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The integration of 3-D cell printing and mesoscopic fluorescence molecular tomography of vascular constructs within thick hydrogel scaffolds

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ABSTRACT

Developing methods that provide adequate vascular perfusion is an important step toward engineering large functional tissues. Meanwhile, an imaging modality to assess the three-dimensional (3-D) structures and functions of the vascular channels is lacking for thick matrices (>2∼3 mm). Herein, we report on an original approach to construct and image 3-D dynamically perfused vascular structures in thick hydrogel scaffolds. In this work, we integrated a robotic 3-D cell printing technology with a mesoscopic fluorescence molecular tomography imaging system, and demonstrated the capability of the platform to construct perfused collagen scaffolds with endothelial lining and to image both the fluid flow and fluorescent-labeled living endothelial cells at high-frame rates, with high sensitivity and accuracy. These results establish the potential of integrating both 3-D cell printing and fluorescence mesoscopic imaging for functional and molecular studies in complex tissue-engineered tissues.

1. Introduction

Despite tremendous progresses in the field of tissue engineering [1], there are still significant challenges in creating thick tissue constructs. Especially, there is a critical need to develop methods that provide adequate vascular perfusion for engineering large functional tissues [2–4]. Thus, creating a dynamically perfused vasculature within 3-D matrix represents an important step toward this goal, and this realistic physiologically relevant system allows us to study the growth and maturation process of the tissue-engineered thick construct.

However, an imaging modality to assess the 3-D structures and functions of the vascular channels is lacking for thicker matrices (>2∼3 mm) [5]. Confocal and two-photon microscopes are useful tools for this research, but they can only image tissues with a depth of less than a few hundred microns. The configuration of 3-D construct in the perfusion chamber makes it even more challenging to access the sample for imaging. Another difficulty arises from the scaffold materials, which can be translucent or opaque depending on the matrix biomaterial and its density, and can become non-transparent as more matrices are produced with tissue growth. This results in optically high-scattering thick tissue samples. Typically, investigations of engineered tissues mostly rely on histological sections. However, time-consuming procedures and sample fixations make this approach not suitable for high-throughput applications and/or obtaining dynamic information of living samples [5]. Thus, it is urgent to develop imaging techniques that can image well beyond the penetration limits of conventional microscopy (several hundred microns) to evaluate in real-time the maturation process and functions of thick tissues up to a few millimeters.

To fulfill this unmet imaging need, we have developed a fluorescence mesoscopic imaging technique based on laminar optical tomography (LOT) [6] principles. LOT is a non-contact laser scanning imaging technique, which harnesses scattered light to probe both absorbing and fluorescent contrast in living tissues. LOT is able to obtain depth-resolved 3-D quantitative images to depths of several millimeters with high sensitivity. Similar to fluorescence molecular tomography (FMT) [7], LOT achieves sensitivity to depth-resolved absorption and fluorescence by exploiting scattering photons emerging from the tissue. In FMT, due to larger source-detector (S-D) separation, more uncertainty from scattering light propagation though the tissue limits the resolution to macroscopic scale, which makes it difficult to visualize small-diameter engineered vasculature. LOT is able to achieve higher resolution than FMT by shortening S-D separation, thus enabling quantitative and

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