Mechanical forces and enzymatic digestion act together to induce the remodeling of collagen fibrils in tumor microenvironment

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1. Validation of the collagen fiber remodeling phenomenon at different matrix concentrations

Three collagen concentrations of 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml were attempted for the reconstruction test under interstitial flow. The orientation of collagen fibrils occurred orientation remodeling in all of the three concentration, as shown in figure S1. However, the degree of reconstruction exists difference. As an example, with the same flow rate of 0.6 um/s, the degree of remodeling is decreasing along with the higher concentration within the three concentrations.

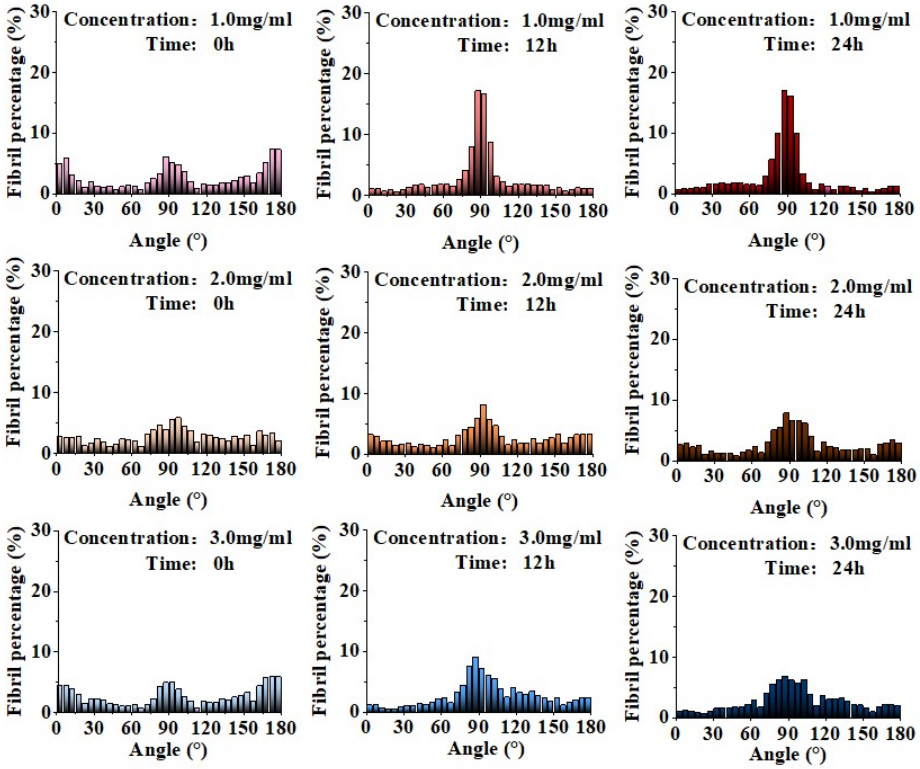

Figure S1 The distribution of collagen fibrils' orientation with three concentration of 1 mg/ml, 2mg/ml, 3mg/ml.

2. Collagenase type 1 was verified participating in collagen fibrils remodeling.

Collagenase type 1(Purchased from Beyotime, Shanghai, China, ST2294) was chosen being transported by the interstitial flow in the TME microfluid system. The degradation was obvious under the recommended concentration of 1 mg/ml with 1 μm/s. The degradation is also obvious along with the pathway. The spectacle was shown in Figure S2 A, the fibril count and distribution of which occur great changes. The distribution and quantifies of fibrils were shown in Figure S2 B-F tracking the process of fibrils cross-link—injecting collagenase 1—injecting peptide chains—injecting collagenase 2 secondary.

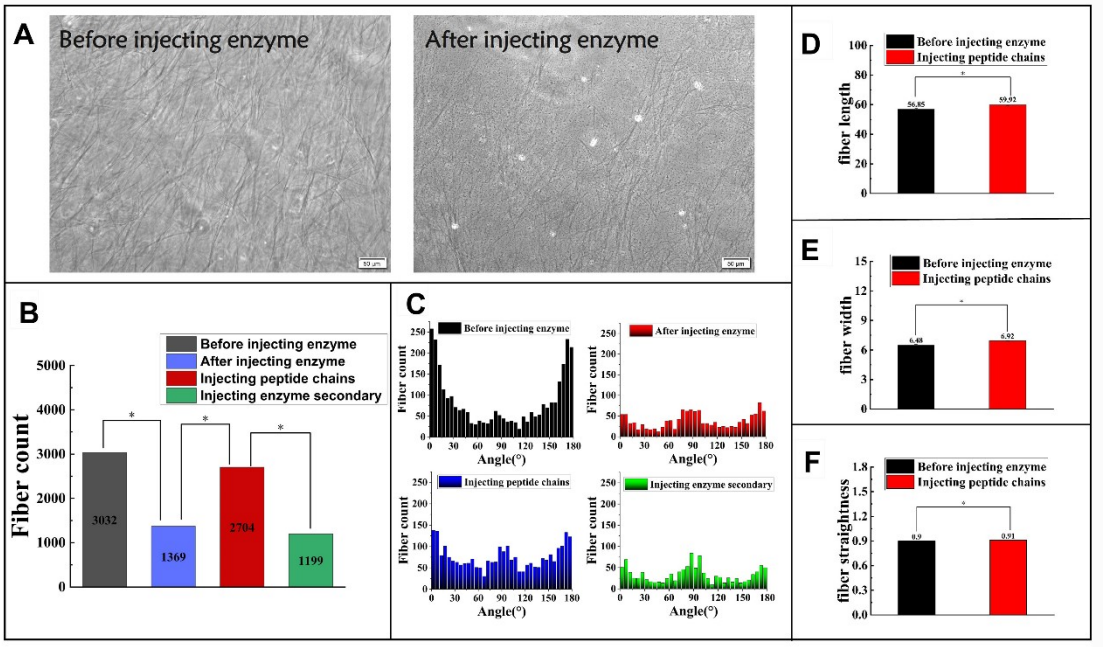

Figure S2 Another enzyme of collagenase type 1 could remodel the collagen fibrils in TME. (A) The experimental scenes before and after enzymatic digestion through flow field transport. (B) The trend of fibril quantification in the process of free orientation collagen fibril preparation—injecting the interstitial flow—adding collagenase—secondary injecting small molecular peptide chains-secondary adding enzyme. (C) the distribution of collagen fibrils in the process of repeated injection of enzyme and small molecule peptides; (D-F) the contrast of fibril length, width and straightness between before injecting enzyme and after injecting peptide chains secondary, respectively. (* represents $p<0.05$).

3. Cell-contractility induced the reorientation of collagen fibrils.

The directed migration induces the orientation remodeling of collagen fibrils was verified in the microfluidic TME system, shown in Figure 6. The dynamics mechanism of the interaction has been reported as early as 2022, which was captured by a long-time tracking of the static 3D culture. In the experiment, collagen1 and MDA-MB-231 also been chosen. The reorientation of collagen fibrils was obvious into bundles induced by the force of cell contractility. The spectacle of interaction was shown in Figure S3.

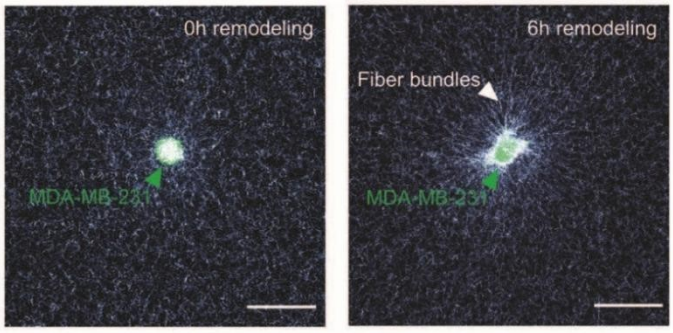

Figure S3 A representative cancer cell (MDA-MB-231GFP) reconstructs collagen to form fiber bundles (white, imaged by confocal microscopy in reflection mode), adapted with permission from the work[1]. Scale bar: 50 μm.

4. The mechanical force from cell migration remodeling collagen fibrils was confirmed in another cell-line of BT549.

Another cell-line of BT549 was cultured in 3D TME of collagen with the directional migration. The scene of evolution was traced 84 hours, as shown in figure S4A. The cells migrated obviously within the period. To highlight the collagen fibrils in the TME, the cells were deducted away (Figure S4B), while the fibrils were highlighted by green (Figure S4C). For greater readability, the local scene was shown in Figure S4A2-C2. The entropy value of collagen fibrils was computed and plotted in Figure S5. The entropy value is drop down, which means the parallelization of collagen fibrils become stronger. From the distribution of collagen fibrils and cells in local scene (Figure S4A2-C2), it could not be ignored of the correlation between cancer cell migration and collagen fibrils, even though it exists different from the scene of cell-line of MDA-MB-231.

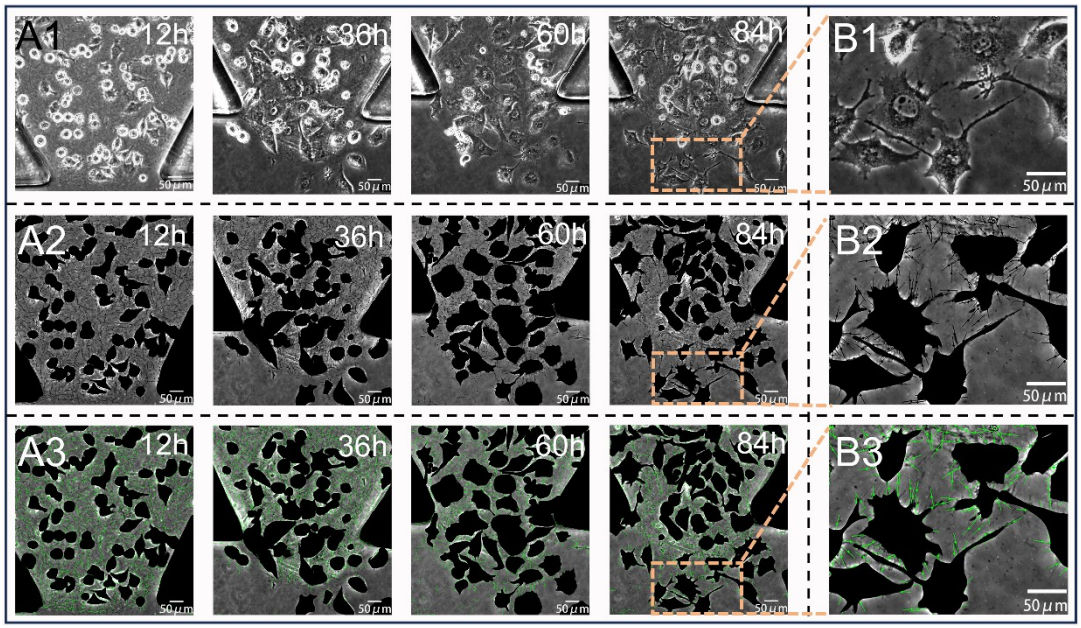

Figure S4 The evolutionary scene of BT549 cultured in 3D collagen.

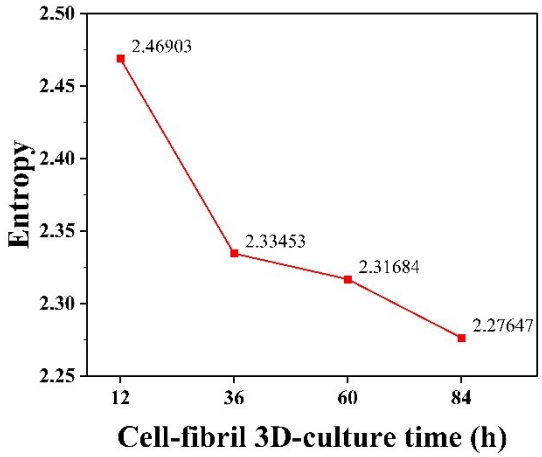

Figure S5 The entropy value of collagen fibrils reduced in the evolutionary process of BT549 cultured in 3D collagen.

References

[1] C. Yang, X. Wang, R. Xie, Y. Zhang, T. Xia, Y. Lu, F. Ye, P. Zhang, T. Cao, Y. Xu, Q. Fan, Dynamically Reconstructed Collagen Fibers for Transmitting Mechanical Signals to Assist Macrophages Tracing Breast Cancer Cells. Adv. Funct. Mater. 2023, 33, 2211807.