Noninvasive monitoring of implant-mediated inflammatory responses by detecting reactive oxygen species in vivo

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Statement of Purpose: Biomaterial implants often trigger various extents of inflammatory responses, accompanied by the accumulation of neutrophils and macrophages. To determine their tissue compatibility, biomaterials are typically implanted in animals; implants and surrounding tissue are then recovered for histological evaluation which is time consuming and can only provide semi-quantitative assessment of immune responses to the biomaterial. Recent studies have found that luminol can be used to assess the release of reactive oxygen species (ROS) and the extent of neutrophil activation during inflammatory diseases.[1] Separate studies have shown that L-012, a new chemiluminescence agent, is much more sensitive than luminol.[2] Since it has been shown that foreign body reactions prompt neutrophil activation to release ROS, we hypothesize that in vivo measurements of ROS can provide an accurate assessment of neutrophil activation and biomaterial biocompatibility.

Methods: To test this hypothesis, both L-012 and luminol were used as chemiluminescence agents. To assess the sensitivity of chemiluminescence agents on ROS measurement, we used activated mice neutrophils isolated from the peritoneal cavities of casein-treated mice. After incubation with different numbers of neutrophils in the presence of L012 (2mM) or luminol (4mM) for 4 min at room temperature, the ROS measurements were initiated by adding 10µL of PMA (6.5nM). Chemiluminescence intensities were recorded continuously for 60 min using Luminescence Reader (Infinite M200, Tecan, Männedorf, Switzerland). To measure the extent of ROS production in vivo, a series of microspheres were fabricated and used in the in vivo studies. These microspheres were made of PLA (D:5-10µm), PEG (D:100nm) and PNIPAM-NH_2 (D:100nm). Microspheres (10% w/v, 50µl/injection) were implanted subcutaneously on the back of Balb/C mice. At the different time points, a 100 µl of L-012 (15mg/ml) was injected intraperitoneally and then chemiluminescence imaging was taken using Kodak In Vivo Imaging system (Carestream Health, Inc., New York). At the end of the studies, the microspheres and surrounding tissues were recovered for histological analyses.

Results: In vitro assay demonstrates that the L-012 probe exhibits high sensitivity towards the ROS in solution. When exposed to hydrogen peroxide in the presence of 5 µm Fe^{2+}, L-012 is found to emit strong chemiluminescent light and chemiluminescent intensities are H_2O_2 concentration dependent. Further study was carried out to determine whether L-012 can detect ROS generated by activated neutrophils. Indeed, neutrophils exhibited a time-dependent increase of chemiluminescence which peaks at ~30 minutes. In addition, there is a linear relationship between L-012 chemiluminescence intensity and cell numbers (Figure 1A). We also find that the ROS sensitivity of L-012 is roughly ~500 fold better than that of luminal when presented to activate neutrophils (Figure 1B). L-012 was subsequently used to detect the extent of biomaterial-mediated neutrophil activation and ROS release. As shown in Figure 1C, we find that the tissue surrounding PNIPAM-NH_2 implants emitted the strongest chemiluminescent light while PEG implants produced the least light. By measuring chemiluminescent light at different time points, we find that the strongest chemiluminescence signals are detected at day 2 and decreased substantially after that. At day 7, chemiluminescence signals are about ~10% of plateau.

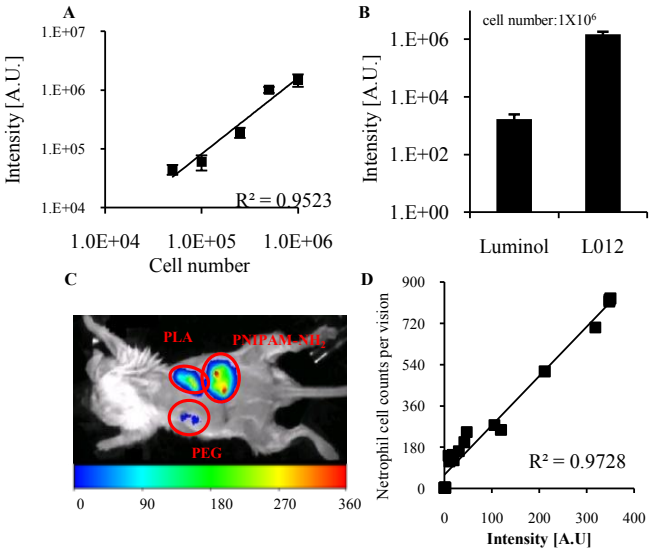

Figure 1

Histological analyses confirm the observed in vivo chemiluminescent measurements. As expected, we find that PNIPAM-NH_2 implants are surrounded with the largest numbers of neutrophils, identified by IHC staining. Substantially less numbers of neutrophils are found to associate with PLA microspheres and very small numbers of neutrophils were recruited to the PEG implants. Finally, we find that there is a good linear correlation between in vivo L012 chemiluminescence and histological neutrophil numbers (Figure 1D).

Conclusions: The results from this study support that L-012 can be used to quantify neutrophils by detecting production of ROS in vitro and in vivo. This novel method provides a means to continuously assess the extent of implant-mediated inflammation responses and foreign body reactions in vivo.

References:

1. Gross,S. Nat. Med. 2009;15:455-461.
2. Imada,I. Anal. Biochem. 1999;271:53-58.