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Investigation of cell dynamics in 3D cell spheroids and cell interaction with 3D printed scaffolds by mOCT

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Abstract: Optical coherence tomography (OCT) is a non-invasive and label-free imaging modality based on the detection of backscattered light in biological samples. As microscopic OCT (mOCT) combines high axial and lateral resolution by using a supercontinuum light source and a high numerical aperture objective, an investigation of biological and printed samples at subcellular level is feasible. The additional excellent depth resolution enables mOCT to be a suitable quality control of 3D printed samples. The use of speckle variance (SV) adds information about cell viability. Here we present the feasibility of investigating cell viability within a cell spheroid and monitoring the cell interaction with bioprinted scaffolds using mOCT.

I. Introduction

Over the past decades, 3D printing has gained increasing importance in medical technology. Tissue-analog structures can now be created by 3D printing of synthetic materials together with biological components. Bioprinting requires careful quality control regarding its morphology and biochemical properties if it should be applied successfully for *in vivo* application. Microscopic optical coherence tomography (mOCT) can contribute to a label-free and non-invasive quality control of three-dimensional structures due to its high axial and lateral resolution as well as its excellent depth resolution. The additional ability to capture cell metabolic activity by using speckle variance (SV), mOCT is a feasible tool to investigate cell viability and their interaction with printed materials.

In addition to the morphological investigation of biological samples, OCT is capable of visualizing dynamic processes. This enables virtual labeling of cells on printed materials. One way to examine dynamic processes is through speckle variance. Movements of individual cells and intracellular dynamics lead to changes in the speckle pattern. Speckle variance OCT is an inter-frame detection technique that measures residual signal power after filtering out static tissue components [1].

II. Material and methods

Microscopic OCT was used for the investigation of three-dimensional HeLa cell spheroids and bioprinted polymer scaffolds colonized with fibroblast cells. A custom-built frequency domain OCT with microscopic resolution (mOCT) based on a Michelson interferometer (Fig.1) was used [2].

II.I. Imaging system

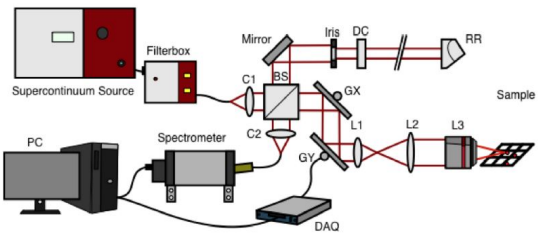

Figure 1: Schematic mOCT setup. C1/C2: collimators; BS: 50:50 beam splitter; GX/GY: galvanometer; L1/L2: beam expansion; L3: microscope objective; DC: dispersion compensation; DAQ: data processing; RR: retroreflector.

A supercontinuum laser (SuperKExtreme EXW-OCT, NKT Photonics, Birkerød, Denmark), which generates a supercontinuum of 450 nm to 2400 nm by nonlinear effects in a photonic crystal fiber, was used as a broadband light source. For mOCT imaging, only the range between 550 nm and 950 nm was used. The collimated beam was divided by a non-polarizing beam splitter (BS014, Thorlabs GmbH, Dachau, Germany) between the reference and the sample arm (50:50). A galvanometer-based 2-axis optical scanner was used to scan the light beam of the sample arm. After the light beam had passed through a telescope system for beam expansion, it was focused through the objective (HCX APO L 10x/0.30 WUVI, Leica Microsystems, Wetzlar, Germany) onto the sample. The scattered light of the sample combined with the reflected light of the reference

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path results in an interference signal measured by a customized and OCT optimized high-speed spectrometer (Thorlabs GmbH, Dachau, Germany), which covers a spectral range from 550 nm to 950 nm. The spectrometer worked at a line scan rate of 127 kHz using 2048 pixels.

For visualizing dynamic changes in the sample the variance *V(x,z)* of the intensity data is calculated at each pixel location within a set of *N* frames using the following formula

$$V(x,z) = \frac{1}{N-1}\sum_{i=1}^{N}\left(I(x,z,i) - \frac{1}{N}\sum_{i=1}^{N} I(x,z,i)\right)^2 \qquad (1)$$

Where *N* is the total number of frames and *I(x,z,i)* is the signal intensity at pixel *(x,z)* and the i^{th} frame.

II.I. Samples

Three-dimensional spheroids were created by embedding HeLa cells in 3D Corning® Matrigel® Matrix. To obtain a controlled size distribution of spherical polyclonal spheroids a seeding density of approximately 18,400 cells/mL was chosen.

Scaffolds were fabricated with melt electrospinning writing method at the Department for Functional Materials in Medicine and Dentistry (FMZ) at the University Hospital of Würzburg and afterward colonized with murine fibroblast cells with a seeding density of 75,000 cells/mL. Polymer poly-ε-caprolactone (PCL) is used for the majority of biological applications as well as for the scaffold used in these experiments.

III. Results and discussion

Fig. 2(A) shows a three-dimensional *in vivo* mOCT image of a typical cell spheroid. In the speckle variance image the variation between the individual cells are visible (Fig. 2(B)). In principle, higher speckle variance should be correlated to higher intracellular dynamics and movements of the cells, which can be indicators of cell vitality.

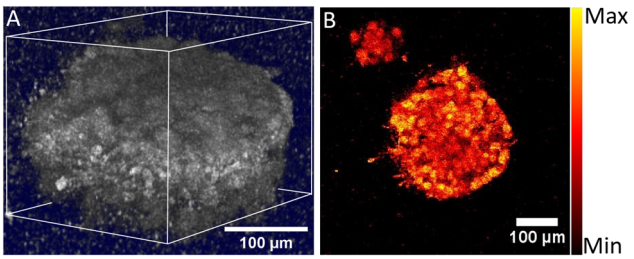

Figure 2: Three-dimensional cell spheroid imaged with mOCT. A: 3D OCT intensity of a single spheroid; B: SV enface image of a single spheroid.

In bioprinted polymer scaffolds colonized with fibroblast, cells were distinguished with high contrast from the scaffold material which gives a static signal. By superposing the intensity image with the speckle variance image, virtual labeling is possible. The cells are clearly distinguishable from the scaffold, seen in Fig. 3. The cells adhere to the scaffold. Since the polymer skeleton is mainly hydrophobic, it forms an unsuitable environment for the cells. This leads to the formation of cell clusters, which makes it difficult to differentiate individual cells. Nevertheless, is PCL due to its heat resistance, its high stability and its biodegradability, particularly suitable for 3D printing and is FDA approved for the *in vivo* use in humans [3]. Further advantages result from the precise formability and high viscosity, which increase the migration and proliferation of the adhering cells [4].

Microscopic OCT provides information about the location of the cells and by analyzing the variance whether the attached cells are still alive. Fig. 3(A) shows a scaffold incubated with fibroblast cells for 7 days and (B) a scaffold incubated for 9 days. The cell growth increases with time and an increased change in the speckle pattern becomes visible as well.

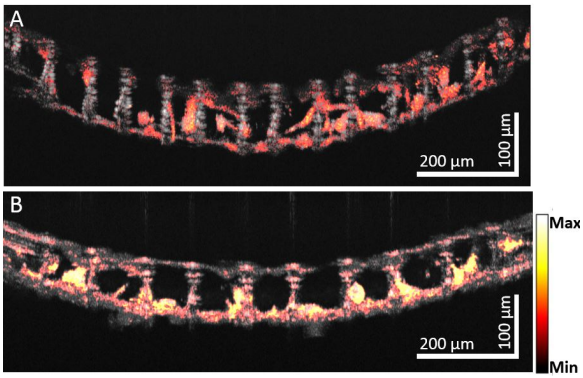

Figure 3: Intensity (gray) and SV (colored) mOCT images of scaffolds colonized by cells. A: scaffold was imaged after 7 days of incubation; B: scaffold was imaged after 9 days of incubation.

IV. Conclusions

Microscopic OCT enables a nondestructive investigation of three-dimensional cell cultures in their morphology as well as their cell viability. It may become an important method for monitoring cell activity and cell interaction with bioprinted scaffolds. 3D volume imaging in real-time and at high depth resolution of mOCT is a distinct advantage compared to conventional optical microscopy methods. An OCT system implemented into a bioprinting device may allow in-situ quality control of scaffold geometry and cell vitality.

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Author's statement

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