Imaging of Biomaterial-Associated Inflammation Using Hydrocyanine Dyes

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Introduction: Implantation of biomaterials and medical devices elicits a dynamic inflammatory response that severely limits the integration and biological performance of various devices in millions of patients each year [1,2]. The inability to directly image inflammatory responses associated with implanted devices constitutes a major roadblock to the evaluation/diagnosis of device-associated inflammation as well as the development of effective therapies. Hence there is a great need for the development of minimally invasive approaches to image inflammation towards implanted biomaterials in vivo.

Reactive oxygen species (ROS) 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]. 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: Mice were randomly divided into 2 groups: (1) sham, an incision was made to create a subcutaneous pocket and nothing was implanted; (2) implant,PET disks (8 mm dia, 2 disks/animal) were implanted intosubcutaneous pockets on either side of the spine. Bioimaging was performed immediately following implantation (day 0), 1, 4, 7 and 14 days post-implantation 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. On day 14, animals were euthanized and the PET disks were carefully explanted with the surrounding tissues intact for histological and immunohistological analyses.

Results: Our bioimaging data showed significant differences in total fluorescence efficiency between the implant and sham groups on days 7 and 14 post-implantation **(Fig. 1)**.

Histological staining on explants with hematoxylin and eosin showed a large number of infiltrating cells localized at the tissue-implant interface. Distinct fibrous capsules were clearly visible by day 4 post-implantation **(Fig. 2)**. Importantly, capsule thickness correlated with fluorescence readouts,

Co-staining analysis for inflammatory cell markers and ROS activity demonstrated that macrophages and neutrophils were primarily responsible for the ROS activity associated with the implant **(Fig. 3)**.

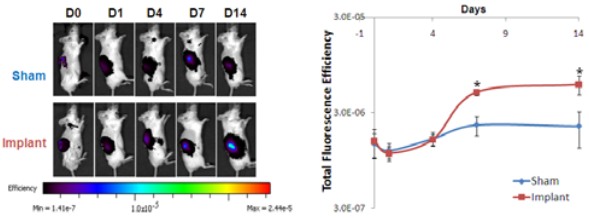

Fig. 1. ROS bioimaging in living mice after hydro-ICG injection on day 0. Biofluorescence was tracked for a period of 2 weeks at 1, 4, 7, and 14 days post-implantation. Data is represented as mean ± SE of seven mice. *p < 0.05, compared between implant and sham groups.

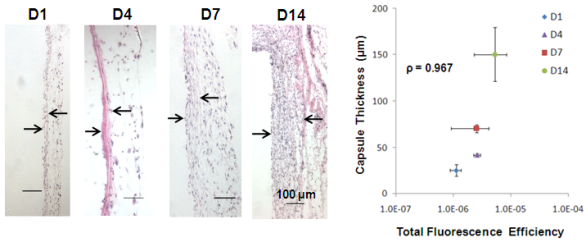

Fig. 2. ROS intensity correlates with fibrous capsule thickness around implant. (a) H&E staining for capsule (arrows) for different implantation times. (b) Strong correlation (ρ = 0.97) between capsule thickness and fluorescence efficiency (n = 3).

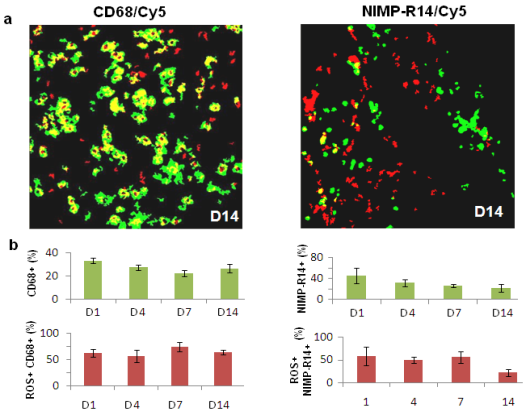

Fig. 3. (a) Immunostained sections of implants (day 14) showing co-localization of macrophages (CD68+) and neutrophils (NIMP-R14) with ROS (Cy5). (b) Quantification of macrophages and neutrophils at the tissue-implant interface. Data is represented as mean ± SE with n = 4.

Conclusions: Our results demonstrate that this imaging technology can be used to detect and monitor implant-associated inflammation and can be correlated to standard methods of implant analysis.

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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