Using Light Sheet Fluorescence Microscopy to Contextualize the Immune Responses to Biomaterials in Trauma Microenvironment

Tran B. Ngo[a], Sabrina Destefano[a], Yijun Su[b], Harshad D. Vishwasrao[c], Jiamin Liu[c], Hari Shroff[b, c], Kaitlyn Sadtler[a]

[a]Section on Immuno-Engineering, National Institutes of Health, Bethesda MD, United States; [b]Laboratory of High-Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, United States; [c]Advanced Imaging and Microscopy Resource, National Institutes of Health, Bethesda MD, United States

Statement of Purpose: The immune system plays a delicate role in response to biomaterial implants, balancing between tissue repair and fibrosis mechanisms. Immune responses diverge in response to different biomaterial classes[1]. Understanding more about the immune-biomaterial response will pave the way towards need- and tissue-specific immunomodulatory biomaterial products. Flow cytometry and traditional histology have been used to study immune cell populations responding to biomaterials, but these methods cannot represent fully the direct interactions between immune cells and the biomaterial implant. Therefore, it is necessary to develop a method to fully visualize intact structures and cells within the tissue to holistically understand the dynamic immune response to biomaterials in an injury. This study utilizes a simple and inexpensive clearing method, iDISCO[2], that permits volume imaging of large samples to visualize the immune response to scaffolds for tissue repair in mouse muscle tissue using cleared tissue dual-view light sheet microscopy[3,4] (ct-diSPIM).

Materials and Method: This study utilized the mouse volumetric muscle loss (VML) model to evaluate the immune response to a biologic (extracellular matrix) or synthetic (polylactic acid) materials implanted in muscle trauma injury. Implantation and tissues were collected after 3 weeks, fixed in 4% paraformaldehyde overnight, washed in 1x PBS/0.01% Triton-X, and bleached in chilled fresh 5% H_2O_2 for 5 hours at 4°C. Tissue samples were blocked before immunostaining with conjugated antibodies – Ly6G (neutrophils), CD11b (myeloid), CD3 (T cells) – at 1:100 dilution (BioLegend) for 2 days. Then, samples were dehydrated and cleared in tetrahydrofuran (THF) and H_2O series: 3 hours each at 20%, 40%, 60%, 80%, 100% and again in 100% tetrahydrofuran overnight. Then, equilibrating in DiBenzyl Ether (DBE), samples were imaged in the microscope chamber filled with DBE.

Results and Discussion: Tissue and ECM-implanted material were clearly imaged using the ct-diSPIM (Figure 1). Both muscle and fat tissue are visible via autofluorescence signal (magenta channel). The implanted ECM-biomaterial was showed to be integrated in the muscle wound bed and covered by an adipose tissue layer that infiltrated into the wound sites. Specific immunolabeling signal indicates an infiltration of both innate and adaptive immune response at the implantation sites: Ly6G for neutrophils, CD11b for myeloid linage cells, and CD3 for T cells. With this imaging method, the morphology of the foreign body response (FBR) to PLA in 3D context, including the fibrotic capsule, wear particles, and implant can be visualized (Figure 2).

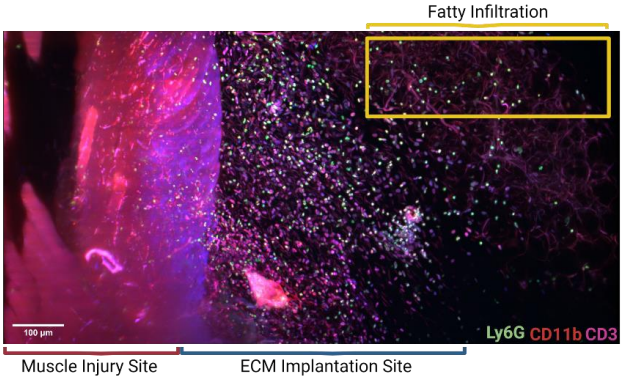

Figure 1. Composite image scan at the muscle injury and implant sites. Immunolabeling signals (green, red) indicate immune cells while autofluorescence signal (magenta) shows tissue structure.

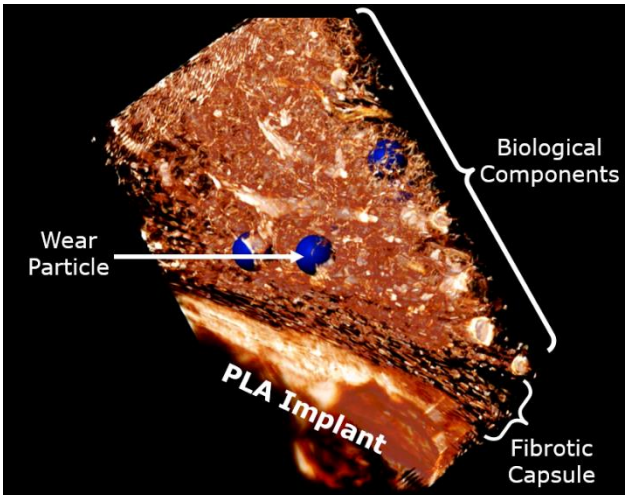

Figure 2. Morphological depiction of the FBR to material implants in 3D-context

Conclusions: This study showcases a method to capture the immune response to biological and synthetic biomaterial scaffolds in three-dimensional context. We adapted a simple, robust, and inexpensive iDISCO protocol to clear quadricep skeletal muscle tissue to clear large and thick tissue specimens. This chemical clearing process also allows immunolabeling and fluorescence-imaging with light sheet microscopy. Additionally, tissue autofluorescence can be used for morphological contextualization of the foreign body response. With this method, specific immune mechanisms can be further characterized, visualized, and compared in various materials to promote healing in damaged tissue.

References:

[1]Sadtler K. *et al.* Biomaterials. 2019; **192**: 405–415
[2]Renier N. *et al.* Cell. 2014; **159:** 896–910
[3]Guo M. *et al.* Nat. Biotechnol. 2020; **38:** 1337–1346
[4]Wu Y. *et al.* Nat. Biotechnol. 2013; **31**: 1032–1038