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Characterization of metabolic changes associated with the functional development of 3D engineered tissues by non-invasive, dynamic measurement of individual cell redox ratios

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ABSTRACT

Non-invasive approaches to assess tissue function could improve significantly current methods to diagnose diseases and optimize engineered tissues. In this study, we describe a two-photon excited fluorescence microscopy approach that relies entirely on endogenous fluorophores to dynamically quantify functional metabolic readouts from individual cells within three-dimensional engineered tissues undergoing adipogenic differentiation over six months. Specifically, we employ an automated approach to analyze 3D image volumes and extract a redox ratio of metabolic cofactors. We identify a decrease in redox ratio over the first two months of culture that is associated with stem cell differentiation and lipogenesis. In addition, we demonstrate that the presence of endothelial cells facilitate greater cell numbers deeper within the engineered tissues. Since traditional assessments of engineered tissue structure and function are destructive and logistically intensive, this non-destructive, label-free approach offers a potentially powerful high-content characterization tool for optimizing tissue engineering protocols and assessing engineered tissue implants.

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1. Introduction

Tissue regeneration is often required after disease or trauma. To facilitate regeneration and restore tissue function, tissue grafts have traditionally been implanted, but often with suboptimal outcomes [1]. Tissue engineering offers the potential to improve repair and regeneration [2,3]. Engineered tissues are typically developed by seeding stem cells on a biomaterial scaffold and promoting proliferation and/or differentiation over the course of weeks or months. To assess tissue development and optimize culture protocols, mass spectrometry (MS), western blots, quantitative polymerase chain reaction (qPCR) techniques, immunohistochemistry (IHC), and electron microscopy have typically been employed to assess the biochemical and structural characteristics of the tissue at specific time points [4-7]. However, the destructive nature of these methods does not allow for dynamic assessments of tissue development over time. Furthermore, such techniques cannot be used to evaluate the integration of engineered tissue with native tissue following implantation. As a result, there is a need for quantitative, repeatable, non-invasive or minimally invasive methods to assess tissue biochemical status and structural integrity within threedimensional (3D) tissues.

Two-photon excited fluorescence (TPEF) microscopy offers a number of advantages over traditional approaches for imaging 3D tissues. In TPEF microscopy, molecules are brought to an excited state by the simultaneous absorption of two photons. Since each of these two photons require half the energy to excite a molecule compared to a single photon, near infrared (NIR) light can be used to excite molecules and produce emission in the visible range [8]. NIR light can penetrate deeper into tissue and the potential for outof-focus photodamage decreases substantially compared to confocal microscopy [8,9]. Furthermore, for practical purposes, two-photon absorption is limited to the focal plane, which provides intrinsic depth sectioning without the need for confocal detection [8]. Using NIR excitation, endogenous fluorescence can be produced most efficiently from cell mitochondria by nicotinamide and flavin adenine dinucleotides (NADH and FAD, respectively) [10,11]. In addition to measures of structural organization, NADH and FAD fluorescence can be used as a sensitive measure of the metabolic status of cells [12,13]. NADH and FAD are cofactors that are directly involved in cellular respiration. They exist in either



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