Bioluminescent Imaging of Biomaterial-induced Reactive Oxygen Species

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Statement of Purpose: The foreign body response to implanted materials is a major challenge in medical device design. This response consists of a cascade of events initiated by implantation, followed by protein adsorption, adhesion and activation of immune cells, and finally the recruitment of fibroblasts and formation of a fibrous capsule[1]. It is thought that reactive oxygen species (ROS) are released by activated phagocytes during this process, and contribute to the oxidative degradation of many biomaterials[2]. While ROS generation has been used to characterize inflammatory cell response to biomaterials in numerous in vitro studies[3,4], the detection of biomaterial-induced ROS in vivo has yet to be demonstrated. Here we develop a bioluminescent imaging approach to examine ROS generated in response to implanted materials within live animals using luminol, a non-toxic probe that luminesces in the presence of ROS, and has recently been used to detect ROS in mice following lipopolysaccharide injection[5]. We compare the response of two materials, polystyrene and alginate, which are known to invoke different immune responses. Finally, we used luminol to detect differences in phagocyte activation in response to materials in vitro.

Methods: Alginate and polystyrene beads (50% slurries) were injected using an 18 gauge needle in an array format into the subcutaneous region on the dorsal side of isofluorane anesthetized SKH1 mice (Charles River, See Fig 1a). After 24 hours, 5 mg of luminol (Sigma-Aldrich) was injected into the intraperitoneal space, and animals were then imaged at 20 minutes after luminol injection using an IVIS spectrum (Caliper Lifesciences) with a 3 minute exposure. For in vitro studies, thioglycollate (Sigma-Aldrich) was injected in the intraperitoneal space of C57BL/6J mice (Jackson Labs), animals were sacrificed after 24h, and peritoneal cells were isolated by lavage. Cells were seeded in DMEM supplemented with 10% bovine serum on tissue culture surfaces either uncoated or coated with alginate, and then treated with phorbol myristate acetate (Sigma-Aldrich) for 10 minutes. Luminol was added and wells were imaged after 20 minutes with a 60 second exposure. All images were analyzed using LivingImage 3.0 Software.

Results: We examined luminol bioluminescence caused by polystyrene and alginate, which are materials that are known to cause high and low levels of phagocytic cell recruitment, respectively. Materials were injected into the subcutaneous region of SKH1 mice, which are hairless and immunocompetent. High levels of luminescence were observed at regions near polystyrene implants, but not near alginate implants or at control, PBS injections

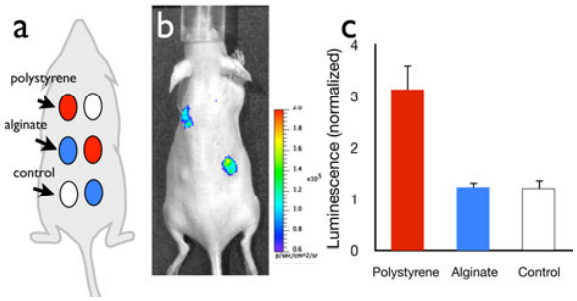

Figure 1. Detection of ROS in live mice with luminol. (a) Schematic of implants, (b) luminol bioluminescence image at 1 day after implantation, and (c) quantification of luminescence.

(Figure 1b). Quantification of total flux revealed that the intensity of luminescence near polystyrene implants was approximately three times higher than the levels near alginate or control injections (Figure 1c). These trends correlated with in vitro experiments, in which peritoneal phagocytes were seeded on polystyrene and alginate surfaces. Here luminol was added to phagocytes that were pre-activated with phorbol myristate acetate. Again, the levels of ROS generated by phagocytes in response to polystyrene was approximately three times higher than the levels produced in response to alginate (Figure 2, a and b).

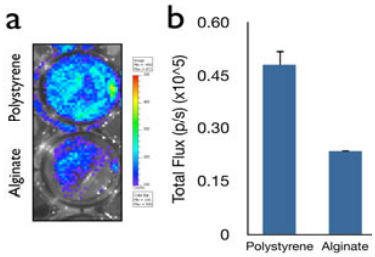

Figure 2. ROS generation by phagocytes in vitro. (a) Luminescent image of phagocytes seeded on polystyrene or alginate, and (b) quantification of luminol bioluminescence.

Conclusions: In this study, we provide evidence that ROS is produced in response to materials within live animals. Using luminol, we detected ROS generation localized to sites of polystyrene, but not alginate, implants. Although only two materials were tested here, the same techniques can be used to test the biocompatibility of many new materials in parallel. A better understanding about the role of ROS in biomaterial-induced inflammation may ultimately aid in the design of novel materials to prevent their formation, and thus reduce the foreign body response.

References: [1]Anderson, JM. Semin Immunol 2008;20(2):86–100. [2]Sutherland, K. J Clin Invest 1993;92(5):2360–7. [3]Ginis, I. Blood 1990;76(6):1233–9. [4]Kaplan, SS. J Biomed Mater Res 1992;26(8):1039–51. [5]Gross, S. Nat Med 2009;15(4):455–61.