Longitudinal Monitoring of Biomaterial-associated Inflammation and Bacterial Infection in a Minimally Invasive Fashion

Shalu Suri [a], Shivaram Selvam [a], Susan M. Lehman [a], Khalilah Reddie [b], Niren Murthy [c], Andrés J. García [a]
[a] Department of Mechanical Engineering, [b] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; [c] Department of Bioengineering, University of California, Berkeley, CA

Statement of Purpose: Implant-associated inflammation severely limits the functional performance of medical devices in vivo (1). Furthermore, device-associated bacterial infections, such as catheter-associated bloodstream and surgical site infections, result in substantial morbidity and mortality and increased healthcare costs. Current methods to detect device associated inflammation and infection rely on end point assays which are inherently invasive, destructive and time consuming. The inability to directly image inflammatory responses to implanted devices constitute a major roadblock to the evaluation/diagnosis of device associated inflammation and infection as well as the development of effective therapies. Reactive oxygen species (ROS) which comprise of oxygen ions, free radicals and peroxides, play a pivotal role in the inflammatory response towards implanted biomaterials. More importantly, the level of ROS could serve as an excellent marker of the severity of inflammation around the vicinity of an implanted device (2). Nitric oxide (NO), a short lived free radical, is naturally produced by macrophages and neutrophils to kill invading bacterial pathogens. Particularly, NO-releasing polymers have shown great promise as anti-bacterial coatings for medical implant applications (3). We therefore hypothesize that by using ROS and NO specific fluorescence probes, we can selectively detect ROS associated with aseptic inflammation and NO released in response to bacterial infections.

Methods: H-Cy5, a ROS sensor, and DAC-S, a NO sensor, were prepared as described previously (4,5). Biofilm PET disks or sterile PET disks were implanted subcutaneously following IACUC-approved procedures in 6-8 wk old male BALB/c mice. Mice undergoing the same surgical procedure but receiving no biomaterial implants were used as sham controls to account for surgery-associated inflammation.

For bioimaging, 0.3 mg/ml of DAC-S and 1 mg/ml of extracellular H-Cy5 were dissolved in 1 ml of phosphate-buffered saline. Fifty microliters of this dye mixture was injected near the vicinity of an implant. ROS and NO bioimaging was performed 30 min after dye injections on 1, 4, 7 and 14 days post-surgery using an IVIS Lumina® bioimaging system (Xenogen). Biofluorescence was then integrated using Living Image® software.

Results: We evaluated both biomaterial-associated inflammation and infection in response to implanted aseptic and biofilm PET disks. As shown in figure 1, mice from both aseptic and biofilm groups exhibited increases in fluorescence intensities for NO sensor form day 4. Significant differences in fluorescence intensities were observed between aseptic and biofilm implant groups at day 14 ($p<0.05$) post-implantation. For H-Cy5 sensor, no significant difference in the fluorescence intensities was observed between the aseptic and biofilm implant groups at all time-points (Figure 1).

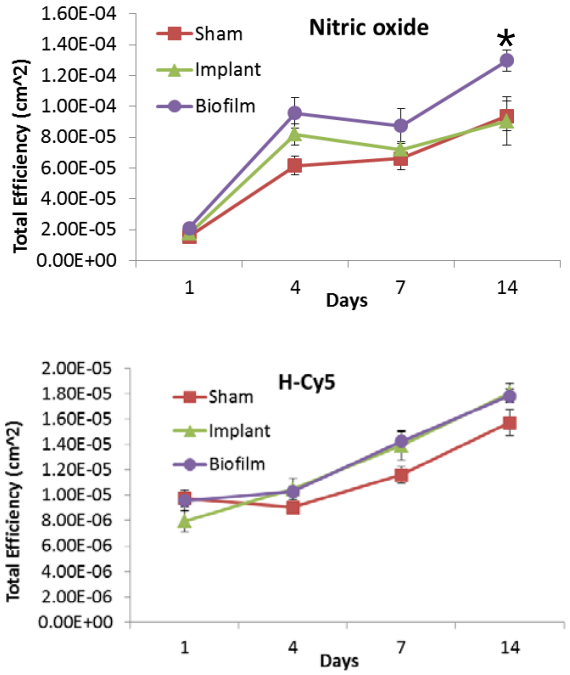

Figure 1: Quantification of NO (top) and H-Cy5 (bottom) fluorescence data from mice receiving dye injections at all time-points in sham, aseptic, and biofilm implant groups (* represents $p<0.05$ between implant and biofilm).

Conclusions: Our results demonstrate that ROS is released as a result of implant-associated inflammation in both aseptic and biofilm implant groups whereas higher levels of NO observed near the vicinity of biofilm implants indicate that NO is produced in response to bacterial infections enabling us to selectively image infection. Taken together, these studies show that NO could serve as a surrogate marker to discriminate between device-associated bacterial infection and aseptic inflammation. This method also enables us to perform quantitative, longitudinal monitoring of inflammation and infection selectively in a minimally invasive fashion overcoming the limitations associated with current methods.

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References:

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