Real-Time, In Vivo Imaging of Biomaterial-Associated Inflammation

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Introduction: The inflammatory response to implanted biomedical devices severely limits the biological performance of various devices in millions of patients each year [1,2]. Whereas numerous anti-inflammatory therapeutics have been explored, the inability to determine when to initiate anti-inflammatory therapeutics and their precise dosing kinetics significantly limits therapeutic efficacy. Hence, developing methods to directly image the inflammatory response around the vicinity of an implant could greatly augment effective therapeutic approaches towards implanted biomaterials.

Reactive oxygen species (ROS) produced by invading macrophages and neutrophils in response to implanted biomaterials have been widely implicated to play a central role in the failure of these medical implants [3,4]. More importantly, the level of ROS is a reliable indicator of the severity of inflammation within the vicinity of an implant and is therefore an excellent diagnostic marker for detecting inflammation.

The hydrocyanines are a new family of fluorescent contrast agents derived from the cyanine family of dyes that possess ideal physical/chemical properties for imaging ROS in vivo [5]. These include excellent stability to auto-oxidation, tunable emission wavelengths, and nanomolar sensitivity to ROS. In this study, we demonstrate that ROS generated near the vicinity of a subcutaneous polyethylene terephthalate (PET) implant can be imaged using a fluorescent ROS sensor, hydro-ICG [5].

Methods: PET disks (8 mm dia.; 2 constructs/animal) were implanted subcutaneously on either side of the spine of a mouse. Briefly, 21 mice were randomly divided into 3 groups: (1) sham, an incision was made to create a subcutaneous pocket and nothing was implanted; (2) implant, a PET disk was implanted into the subcutaneous pocket; (3) implant + LPS, a PET disk was implanted and LPS (100 µl at 1 mg/ml) was added into the subcutaneous pocket.

Bioimaging was performed immediately following implantation (day 0) by injecting hydro-ICG (30 µl at 1 mg/ml) subcutaneously near the vicinity of the implant. Thirty minutes after the injection, the whole body of the animal was scanned in an IVIS® imaging system and the biofluorescence was integrated. At different days following implantation, biofluorescence was measured in anesthetized animals. Following euthanasia, the PET disks were carefully explanted with the surrounding tissues intact for histological, immunohistological and BioPlex-based cytokine analyses.

Results: Our bioimaging data showed significant differences in total fluorescence efficiency between the implant and sham groups on days 1 and 4 post-implantation and between implant + LPS and sham groups on days 1, 4 and 7 respectively **(Fig. 1)**.

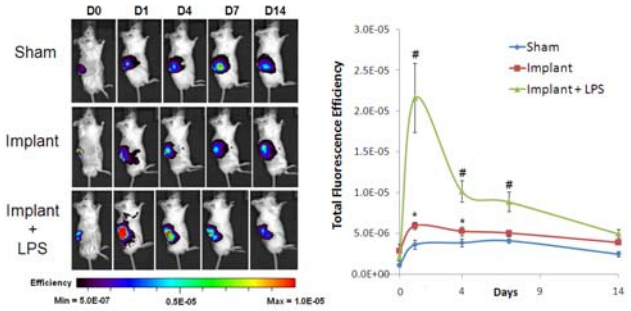

Fig. 1. ROS bioimaging data in living mice after hydro-ICG injection on day 0. Biofluorescence was tracked for a period of 2 weeks at 1, 4, 7, and 14 days post-implantation. Data is represented as mean ± SE of seven mice. *$p < 0.05$, compared between implant and sham groups and #$p < 0.05$, compared between implant + LPS and other two groups.

Histopathology staining on explants obtained 14 days post-implantation with hematoxylin and eosin showed a large number of infiltrating cells localized at the tissue-implant interface. Immunofluorescence staining for the macrophage marker, CD68, confirmed the presence of macrophages present on the implant surface and implant-associated tissues 14 days post-implantation. Cytokine analysis detected the presence of IL-1β and the monocyte chemotactic protein-1 (MCP-1) in the implant and implant + LPS groups **(Fig. 2)**.

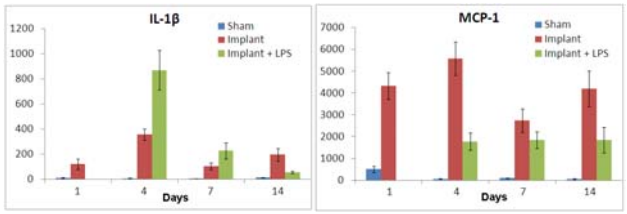

Fig. 2. Cytokine expression in sham, implant and implant + LPS groups. Protein concentration in each sample was normalized to 1 mg/ml. Data is represented as mean ± SE with n = 3 to 6 mice.

Conclusions: Our results demonstrate that ROS produced in response to a biomaterial-associated inflammation can be detected and imaged near the vicinity of an implant using a fluorescent ROS sensor. These studies will significantly enhance our understanding of host responses to implanted biomaterials and will subsequently lead to non-invasive therapeutic strategies to further improve implant biocompatibility.

References : [1] Anderson et al. *Semin Immunol.* 2008;20:86-100; [2] Schutte et al. *Biomaterials* 2009;30:160-168; [3] Lee et al. *J Biomed Mater Res* 2000; 49:25-35; [4] Hooper et al. *J Biomed Mater Res* 2000;50:365-74. [5] Kundu et al. *Angew Chem Int Ed Engl.* 2009;48:299-303.

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