

Supporting Information

Extracellular Calcium ion Concentration Regulates Chondrocyte Elastic Modulus and Adhesion Behavior

Xingyu Shen ¹, Liqiu Hu ¹, Zhen Li ², Liyun Wang ³, Xiangchao Pang ⁴, Chun-Yi Wen ⁵ and Bin Tang ^{1,*}

¹ Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen, 518055, China; 11749079@mail.sustc.edu.cn (S.X.); 11930748@mail.sustech.edu.cn (H.L.)

² School of Chemistry and Environmental Engineering, Jiangsu University of Technology, Changzhou 213001, China; lizhen198458@163.com

³ Center for Biomechanical Research, Department of Mechanical Engineering, University of Delaware, Newark, DE, 19716, USA; lywang@udel.edu

⁴ College of Materials Science and Engineering, Central South University of Forestry and Technology, Changsha, 410004, China; T20172373@csuft.edu.cn

⁵ Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong, 999077, China; chunyi.wen@polyu.edu.hk

* Correspondence: tangb@sustech.edu.cn

Table

Table S1. The precise values of the results.

Ca ²⁺ concentration / mM	1.75	2.25	2.75	3.25	3.75	Control
Young's modulus / KPa	1202 ± 250	1521 ± 75	1644 ± 396	1402 ± 211	1510 ± 155	
F _{max} / nN	1103± 439	1298 777	1632 ± 724	1295 ± 526	1208 ± 633	130 ± 90
W _d / 10 ⁻¹⁵ J	1.3 ± 0.8	1.8 ± 0.6	2.3 ± 0.9	1.9 ± 0.9	1.6 ± 0.9	0.1 ± 0.1
F _{max} / nN (CWHM-12)	308 ± 172	300 ± 200	410 ± 217	304 ± 149	381 ± 203	
W _d / 10 ⁻¹⁶ J (CWHM-12)	3.6 ± 2.8	4.4 ± 4.2	3.8 ± 2.8	3.8 ± 2.7	4.6 ± 3.1	
(F _{max} - F _{max} (CWHM-12))/ F _{max}	72%	77%	75%	76%	68%	
(Wd - Wd(CWHM-12))/Wd	72%	76%	83%	80%	71%	

Table S2. Primers for qPCR.

Gene	Sequences (5'-3')
	Forward primer: GCTCCTCCTGAGCGCAAGTAC

Gene	Sequences (5'-3')
β -actin	Reverse primer: GGACTCGTCATACTCCTGCTTGC
<i>col2a1</i>	Forward primer: CGCCGCTGTCCTCGGTGTC Reverse primer: AGGGCTCCGGCTTCCACACAT
<i>acan</i>	Forward primer: TGGGAACCAGCCTATACCCCAG Reverse primer: CAGTTGCAGAAGGGCCTTGTAC
<i>myosin</i>	Forward primer: CAGCAAGCTGCCGATAAGTAT Reverse primer: CTTGTCGGAAGGCACCCAT
<i>integrin beta 1</i>	Forward primer: CCTACTTCTGCACGATGTGATG Reverse primer: CCTTGCTACGGTTGGTTACATT
<i>integrin beta 3</i>	Forward primer: GTGACCTGAAGGAGAATCTGC Reverse primer: CGGAGTGCAATCCTCTGG
<i>collagen VI</i>	Forward primer: ACAGTGACGAGGTGGAGATCA Reverse primer: GATAGCGCAGTCGGTAGG
<i>Collagen I</i>	Forward primer: CGCCGCTGTCCTCGGTGTC Reverse primer: AGGGCTCCGGCTTCCACACAT

Figure

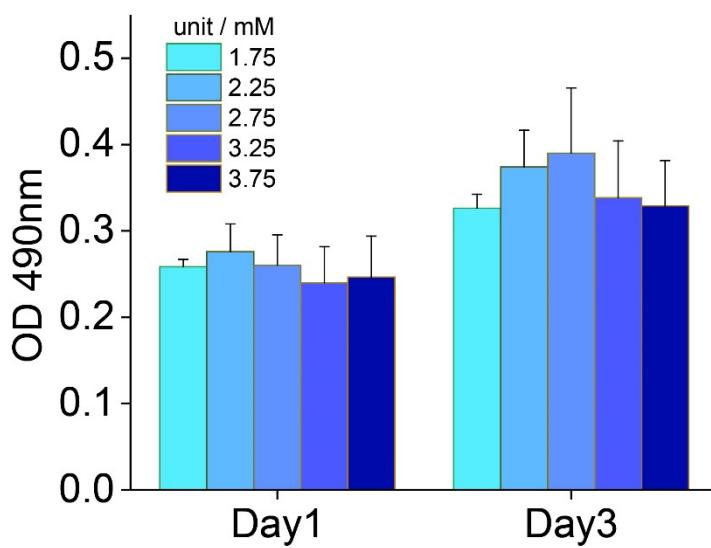


Figure S1. MTT assay shows the proliferation of chondrocytes at day 1 and day 3. No statistic difference in each groups.

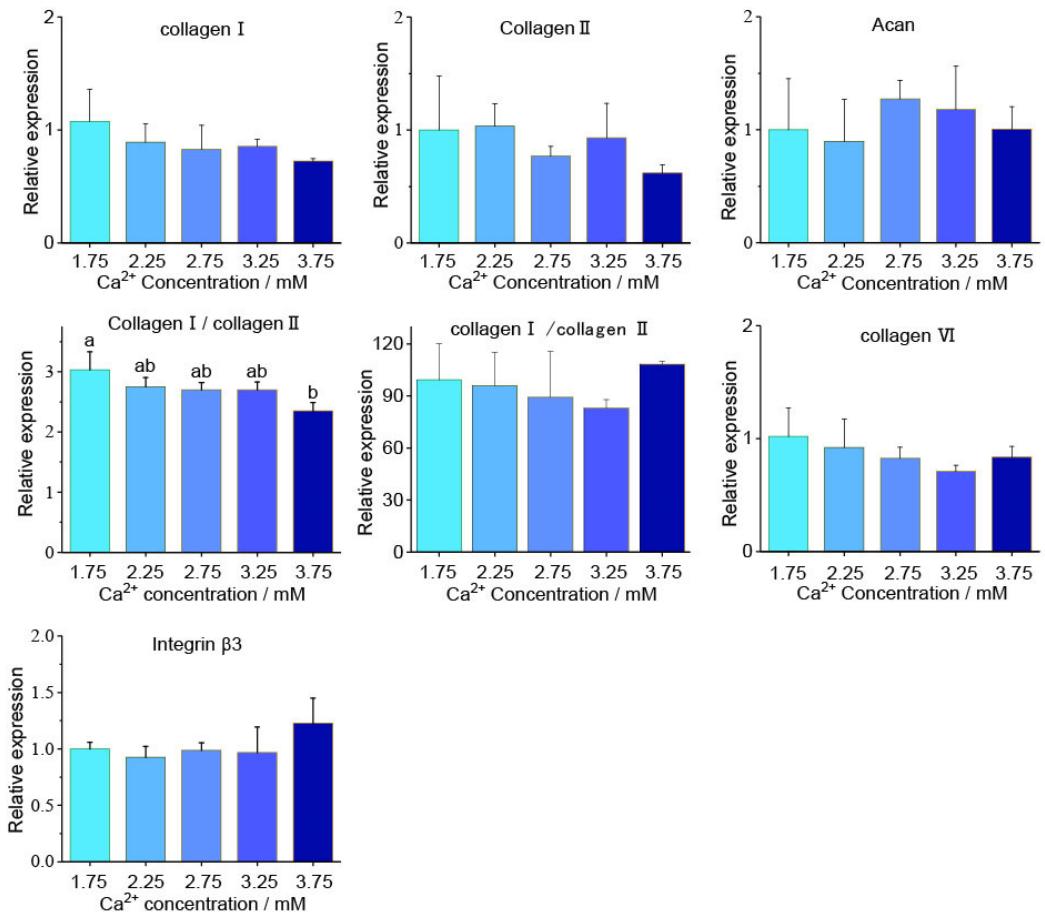


Figure S2. The expression of *collagen II*, *collagen I*, *collagen VI*, *integrin $\beta 3$* , and *aggrecan* has no significant difference.

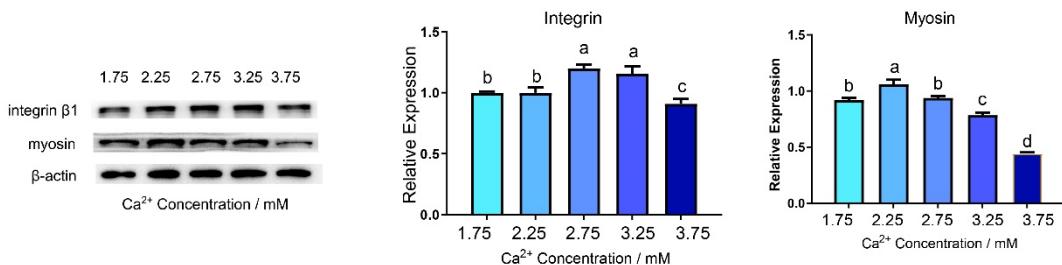


Figure S3. Western blot shows that the trend of *myosin II* and *integrin $\beta 1$* expression level is similar to that of qPCR and immunofluorescence results.

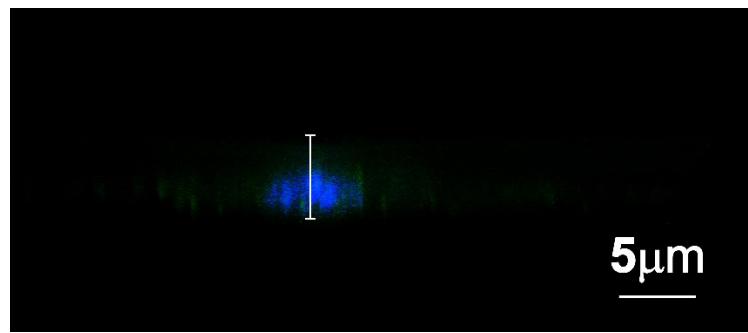


Figure S4. The height of chondrocytes was about 6 μm measured by confocal laser scanning microscope. Blue: DAPI; green: phalloidin.