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Dr. Nandita Singh
Senior Science Editor: JoVE

Dear Dr. Singh,

Please find attached the manuscript we are submitting as an invited methods article to the Journal of Visualized Experiments (JoVE), entitled "A macrophage reporter cell assay to examine Toll-like receptor-mediated NF-kB/AP-1 signaling on adsorbed protein layers on polymeric surfaces". The attached manuscript was authored by Laura A. McKiel, Kimberly A Woodhouse and Lindsay E. Fitzpatrick (corresponding author) and is a methods paper that describes the use of a macrophage report cell line to investigate the contribution of damage-associated molecular patterns and Toll-like receptors on various polymer surfaces. The many of methods have previously been described in an original research article "McKiel LA & Fitzpatrick LE. Toll-like Receptor 2-Dependent NF-κB/AP-1 Activation by Damage-Associated Molecular Patterns Adsorbed on Polymeric Surfaces. *ACS Biomater. Sci. Eng.* 4, 3792–3801 (2018)." All authors have seen and approved the submission of this manuscript.

The performance of implanted biomedical devices is heavily dependent on the host response that occurs at the biomaterial-tissue interface. However, the molecular mechanisms that determine macrophage activation and inflammatory responses to biomaterials are not fully understood. Toll-like receptors (TLR) play a critical role in host defense by initiating sterile inflammatory responses to damage-associated molecular patterns (DAMPs), released by damaged and stressed tissues. While the role of TLR in sterile inflammatory host responses to solubilized polymer molecules, nanoparticles and phagocytosable microparticles is relatively well established, the current literature on the role of TLR signaling in macrophage responses to solid, non-phagocytosable biomaterials is limited. Therefore, we chose to examine TLR signaling in macrophages using an *in vitro* model for generating DAMP-containing adsorbed protein layers. This manuscript provides detailed methods on generating poly(methyl methacrylate), poly(dimethylsiloxane) and fluorinated poly(tetrafluoroethylene) surfaces, generation of 3T3 fibroblast lysate as an in vitro model of a cell-derived, complex DAMP-containing protein mixture, and cell culture protocols for indirectly measuring NF-κB/AP-1 activity in macrophages cultured on protein adsorbed surfaces using a colourimetric alkaline phosphatase activity assay. Representative results are provided as well. We believe the focus and scope of this manuscript is well suited for publication in JoVE.

We look forward to receiving your feedback about the suitability of this manuscript for publication. Should you require further information, please do not hesitate to contact me directly.

Yours sincerely,

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