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Scanning Acoustic Microscope for Visualizing 3D Cell Clusters Embedded in Hydrogel Systems

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1. Introduction

Tissue engineering has rapidly advanced, allowing the creation of 3D biological constructs that closely resemble human tissues, with applications in wound healing, surgical dressings, and drug delivery. Central to this process is encapsulating cells in nutrient-rich hydrogels, enabling their maturation into functional tissues. Despite the progress in this field, a major challenge remains, i.e., the need for non-destructive characterization tools that can accurately inspect biological cells or cell clusters in 3D, on a macroscopic scale, with quick data acquisition and in real-time[1)]. Scanning acoustic microscopy (SAM) imaging has emerged as a promising non-destructive technique[2, 3)] to address this gap by providing crucial insights into the development of cells in tissues or tissue-like constructs[4-6)]. We used SAM to visualize cellular hydrogels, specifically Gelatin Methacrylate (GelMA) hydrogels containing fibroblasts. We have estimated the cell density at different depths in the sample and measured cellular density. This information provides valuable insights into this technique's potential and limitations in engineered tissue constructs.

2. Materials and methods

Mouse embryonic fibroblast cells cultured in media were embedded in 5% (w/v) GelMA solution. A polymethylpentene (TPX) film (150µm) was used as substrate to avoid strong interface reflections. A silicon gasket with holes (Grace Bio-Labs) was secured firmly on top of TPX using tapes to form wells for casting gel (**Fig. 1 c**). An 800µm thick layer of the cell-laden GelMA was cast in the empty circular wells and UV cured (395nm, 10s) to form hydrogel. A 30MHz spherically focused polymer transducer (Olympus) was used for SAM imaging and PBS as media for cell viability. The backscattered signals were recorded and a log20 (dB) transformation was applied to enhance the visibility of low-intensity scatterers, to include weak scatters. The recorded data was processed in MATLAB and Fiji. During the image analysis, a rectangular region was defined within each circular well to focus on the center of the sample, avoiding scattering interference from the walls. To benchmark the sample a confocal optical microscope (Nikon Ti2e) was used with 60x, 1.3NA silicon oil objective.

4. Results and discussions

SAM imaging revealed small but detectable scattering signals from the cells embedded in the hydrogel matrix (**Fig. 1a**). Despite low scattering

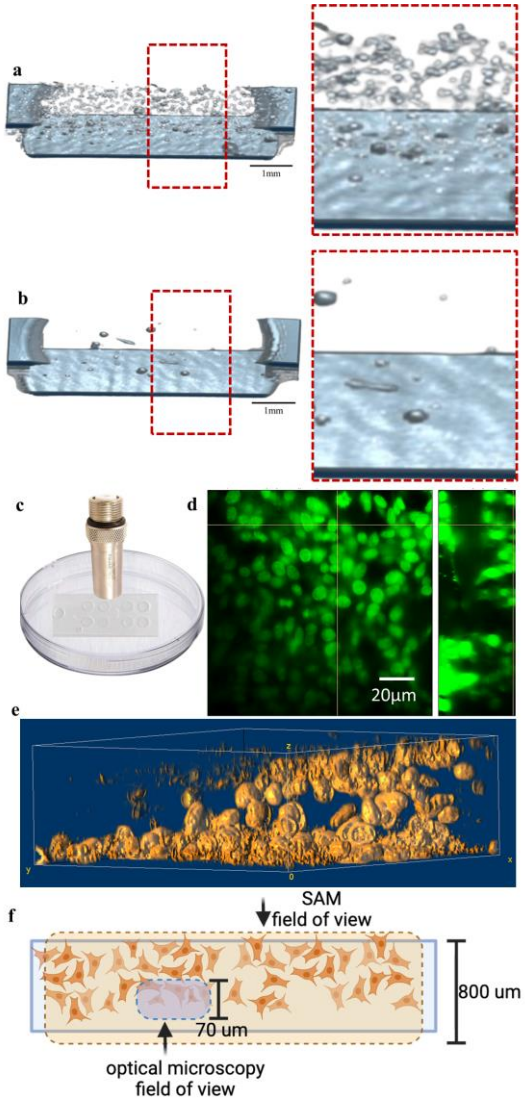

Fig. 1 Visualization of a) cell laden GelMA hydrogel, b) control hydrogel with no cells, c) SAM imaging setup, d) optical confocal image stained for cell nucleus (Sytox green) and corresponding side view, e) 3D rendered optical image, and f) comparing the field of view of SAM and optical microscope for imaging the sample.

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intensity, fibroblast cells were successfully visualized after 256 ensemble averages and 20 dB additional signal amplification. Reflections from the elastomer silicon helped localize the cells within the hydrogel. Cell scatterers were distributed throughout the hydrogel's inner volume, away from the well boundary (**Fig. 1a**). The control sample, exhibited minimal scattering (**Fig. 1b**) with some scattering artifacts, likely due to impurities, or air bubbles. The spatial distribution and clustering of the cell-laden hydrogels were benchmarked with the side, top, and 3D views with confocal optical microscope (**Fig. 1d,e**).

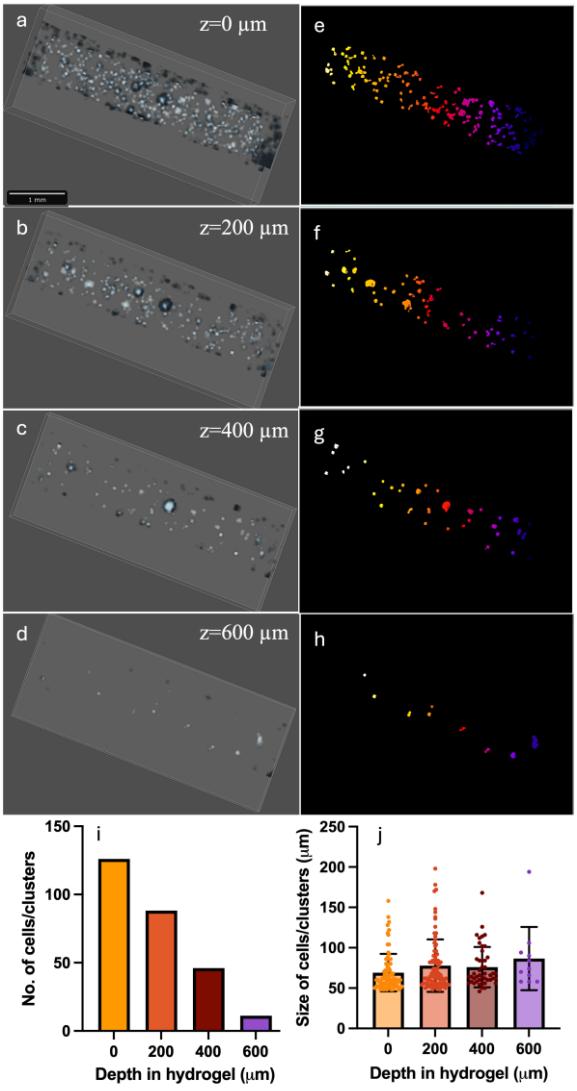

Fig. 2 z (depth) -slices of the cell laden hydrogel up to 600 µm- a-h) rendered SAM image e-h) corresponding detected cells/clusters. Plot of the i) number of cells and j) size distribution at the different depths.

SAM was used to measure the cell density in different depths (**Fig. 2a-h, i**). The cell cluster density at different depths of 0, 200, 400, and 600 µm is evaluated to be 20, 15, 7.6, and 1.6 cell clusters/mm^2. The size of the resolvable objects (cells/cell clusters) had a mean of 71 ± 32 µm (**Fig. 2j**). Since the size of the cells ranges from 30 - 40 µm, it can be concluded that we can see both cells and small clusters with SAM.

5. Conclusion

SAM is an effective non-destructive imaging technique for the characterization of 3D tissue-engineered constructs. These results provide valuable insights into the structural development of cellular hydrogels and highlight the capabilities of SAM in visualizing biological constructs in real time for applications in tissue characterization in wound healing and cancers[7, 8, 9].

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