Development of a Novel Optical Probe for In Vivo Detection of Biomaterial-Associated Apoptosis

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Statement of Purpose: Several imaging probes have been established in recent years to monitor foreign body reactions by targeting various inflammatory cells and cellular products.[1, 2] However, these probes cannot be used to investigate the events after biomaterial-mediated inflammatory responses, such as apoptosis. Previous studies have demonstrated that biomaterial-adherent cells can undertake biomaterial-dependent apoptosis both in vitro and in vivo, and adherent inflammatory cell apoptosis has been considered as an important mechanism for the resistence of an implant device to bacterial infection.[3] Therefore, There is a need for the development of a fast and accurate method to estimate biomaterial-associated apoptosis. In this study, a apoptosis-detecting optical probe was developed, to assess the extent of implant-associated apoptosis using in vivo imaging.

Methods: Apoptosis-detecting optical probes were prepared by incubating eight-arm PEG-amine (M_w:10k) with an amine-reactive near infrared dye:Oyster-800 in PBS buffer. The eight-arm PEG-Oyster-800 was dialyzed and then lyophilized. The remaining amine groups of the eight-arm PEG-Oyster-800 were reacted with SM(PEG)2 to introduce maleimide group onto the PEG-Oyster-800. Apoptosis-affinity peptides (CQRPPR) were incubated with the above-prepared maleimide-PEG-Oyster-800. The apoptosis-detecting probe was purified with dialysis against DI water, lyophilized and stored at refrigerator for further use. Female Balb/c mice (20-25 gram) were used in this investigation. SiO_2 and polyethylene glycol (PEG) particles were used as implant materials. Whole body images of mice were performed using Carestream *In Vivo* FX Pro. Regions of interests were drawn over the implantation locations, and the mean fluorescence intensities for all pixels were calculated using Carestream Molecular Imaging Software. All results will be expressed as mean ± Standard error of the mean (SEM). One-way analysis of variance (ANOVA) and student t-test were performed to compare the difference between groups. A value of $p \leq 0.05$ was considered to be significant.

Results: An apoptosis-detecting optical probe was first prepared by conjugating a near infrared dye with a apoptosis-affinity peptide using PEG as a carrier since PEG is biocompatible an long time circulation in blood. In vitro tests demonstrate that the probes preferentially bind to apoptotic cells but not to live cells and there is a linear relationship between fluorescent intensity and number of apoptotic cell, indicating that this probe may be used to quantify the apoptotic cell. MTS assays reveal that the probe has no significant cytotoxicity to cells, suggesting that the above probes possess cell compatibility for further *in* vivo animal study. For in vivo studies, SiO_2 and PEG particles were used as model materials and implanted subcutaneously in mice. 7 days after implantation, the apoptosis-detecting probes were administered *i.v* via ocular route. The animals were subjected to whole body imaging one day after probe injection. It was found that different materials prompt various extents of probe accumulation in the order: SiO_2 > PEG (Fig.1, left panel), Quantitative analysis revealed that SiO_2 implant triggered almost 3 times higher fluorescent intensity than PEG implant (Fig.1, right panel). To validate the results, histological analyses also revealed that the accumulation of apoptotic cells follows the same trend. By correlating the results obtained, a linear relationship between biomaterial-associated probe accumulation and localized apoptotic cells was found. The results indicate that the apoptosis-detecting probe can be used to quantify implant-associated apoptotic cell accumulation at the implant site.

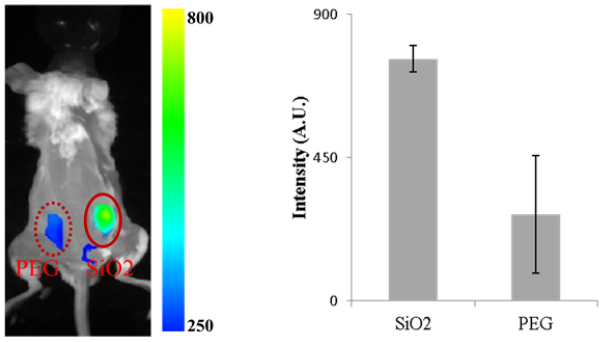

Fig.1 Representative in vivo fluorescence imaging (left panel) and quantitative analysis (right panel) of the fluorescence intensity. SiO_2 and PEG particles were implanted subcutaneously on the back of mice. 7 days after implantation, the probe was injected *i.v.* via ocular route. 24 hours after probe injection, imaging was captured.

Conclusions: Our results support that the apoptosis-detecting probe and *in vivo* fluorescence imaging technique may serve as a powerful tool to monitor and quantify the extent of implant-associated apoptosis.

References:

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[3] Anderson JM, Rodriguez A, Chang DT. Semin Immunol. 2008;20:86-100.