Chronic Label-free Volumetric Photoacoustic Microscopy of Melanoma Cells in Three-Dimensionally Porous Scaffolds

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Statement of Purpose: Despite dramatic achievements in tissue engineering, visualizing live cells inside scaffolds is still challenging. Microscopic imaging systems capable of providing volumetric information of cells are quite rare. Scanning electron microscopy, fluorescence optical microscopy, micro-computed tomography, optical coherence tomography and magnetic resonance imaging all have their limitations, including sample deformation, light scattering, ionizing irradiation, low spatial resolution, long image acquisition time, and among others. The emerging photoacoustic microscopy (PAM) detects acoustic waves generated from the objects that absorb pulsed or intensity-modulated laser irradiation [1, 2], and has been used extensively in imaging vasculatures *in vivo*. However, there has been no attempt of using PAM for tissue engineering. In the present study, we used melanoma cells which contain natural dark pigments as a model cell line, and demonstrated PAM of the spatial distribution, temporal proliferation, and quantification of cells in three-dimensional (3D) poly(D, L-lactide-*co*-glycolide) (PLGA) inverse opal scaffolds.

Methods: PLGA inverse opal scaffolds were fabricated using a microparticle templating method.[3] B16.F10 melanoma cells were seeded into the scaffolds and cultured in phenol red free medium to reduce possible interference. Scaffolds were removed from the culture medium, placed in a PDMS mold containing warm PBS (37 °C) with 1% P/S, and imaged with PAM. The PAM was equipped with a dye laser pumped by a Nd:YLF laser (7-ns laser pulses). A focused ultrasonic transducer with 50 MHz central frequency achieved 45 μm lateral resolution, 15 μm axial resolution, and more than 3 mm penetration depth. Photoacoustic (PA) data were collected and processed by user-defined LabView and MATLAB programs. Cell proliferation was also conducted in parallel using a traditional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: Our inverse opal scaffolds had a uniform and well-arranged pore structure (not shown), which could provide good interconnections throughout the whole scaffolds to facilitate cell migration and nutrient/waste transport. The PA coronal and sagittal maximum amplitude projection (MAP) images in Figure 1A clearly show the cells growing in a scaffold, with the capability of penetrating the whole cell/scaffold construct with a thickness of around 1.2 mm. Figure 1B shows a 3D depiction of the melanoma cells in the scaffold. Individual cells or cell clusters could be identified (black dots or patches) in both the 2D and 3D images. Interestingly, PAM system could still provide acceptable resolution at such a deep penetration depth. Melanoma cells were seeded into inverse opal scaffolds using a spinner flask. At days 1, 3, 7, and 14 post-seeding, the scaffolds with cells were taken out from the media and immersed in warm PBS at 37 °C for PAM imaging. Time-course PA coronal MAP images of the entire scaffold clearly show the growth of melanoma cells inside the scaffolds (Fig. 1C). We further utilized the collected PA volumetric data to quantify the cell numbers, which would be a critical feature of PAM. The cell numbers calculated from the PA volumetric data were plotted against different time points (Fig. 1D). From the calibration curve, the average cell numbers at days 1, 3, 7, and 14 were calculated to be 4.9×10^4, 2.8×10^5, 3.4×10^5, and 2.7×10^5 per scaffold, respectively. Interestingly, the overall profile obtained from a parallel MTT assay had a trend similar to that from PAM: the average numbers of cells in the scaffolds for the MTT group at days 1, 3, 7, and 14 were calculated to be 7.1×10^4, 2.2×10^5, 3.3×10^5, and 2.6×10^5, respectively. The viability of melanoma cells remained high (>70%) after continuous PAM monitor for 2 weeks.

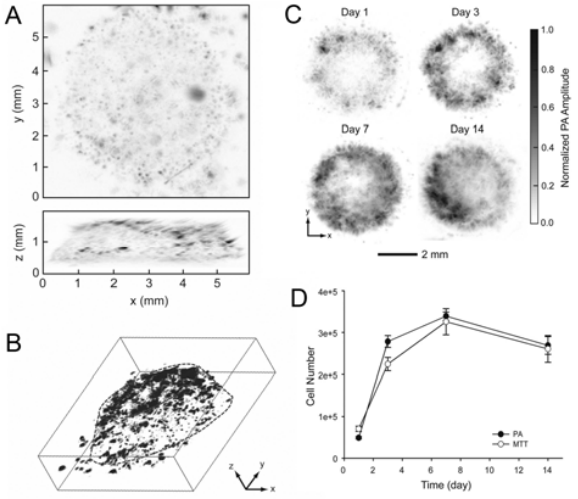

Figure 1. A) PAM MAP images from the top and side of a scaffold with melanoma cells. B) A 3D reconstructed view of melanoma cells grown in a scaffold. C) Chronic PAM images of a typical scaffold grown with melanoma cells over 14 days of culture. D) Melanoma cell proliferation profiles obtained by quantifying the PA volumetric data and the parallel MTT assay.

Conclusions: We have demonstrated that PAM could be a powerful tool for investigating cell distributions in 3D scaffolds in a non-invasive manner. Melanoma cells were used as a model cell line due to their intrinsic dark pigment. The whole scaffold (1.2-1.5 mm in thickness) containing melanoma cells could be imaged and resolved in a 3D fashion. We were also able to chronically image the same cell/scaffold construct at different time points by PAM. It was confirmed that the continuous quantitative data obtained from PAM matched well with those measured using the biochemical MTT cell viability analysis.

References: [1] Kim, C., Chem. Rev. 2010, 110, 2756. [2] Xu, M., Rev. Sci. Instrum. 2006, 77, 41101. [3] Choi, S.-W., Langmuir 2010, 26, 12126.