Matrix viscoelasticity drives cell cluster formation to counteract cellular senescence

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Table 1 Different viscoelastic PAM/Alg matrix formulation

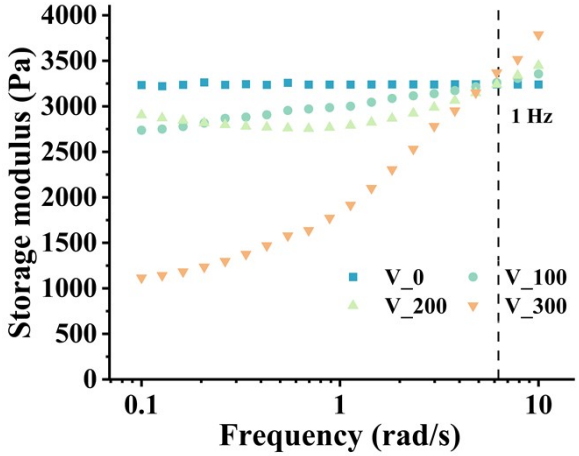
Groups	**40%AM**	**2%BIS**	**UP H_2O**	**5%Alg**	**TEMED**	**10%APS**
V_0	2ml	500μl	7.4ml	0ml	4μl	100μl
V_100	2ml	400μl	3.6ml	4ml	4μl	100μl
V_200	2.2ml	200μl	1.6ml	6ml	4μl	100μl
V_300	3.2ml	40μl	0ml	6.8ml	4μl	100μl

SI Table. 1. The preparation method of viscoelastic matrix is mixed into glue in one step according to the above ratio.

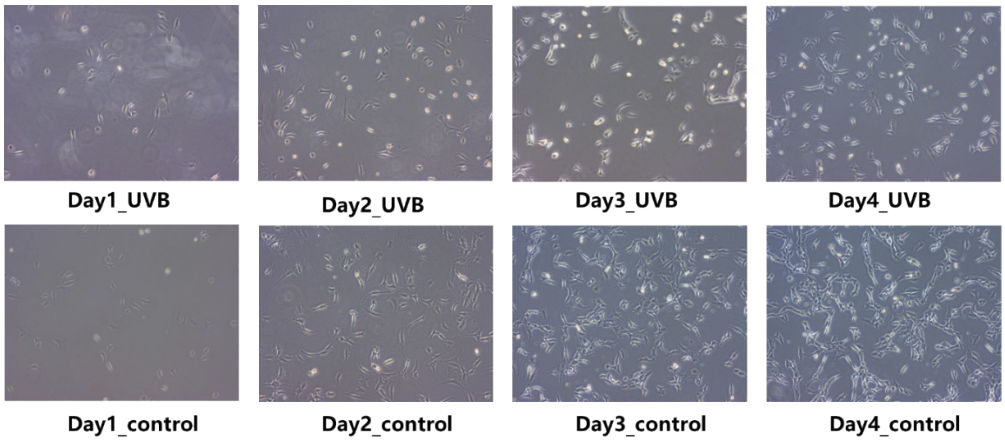
Table 2 Primer sequences for RT-qPCR

引物名称	引物序列
GAPDH-F	CAGTGGCAAAGTGGAGATTGTTG
GAPDH-R	TCGCTCCTGGAAGATGGTGAT
Cdkn1a-F	TCCCGACTCTTGACATTGCT
Cdkn1a-R	AGTATGGGGTGGGGGAAAAG
Cdkn2a-F	GGTGATGATGATGGGCAACG
Cdkn2a-R	GAGAAGGTAGTGGGGTCCTC
Cxcl12-F	CAAGTGTGCATTGACCCGAAATT
Cxcl12-R	GAAGAGGGAGGAGCGAGTTACAA
Rock2-F	TGCAATACACTCCATGGGCTTA A
Rock2-R	GATTTCAGAACCTCGGGCGATAT
Ctnnb1-F	AACCTTTCAGATGCAGCGACTAA
Ctnnb1-R	GCTGCACAGGTGACCACATTTAT
Gja1-F	ATCGCGTGAAGGGAAGAAGC
Gja1-R	TCGCTGGCTTGCTTGTTGTA

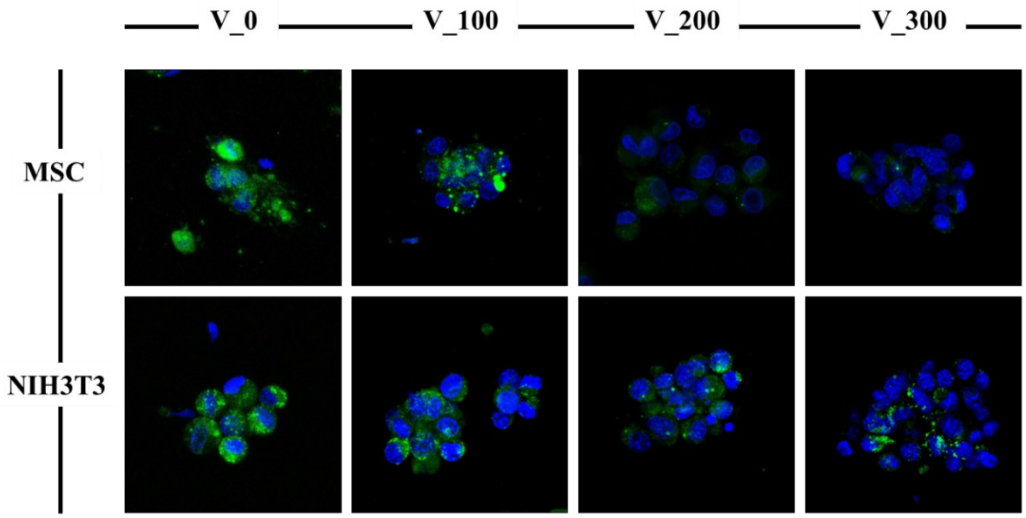
SI Table. 2. The primer sequence of the gene designed in the experiment.



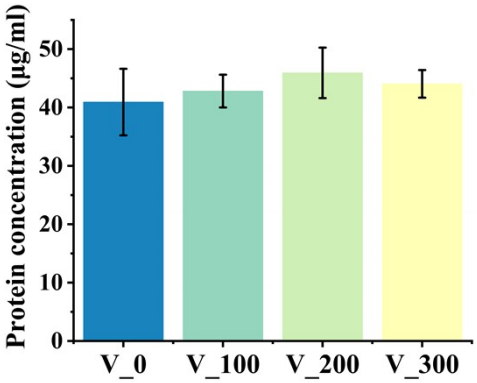
SI Fig. 1. The variation of storage modulus with frequency for all groups.



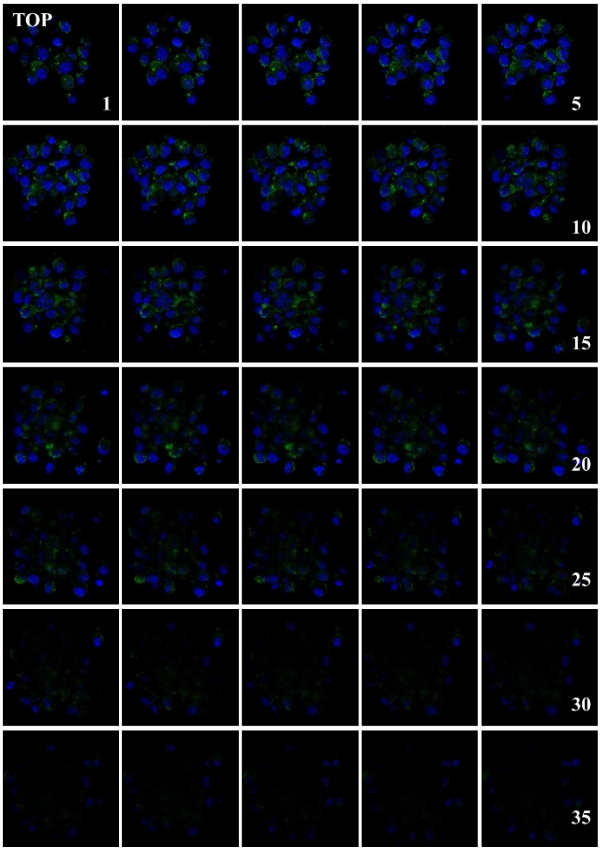
SI Fig. 2. Preliminary experiment on the establishment of UV-induced cell senescence model. NIH3T3 cells were inoculated on the culture plate, and the experimental group was subjected to ultraviolet irradiation experiment from the second day, and the cell status was observed and recorded daily through the optical microscope.



SI Fig. 3. Validation of H_2O_2-Induced Cellular Senescence Model in MSCs and NIH-3T3 Cells. The concentration of H_2O_2 was determined to be 800 μM based on preliminary experiments. Cells were exposed to this concentration for 4 hours, followed by a 24 h incubation in fresh medium before performing SA-β-gal staining to assess cellular senescence.

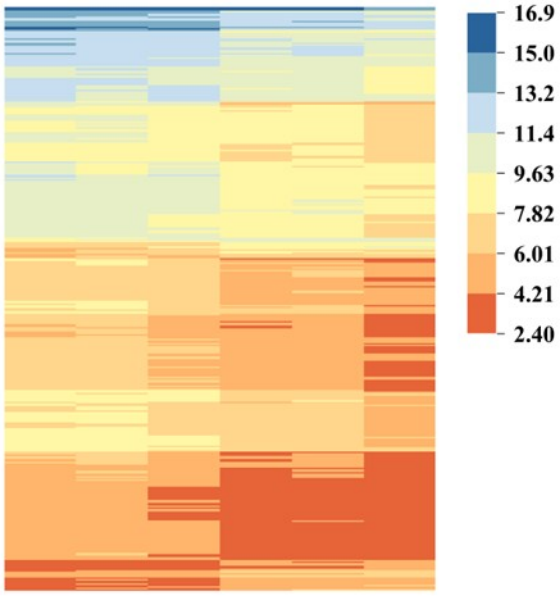


SI Fig. 4. Protein content of materials with different groups. Protein concentration comparison of materials with different groups after soaking in equal volumes of culture medium for 2 days; equal weights of samples were lysed with 1% Triton X-100 at a 1:5 ratio, followed by BCA assay measurement.

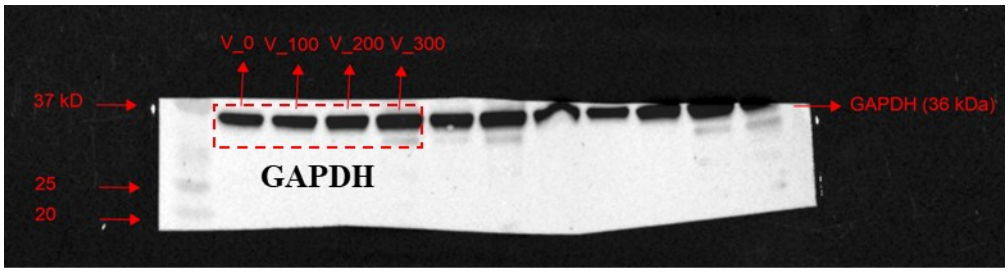


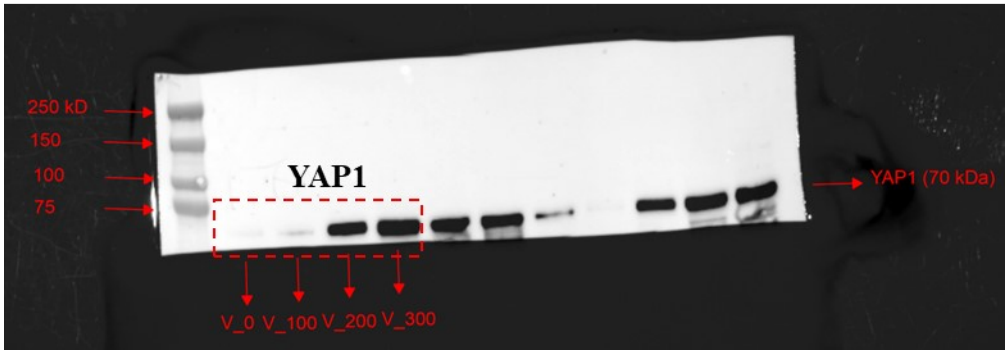
SI Fig. 5. Cell senescence in different spatial layers of spherical cell aggregates of

group V_300.



SI Fig. 6. Volcano map of all different genes in group V_0 and group V_300.





SI Fig.7. Original blots.