EFFECTS OF THE COLLAGEN COMPOSITION ON THE MECHANICAL MICROENVIRONMENT OF BREAST CANCER CELLS.

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Introduction

Mechanics plays a key role in cell function. In the literature, forces exerted by the cells in their extracellular matrix have been recently investigated to assess cell properties (focal adhesion, cell differentiation, and invasiveness) or to evaluate their potential metastatic and malignancy [1,2]. In particular, cancer cells have been shown to exert higher traction forces compared to healthy ones [2]. Thus, the analysis of the mechanical and structural microenvironment that favors the migration, proliferation and differentiation of metastatic cells is crucial to develop new cancer treatment strategies. The cancer developed from breast tissue is the most commonly diagnosed cancer type, accounting for 1 in 8 diagnoses worldwide. Therefore, this work aims to combine confocal imaging, and Focus Ion Beam with Scanning electron microscope (FIB-SEM) to evaluate the mechanical response of breast cancer cells in collagen hydrogels with different collagenous composition.

Materials and Methods

In this study, MDA-MB-231 cells were investigated as a common line to model late-stage breast cancer. They were initially cultured and stained using the commercial fluorescent dye CellTracker™. In the analysis of their mechanical behavior at different microenvironment, several extracellular matrices were mimicked by means of hydrogel substrates. They were prepared at different concentrations of only rat-tail collagen, only bovine-skin collagen or mixed 50/50 at 0.8, 1.5 and 2.3 mg/ml. The Traction Force microscopy (TFM) methodology was applied, which consisted of embedding 0.2 micron-sized fluorescent beads in the collagen gels and imaging their 3D spatial movements between the *stressed* and *relaxed* states of cells cultured on the gel [3]. This cell relaxation was achieved *in situ* by supplying Cyto-D, a cell-permeable inhibitor of actin polymerization. The particles' displacement field and the traction force of cells were calculated computationally using the free Matlab™ toolbox TFMLab [3]. Confocal images of each analyzed cell were taken using a LEICA™ Stellaris 8 microscope and a 20x Glyc objective.

Lastly, the microarchitecture of the different collagen hydrogels was characterized by the FIB-SEM ZEISS™ Crossbeam 550. After fixation, an osmium impregnation treatment (OTO) was applied to hydrogels samples to increase the back-scatter electron signal and the image contrast [4]. Images were taken at a voxel size of 9.5x9.5x19 nm and analyzed in the software AVIZO™.

Results

An example of a displacement field calculated via TFMLab is shown in Figure 1A. The results reported a significantly smaller displacement field as we increase the collagen concentration. A greater degradation of the collagen structure was also found in the hydrogels prepared exclusively with bovine collagen. In this composition, a weaker microarchitecture was also reported by FIB-SEM (example in figure 1B).

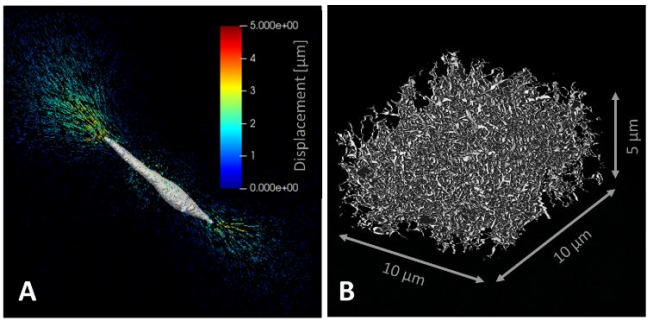

Figure 1: A) Displacement field after cell relaxation calculated by TFM; B) example of a 3D reconstruction of the collagenous structure of the hydrogel from the FIB-SEM images.

Discussion

In the concentrations analyzed in this study (up to 0.8 mg/ml of collagen), the cells seem to have greater migration in softer microenvironments with more bovine-skin proteins. This outcome could be related to their higher concentration of enzymatically degraded collagen fragments. While, the stiffer environment (2.3 mg/ml of collagen) prevent their expansion, mobility and cell survival, intermediate conditions (1.5 mg/ml of collagen) optimize their proliferation.

References

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