Development of novel imaging probes for the detection of polarized macrophage subsets during foreign body reactions

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Introduction: It is well known that macrophages (MΦ) are pivotal cells in the foreign body response, contributing to both the normal healing of tissues and the pathogenesis of implant failure. Recently, this contrasting role has been attributed to the plasticity of MΦ functional phenotypes, existing in a spectrum from M1 to M2 cells. Despite of the improvements in understanding inflammatory responses, the interaction and mutual influence between polarized MΦs and inflammatory/ fibrotic responses are mostly unclear, partially due to the lack of method to monitor the polarity of MΦs in real time. Here, we discuss the development of novel *in vivo* imaging probes, targeted to M1/M2 cells, which may be used in conjunction to determine the level of response. These probes have great potential to monitor the dynamic process of MΦ-mediated responses to biomaterial implants.

Methods: Recent studies show that activated M1 MΦ, through lipopolysaccharide (LPS), express high levels of folic acid receptors, which have a high affinity for folate (FA) (1). On the other hand, M2 MΦ show an up-regulation of mannose (MN) receptor when activated by IL-4 and IL-13 (2). To image activated M1 or M2 MΦ, folate- and mannose-conjugated near-infrared (NIR) probes were fabricated by covalently linking peptide ligands with NIR dye-labeling 8-armed PEG core, as described previously (1). For simultaneous imaging FA was linked to IR830 dye and MN linked to IR680 dye. The ability of these probes to image activated MΦ was investigated both *in vitro* and *in vivo*. To determine probe efficiency MΦ M1 and M2 cells were isolated from murine bone marrow and separately differentiated by stimulation with LPS (M1) or IL-4, IL-13 (M2) as previously described (3). The toxicity and efficiency of the imaging probes to detect either M1 or M2 cells was then determined. *In vivo* imaging was then investigated using Balb/c mice and subcutaneous implantation of either pro-inflammatory poly(lactic acid) (PLA) or biocompatible N-isopropylacrylamide (NIPA) particles to determine the relative degree of M1 and M2 responses.

Results: Using Fourier transform infrared analysis (FTIR), we confirmed the conjugation of FA and MN moieties into PEG. Characteristic IR absorption peaks were observed for FA (1600 and 1680cm^{-1}), MN (1390 and 640cm^{-1}), and PEG (1100 and 950cm^{-1}), for each probe respectively (Figure 1 A,B). In addition both probes were determined to have minimal to no toxicity up to 62.5 µg/ml in an *in vitro* test against 3T3 fibrobalsts (Figure 1 C). Incubation of probes with either M1 or M2 MΦ cells shows good cellular specificity (Figure 1 D,E). Probes were incorporated with differentiated MΦ at various densities and found to bind to M1 (FA, but not MN) and M2 (MN, but not FA) in a near linear fashion. MΦ lineage was confirmed though immunohistochemical staining with CD80 (M1) and CD206 (M2) antibodies prior to *in vitro* studies. Preliminary *in vivo* results similarly reveal that the M1 and M2 probes may be used to specifically target MΦ around a biomaterial implant and potentially determine the relative degree of the MΦ response. As expected we observe a higher M2 response to NIPA particles and a higher M1 response to pro-inflammatory PLA particles (Figure 1 F).

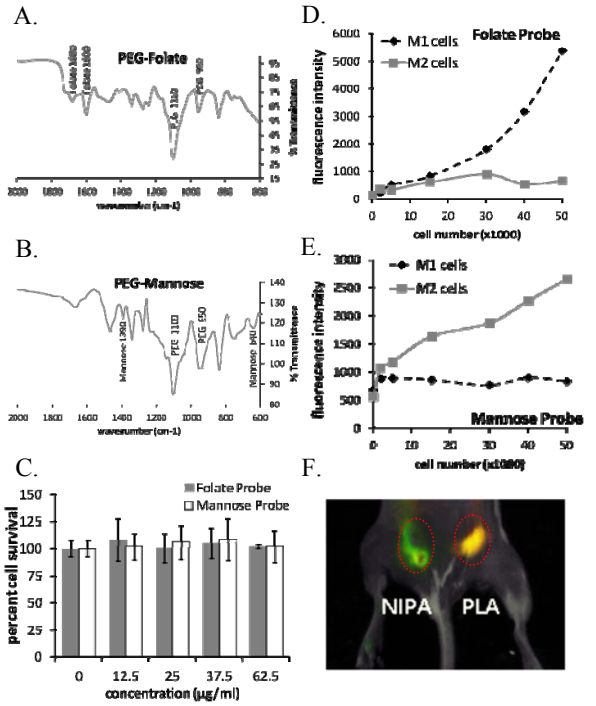

Figure 1. (A,B) FTIR spectral characterization of PEG imaging probes. (C) Toxicity results to 3T3 fibroblasts. (D,E) *In vitro* characterization of probe specificity for M1 or M2 cells. (F) Overlay of *in vivo* imaging of M1 (red) and M2 (green) probes to NIPA and PLA implants (yellow color is the overlay of red and green showing both M1 and M2 cells).

Conclusions: These results demonstrate that NIR imaging probes can be fabricated to monitor polarity changes between M1 and M2 cells. These probes may be used to assess the dynamics of polarization around a biomaterial implant in real-time. Improved knowledge of this process may lead to novel strategies to screen inflammatory properties and improve regeneration capabilities of biomaterials by eliciting a preferential MΦ response.

References:

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