**Supplement Material**

**Substrate stiffness-dependent regulatory volume decrease and calcium signaling in chondrocytes**

Running title: substrate stiffness regulates chondrocyte volume and calcium signaling

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Table I. The RVD parameters of chondrocyte during swelling and recovering in hypo-osmotic medium (180 mOsm).

|  |  |  |  |
| --- | --- | --- | --- |
| RVD responsive parameters | Stiff (n=29) | Medium (n=27) | Soft (n=32) |
| Initial diameter, d0 (μm) | 15 ± 3 | 13 ± 3 | 12 ± 2 |
| Maximum diameter, dM (μm) | 19 ± 3 | 16 ± 2 | 16 ± 3 |
| Swelling time TS (min) | 13 ± 2 | 9 ± 2 | 8 ± 1 |
| Percentage increase of d (%) | 22 ± 4 | 29 ± 3 | 33 ± 4 |
| Recovery time TR (min) | 20 ± 4 | 31± 5 | 34 ± 4 |
| Percent of recovery diameter (%) | 75 ± 9 | 69 ± 6 | 61 ± 8 |
| Responding time TRes (min) | 33 ± 4 | 40 ± 4 | 41 ± 4 |

n is the number of measured cells. All data are shown as mean ± s.d

Table II. The elastic and viscoelastic parameters of chondrocytes on varying substrate stiffness in iso-osmotic medium (320 mOsm)

|  |  |  |  |
| --- | --- | --- | --- |
| Mechanical parameters | Stiff (n=32) | Medium (n=27) | Soft (n=36) |
| *Eelastic* (kPa) | 1.6 ± 0.1 | 1.1± 0.2 | 1.0± 0.1 |
| *τ*ε (s) | 4.5± 0.2 | 3.5± 0.2 | 3.2 ± 0.1 |
| *τ*σ (s) | 7.4 ± 0.3 | 5.4 ± 0.3 | 5.1 ± 0.2 |
| *E*R (kPa) | 0.5± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 |
| *E*0 (kPa) | 0.8± 0.1 | 0.5 ± 0.1 | 0.4± 0.1 |
| *μ* (kPa⋅s) | 1.4± 0.1 | 0.7± 0.1 | 0.6± 0.1 |

n is the number of measured cells. All data are shown as mean ±s.d

Table III. The relative percentage increase of the mechanical parameters of chondrocytes on varying substrate stiffness in hypo-osmotic medium (180 mOsm) compared to the control group in iso-osmotic medium (320 mOsm).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *Eelastic* (kPa) | *E*R (kPa) | *E*0 (kPa) | *μ* (kPa⋅s) |
| Stiff | 24% | 29% | 30% | 33% |
| Medium | 33% | 19% | 18% | 19% |
| Soft | 43%  | 19% | 13% | 16% |

Table IV. The calcium responsive rate of chondrocytes on varying substrate stiffness induced by 4αPDD or GSK205 in iso-osmotic medium.

|  |  |
| --- | --- |
|  | Calcium responsive rate (%) |
| 4αPDD treated | GSK205 treated |
| Stiff | 80 ± 5 | 35 ± 4 |
| Medium | 54 ± 4 | 39 ± 3 |
| Soft | 53 ± 6 | 39 ± 4 |

All data are shown as mean ± s.d

Table V. The relative percentage increase or decrease of the amplitude and frequency of Ca2+ oscillations in chondrocytes on varying substrate stiffness induced by 4αPDD or GSK205 in iso-osmotic medium.

|  |  |  |
| --- | --- | --- |
|  | 4αPDD- induced relative increase | GSK205- induced relative decrease |
|  | Amplitude (F/F0) | Frequency (peaks/min) | Amplitude (F/F0) | Frequency (peaks/min) |
| Stiff  | 50% | 54% | 59% | 52% |
| Medium | 44% | 43% | 46% | 37% |
| Soft | 24% | 33% | 46% | 24% |

Table VI. The percent of chondrocytes on variable stiffness substrates exhibited both RVD response and Ca2+ oscillations in hypo-osmotic (180 mOsm) medium after treatment of 4αPDD or GSK205.

|  |  |
| --- | --- |
|  | Calcium responsive rate (%) |
| 4αPDD treated | GSK205 treated |
| Stiff  | 43 ± 5 | 24 ± 3 |
| Medium | 51 ± 5 | 29 ± 2 |
| Soft | 49 ± 5 | 33 ± 5 |

All data are shown as mean ± s.d

Table VII. The relative percentage increase or decrease of the amplitude and frequency of Ca2+ oscillations in chondrocyteson varying substrate stiffness induced by 4αPDD or GSK205 during cells welling in hypo-osmotic (180 mOsm).

|  |  |  |
| --- | --- | --- |
|  | 4αPDD- induced relative increase | GSK205- induced relative decrease |
|  | Amplitude (F/F0) | Frequency (peaks/min) | Amplitude (F/F0) | Frequency (peaks/min) |
| Stiff | 22% | 18% | 43% | 44% |
| Medium | 32% | 26% | 59% | 55% |
| Soft | 44% | 37% | 70% | 66% |

Table VIII. The relative percentage increase or decrease of the amplitude and frequency of Ca2+ oscillations in chondrocytes on variable stiffness substrates induced by 4αPDD or GSK205 during cell recovering in hypo-osmotic (180 mOsm) medium.

|  |  |  |
| --- | --- | --- |
|  | 4αPDD- induced relative increase | GSK205- induced relative decrease |
|  | Amplitude(F/F0) | Frequency(peaks/min) | Amplitude(F/F0) | Frequency(peaks/min) |
| Stiff | 37% | 31% | 65% | 54% |
| Medium | 25% | 21% | 53% | 47% |
| Soft | 23% | 23% | 52% | 47% |