case, not only the viability but also the proliferation rates of cells were not affected by the intracellularly delivered PVA-Gd. Furthermore, these PVA-Gd conjugates were retained stably in the cytosolic compartment up to 9 days, indicating that these PVA-Gd conjugates are safe materials for the cells.

MRI studies were performed to examine the ability of these PVA-Gd conjugates to enhance the contrast in the MRI image of the cells. At high concentration, enhanced image of the cells could be observed.

Conclusions: PVA-Gd conjugates were synthesized and evaluated as MRI contrast agents. The WST-1 cell proliferation assay showed that the toxicity of PVA-Gd conjugates was quite low. Moreover, these PVA-Gd conjugates have high ability as novel contrast agent for cell labeling. These results suggested that the ability of these PVA-Gd conjugates to be employed in the study of biological phenomena using MRI.

References: Yamaoka, T.; Tabata, Y.; Ikada, Y. "Comparison of Body Distribution of Poly(vinyl alcohol) with Other Water-soluble Polymers after Intravenous Administration" *J. Pharm. Pharmacol.*, 1995, *47*, 479-486.