DEVELOPMENT AND EVALUATION OF LABELED ISLET CELLS USING IRON OXIDE NANOPARTICLES AND DNA HYBRIDIZATION

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Introduction: Type I diabetes is a disease in which the pancreas does not produce insulin because of autoimmune destruction of insulin-producing pancreatic b-cells of the islets of Langerhans. Insulin is a hormone that is important in control of the level of glucose in blood. Thus, decreased insulin secretion leads to hyperglycemia. Transplant of islets is a promising treatment for type I diabetes mellitus, but there are problems with this technique, including difficulty confirming the presence of transplanted islets noninvasively. For this reason, direct imaging of grafted islets is an emerging topic in the field of islet transplantation. MRI is a powerful method for imaging deep regions of the body and can provide noninvasive, high-resolution, three-dimensional images. However, transplanted islets need to be labeled using a contrast agent for MR imaging. In this study, we developed a labeling method for islet cells with superparamagnetic iron oxide particles (SPIOs) based on DNA hybridization, which has been successfully applied to cell surface modification, as shown in Fig. 1. Single-stranded DNA (ssDNA) with a specific sequence was introduced onto the cell surface using a ssDNA-poly(ethylene glycol)-phospholipid conjugate (ssDNA–PEG–lipids)[1,2]. ssDNA with a sequence complementary to that of the ssDNA on the cell surface was positioned on the surfaces of the SPIOs, thus allowing the particles to be immobilized on the cell surface through DNA hybridization. The labeling efficiency of this method was evaluated by MRI, TEM and microscopy.

Materials and Methods: Surfaces of SPIOs were modified via a Michael reaction of oligodeoxyadenylic acid with a 5'-end thiol group ($(dA)_{20}$-SH) with maleic acid functional groups on the SPIOs[3]. The SPIOs were immobilized through DNA hybridization on islet cells pretreated with an oligothymidylic acid-poly(ethylene glycol)-phospholipid conjugate ($(dT)_{20}$-PEG-DPPE). Islets for TEM samples were washed with PBS and fixed with 2% glutaraldehyde solution. The fixed islets were incubated in 1% osmic acid for one hour, followed by dehydration. Samples were embedded in epoxy resin and ultrathin sections were prepared for TEM observation of the location of SPIOs in islet cells. All MRI experiments were performed on a 1.5T whole body scanner (Magnetom Sonata, Siemens AG, Erlangen, Germany) using a dedicated phased array receiver coil for high resolution MRI. Agarose gels (1wt%) in Pyrex glass tubes containing different concentrations of $(dA)_{20}$-modified SPIOs were prepared for T2-relaxivity measurements. MR images were taken using a turbo spin echo sequence (TR: 5000ms; TE: 13.1, 26.2, ... ,419.2ms; slice thickness: 2mm; pixel size: 0.5x0.5mm) and T2 values were determined from MR images of each gel. These values were plotted against concentrations of iron atoms in the gels. The relaxivity (r^2) was evaluated from the slope of the linear regression line of the experimental values for T2 plotted as a function of iron concentration. For in vitro MRI, 25 to 30 islets (SPIO-labeled islets or non-labeled) were immobilized in the center of an agarose gel (1wt%) slice of 2 mm thickness and 15 mm in diameter. MR images were taken using a T2*-weighted gradient echo sequence (TR: 100ms; TE: 45ms; slice thickness: 2mm; pixel size: 0.25x0.25mm). For in vivo MRI, a syngeneic model (C57/B6 to C57/B6) of islet transplantation was used, in which naïve islets or SPIO-labeled spheroids were infused into the right hepatic lobe of the liver through the portal vein. Within 30 min after transplantation, graft location was monitored by in vivo MRI of the mouse liver using a susceptibility weighted 3D FLASH sequence (TR: 28ms; TE: 21ms; slice thickness: 0.5mm; pixel size: 0.25x0.25mm).

Results and Discussion: A plot of 1/T2 values against iron concentrations gave an estimated relaxivity of 188 $mM^{-1}S^{-1}$ from the slope of the linear regression (Fig. 2). The r^2 value of $(dA)_{20}$-modified SPIOs was comparable to that for commercially available contrast agents, indicating that this approach is suitable for MR imaging. Single cells obtained by trypsinization of islets were labeled with SPIOs. The SPIO-labeled cells (2000 cells) aggregated to yield a spheroid with the same size and appearance as that formed by naïve islets (Fig. 3a). Thin sections of these spheroids showed that SPIOs were present in the endosomes of most of the cells in the spheroids and notably even in cells present in the core area of the spheroid (Fig. 3b). In contrast, in intact islets modified with SPIOs, the SPIOs were found only in cells located in the outermost layer of islets. In T2*-weighted MR images (Fig. 3c) and optical micrographs (Fig. 3d) of spheroids formed from SPIO-labeled cells, the individual spheroids were identified as dark spots. Comparison of these two types of images showed that the dark spots in the MR image corresponded well to spots in the optical micrograph. In vivo imaging confirmed the presence of islets in mouse liver.

Conclusion: We developed a method for reliable labeling of islets with SPIOs via a specific interaction based on DNA hybridization. ssDNA-modified SPIOs can be easily anchored on the cell membranes of islets and subsequently transferred to endosomes inside cells. These SPIO-labeled islets can then be detected as dark spots on MR images. Use of a reaggregation method enabled labeling of internal islets in an aggregated spheroid, in addition to cells in the outer layer. Thus, this method improves the hypointensity of dark regions and gives high contrast images in MRI performed in vitro and in vivo. The method is also likely to be applicable for use with other cells, such as stem cells, and for labeling at multiple sites by changing the DNA sequence.

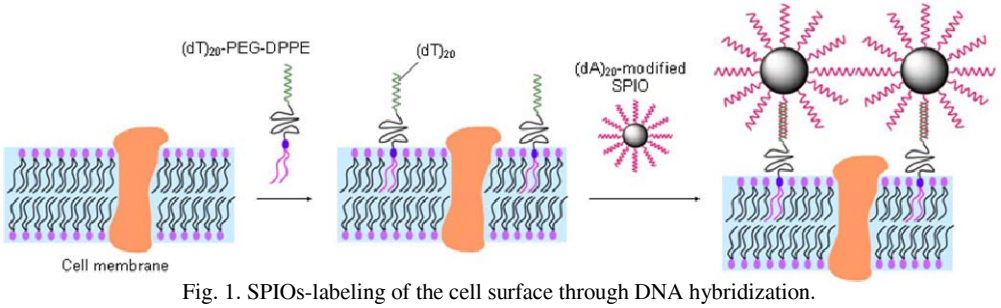

Fig. 1. SPIOs-labeling of the cell surface through DNA hybridization.

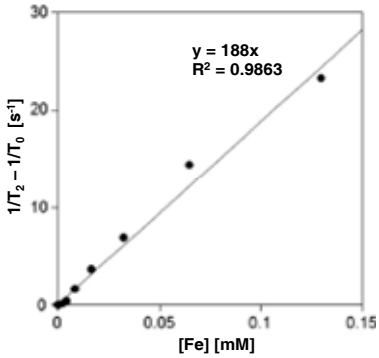

Fig. 2. T2 Values were plotted against iron concentrations which were calculated from concentrations of SPIOs.

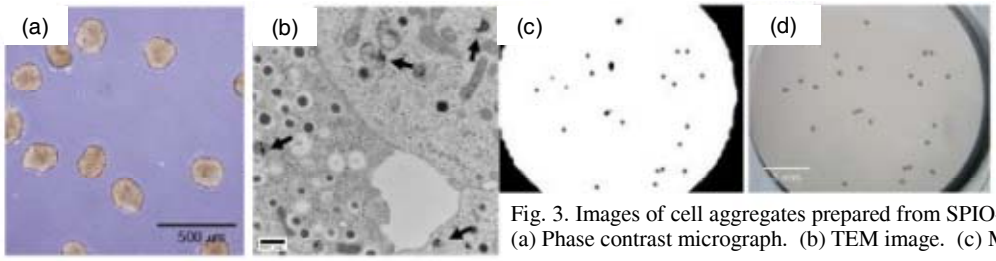

Fig. 3. Images of cell aggregates prepared from SPIO-labeled islet cells.
(a) Phase contrast micrograph. (b) TEM image. (c) MR image. (d) Stereo micrograph.

References: [1] Takemoto N et al., Bioconjugate Chem., 2011; 22: 673-678. [2] Sakurai K et al., Biomaterials, 2013; 34: 361-370.
[3] Tanaka K et al., Bioorg. Med. Chem. Lett., 2008; 18: 5463-5465.