

ABSTRACT

Complex wounds of all types, ranging from infection and diabetes to cancer and burns, pose a substantial worldwide healthcare challenge. While wounds and the corresponding wound healing response can vary widely in mechanism, one common feature of all wound types is the importance of the cell in its etiology and treatment. Despite this known importance, it is largely still conceptual in any clinical application due to the lack of demonstrated methods for characterizing clinically relevant wound models at the cellular scale. Fluorescence imaging has emerged as a very promising tool for such studies largely due to its multi-parametric intrinsic sensitivity for known components of wound healing and unparalleled resolution of both temporal and spatial scales. The goal of this work is to leverage fluorescence and intrinsic optical signals from cellular components and the associated extracellular matrix to assist in the pathological understanding of the wound response in three representative yet distinct wound models of great clinical interest.

Three types of wound models are represented in this work, each of which has clinical challenges to either diagnosing or treating the wound through clinical or surgical intervention. They were chosen for being representative, distinct and yet complementary problems where an accessible research model with validated clinical relevance exists. These three wound types include cancer, where tumors burden and infiltrate healthy tissue; neurodegenerative wounds where, similarly, aggregates of misfolded proteins result in a loss of function; and thermal wounds, where the dependence on severity possesses an indeterminate healing window and affects the degree of clinical intervention. Specifically, glioblastoma multiforme is the most malignant primary brain cancer, where the presence of glioblastoma stem cells resistant to therapy results in tumor repopulation. Techniques in advanced fluorescence microscopy offer the ability to visualize the specific metabolic plasticity of different human phenotypes of these cells that correlates with survival in a mouse model. Alzheimer's disease and Parkinson's disease also affect normal brain metabolism and the presence of plaques and toxic aggregates alters elements of the brain microenvironment. These imaging techniques can visualize metabolic changes associated with Alzheimer's and Parkinson's and coupled with complementary optical techniques like second harmonic generation imaging (SHG) and polarized light microscopy, any associated remodeling of collagen in the extracellular matrix can be assessed label-free and nondestructively with cell-level resolution. Finally, we aim to further understand the role of collagen in human burns and assess the potential for clinical visualization of damaged collagen by validating the specific labeling using a novel fluorescent-labeled collagen-mimetic peptide with two-photon fluorescence and SHG microscopy. Specific insights to the wound response in each of the models is accomplished through the various techniques in fluorescence microscopy outlined in this work. Fluorescence imaging offers the ability to bridge biology and pathology and potentially inform or enhance clinical intervention.