Biomaterials Science

Electronic Supplementary Information (ESI)

3D *in vitro* co-culture disc for spatiotemporal image analysis of cancer-stromal cell interaction

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1. Preparation of A549 spheroids in the spheroid forming plate

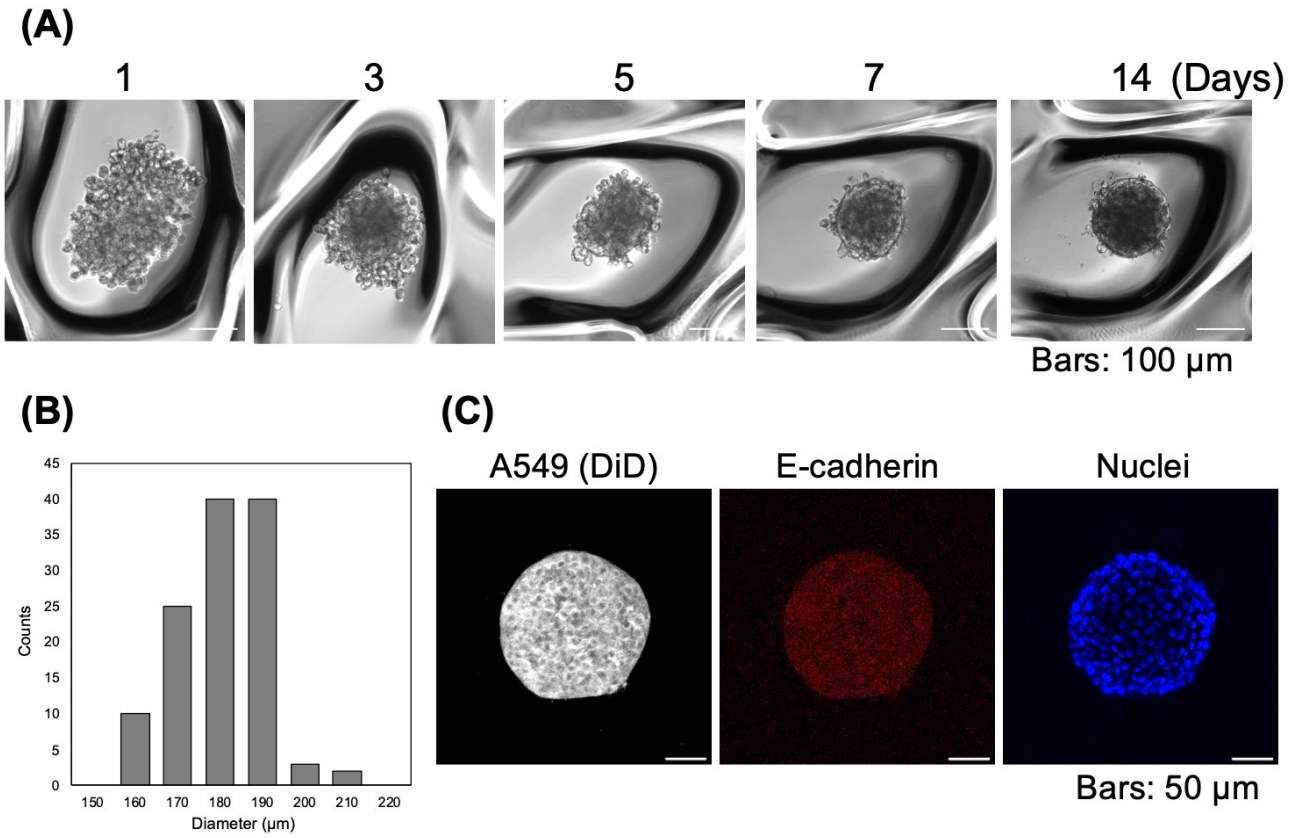

Figure S1. Preparation of A549 spheroids in the spheroid forming plate. (A) A549 spheroid formation in the low cell adhesive spheroid forming plate. **(B)** Size distribution of prepared A549 spheroid after 7-day incubation. **(C)** Immunostaining of A549 spheroid after 7-day incubation. A549 cells were stained by DiD (white signal). E-cadherin (red signal) was detected by immunostaining. The nuclei (blue signal) were stained by Hoechst 33342. Bars: 50 μm.

2. Culture of NHLFs in the collagen gel on glass bottom dish

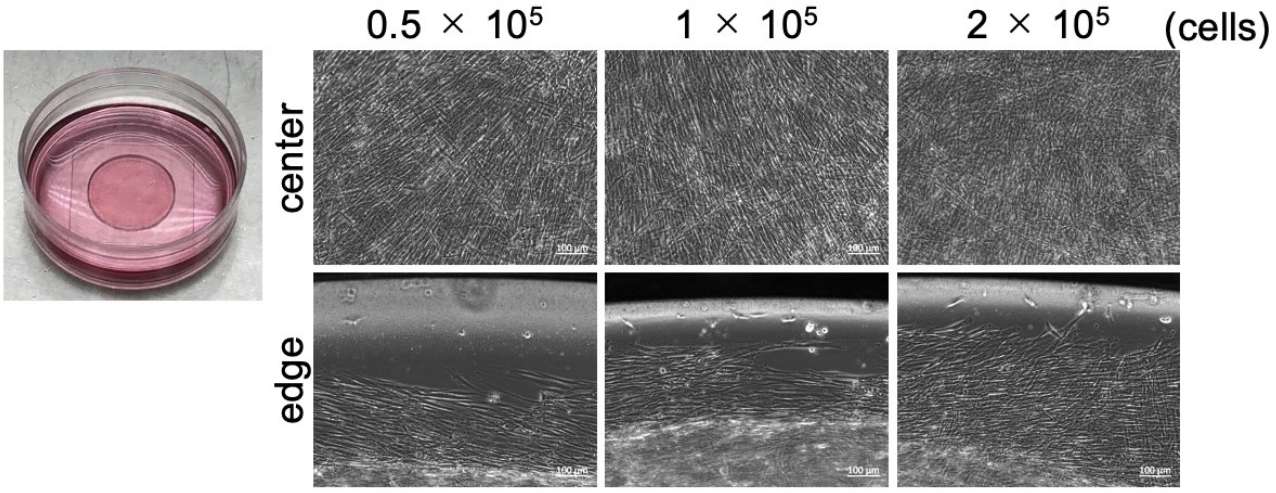

Figure S2. Culture of NHLFs on the glass bottom dish. (Left) Appearance of glass bottom dish. On the center groove (φ14 mm), the NHLFs in the collagen solution were applied to form collagen gel. **(Right)** Center and edge part of glass bottom dish after 10 days incubation.

3. Long term co-culture of A549 spheroid and NHLF in the 3D in vitro co-culture disc

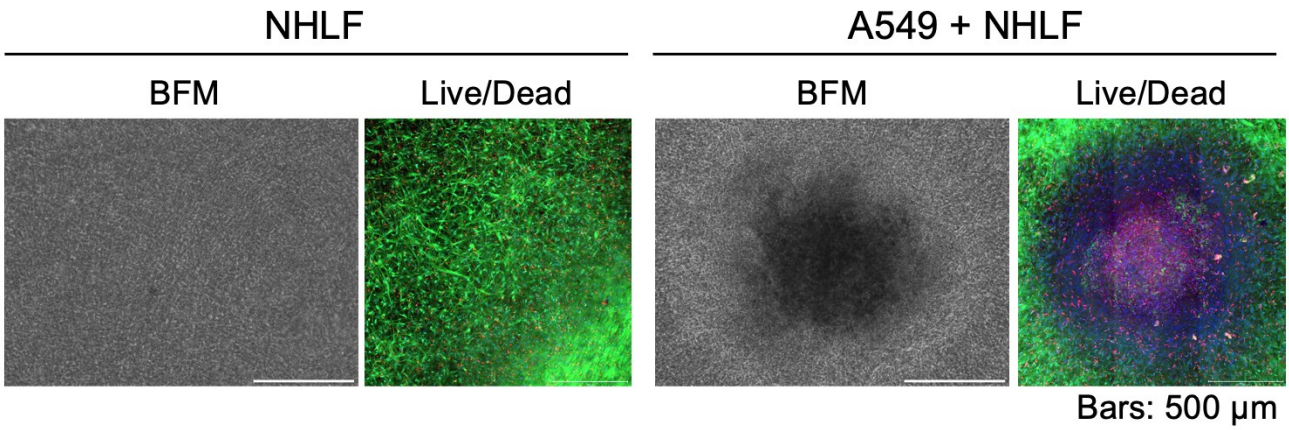

Figure S3. Long co-culture of A549 spheroid and NHLF in the 3D *in vitro* co-culture disc. After 30 days co-culture, the cells were observed by BFM or confocal laser scanning microscope images of discs stained with the LIVE/DEAD™ kit. By the LIVE/DEAD™ staining, living cells was shown with green fluorescent signal due to Calcein-AM and dead cells were stained EthD-1 with red fluorescent. The nuclei (blue signal) were stained by Hoechst 33342.

4. αSMA gene expression under nicotine treatment

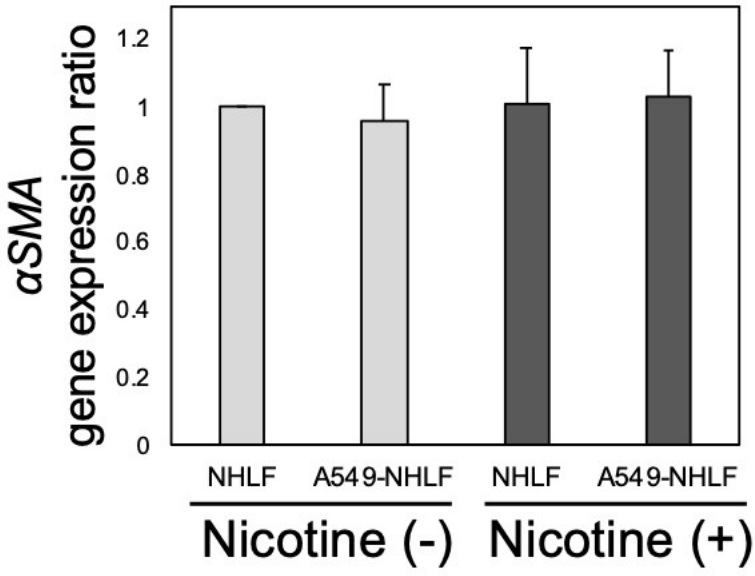

Figure S4. αSMA gene expression ratio (compare to non-treated NHLF) under nicotine treatment determined by RT-qPCR. The cells were collected from collagen gel of disc (n=3). [Nicotine] = 10 μM.

5. Caption for MovieS1

Movie S1. Time-lapse imaging of A549-NHLF interaction in the 3D *in vitro* co-culture corresponding to Figure 4.