Supporting Information

Mimicking loading-induced cartilage self-heating in vitro promotes matrix formation in chondrocyte-laden constructs with different mechanical properties

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Morphological characterization of scaffolds in hydrated state – Micro-CT scans

Hydrated scaffold was put inside a 2 ml Eppendorf tube and carefully frozen in liquid nitrogen while avoiding direct contact of scaffold and nitrogen. Then the frozen samples were vacuumed dried for 72 hours and scanned afterwards for morphological characterization in dried state. The measurement parameters were set as 40 kV for voltage, 100 mA for current, 18 mm for spatial resolution, 400 mS for exposure time and 0.42^{o} for rotation steps without using any filter.

Mechanobiological study – Modes of stimulation

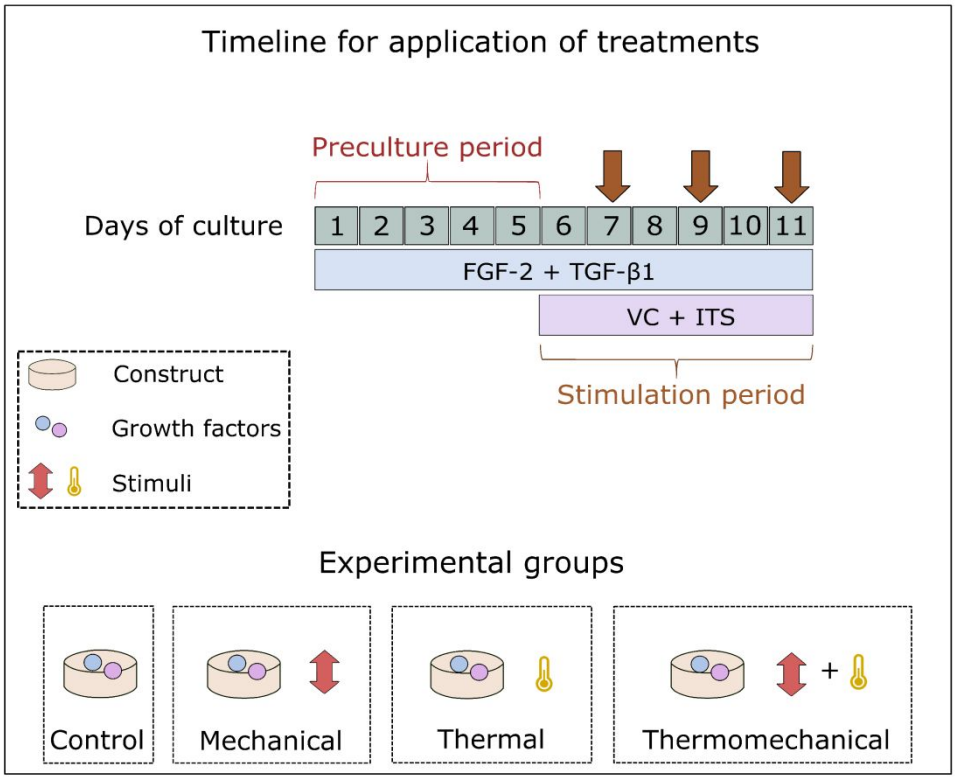

Figure S1 - A schematic illustration of the mechanobiological study timeline is shown. On day 0 (Not shown), chondrocytes are seeded on scaffolds and remain at free swelling condition up to Day 5. On Day 6, foetal bovine serum is removed from the medium, while vitamin C and Insulin transferrin selenium are introduced. All constructs were subjected to dynamic stimulation on Days 7,9 and 11. Constructs were then collected for gene expression (n=3) and biochemical analysis (n=3).

Chondrocyte viability following different forms of stimulation

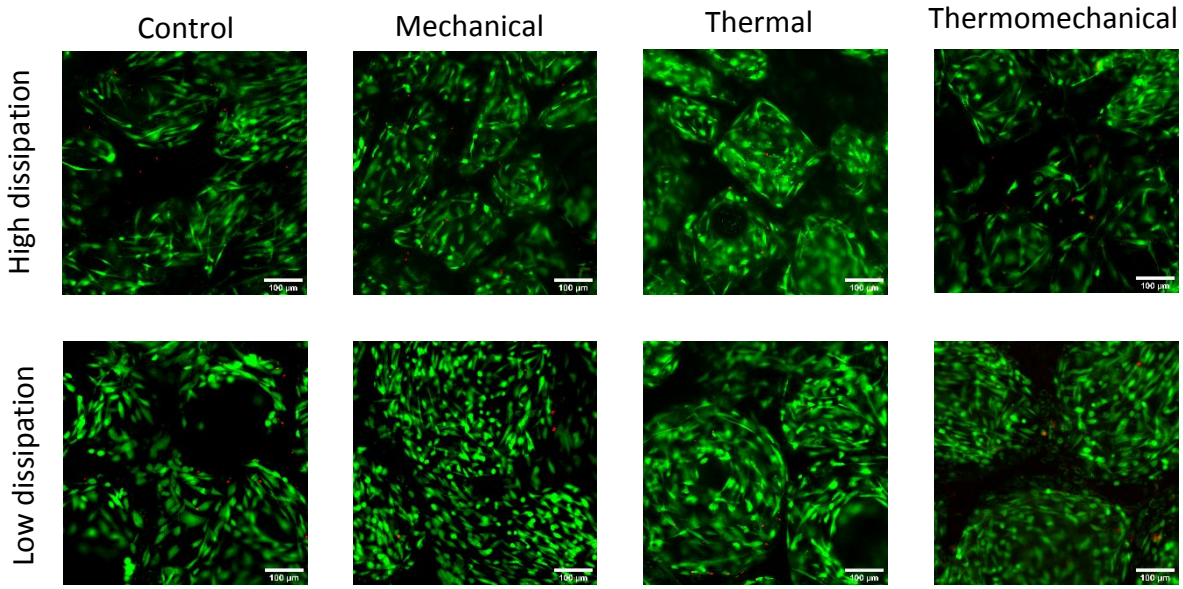

Figure S2 – Typical Live/Dead staining for samples with different levels of energy dissipation and following different forms of stimulation. Green stains for Live cells. Scale bar at 100 μm.

Chondrocyte metabolic response to physiologically relevant thermomechanical stimulus

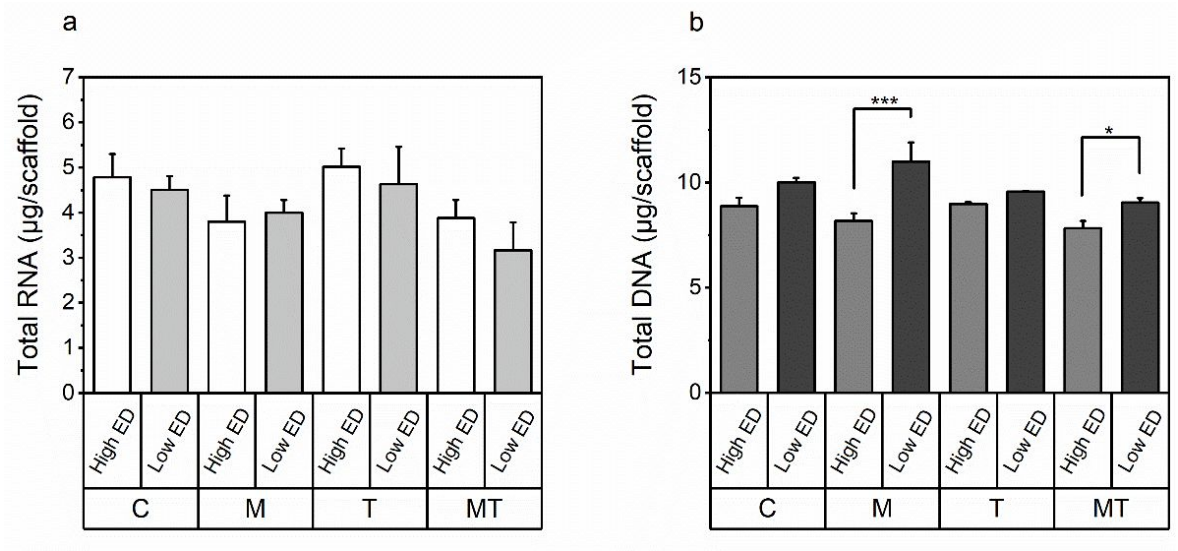

Figure S3 – a) Total RNA and b) Total DNA content for the different groups of scaffolds following different forms of stimulation. Total amount of RNA per construct was determined and compared between the different groups of scaffolds. Overall, no significant changes were observed following the application of different types of stimulation. It is, however, noticeable that there is always a direction towards reduced RNA content in the groups containing dynamic mechanical loading. This trend was evident regardless of the level of energy dissipation. Furthermore, construct cellularity, as quantified from measuring DNA content in each sample, was mainly unaffected by the different forms of stimulation and slightly enhanced in the constructs with higher pore size (lower dissipation level). Additionally, dynamic

thermomechanical stimulation resulted in lower DNA mean content when compared to free swelling controls.

Collagen type 2 expression during the 5-day preculture period as well as after the last loading cycle was ceased.

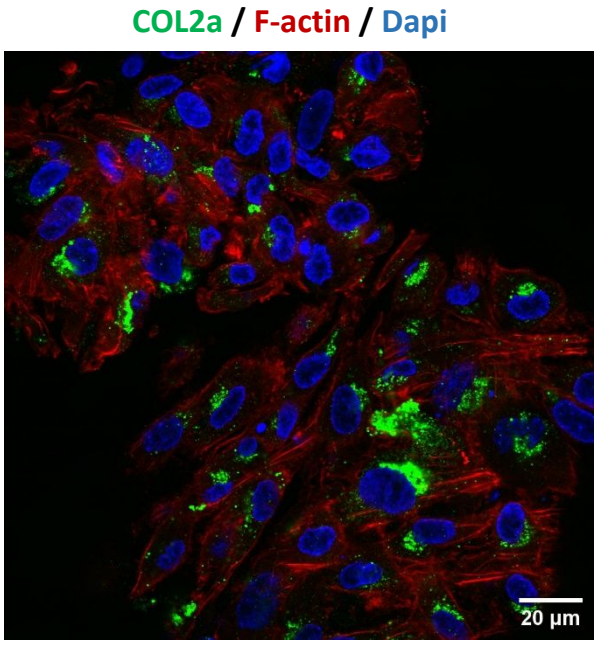

Figure S4 - Representative 3-color immunofluorescence staining of pHEMA-based hydrogels at 20x, depicting accumulation of collagen type 2, F-actin and Dapi during the preculture period (top image), at 63x.

RNA extraction and PCR

Different annealing temperature and concentration were used for primers to optimize the process resulting in efficiency range of 88 to 108%. The PCR amplification was carried out in duplicate for each sample by StepOnePlus Real-Time PCR platform (Applied Biosystems). The thermal cycling condition was defined as an initial 95°C step for 1 min followed by 40 cycles of 95°C for 5s and corresponding annealing-extension temperature of gene (60 to 65°C, Table S4) for 30s. Gene expression data were analyzed using the comparative ΔΔCt method [10] with B2M as the reference gene. Corresponding free swelling hydrogels in each group were used as the biological reference for the stimulated hydrogels (n=3).

Table S1. Primers data used for qRT-PCR

Gene	Primer concentration (nM)	Efficiency (%)	Sequence 5'-3'
RPL13a	175	104	**F:**TAAACAGGTACTGCTGGGCCG **R:**CTCGGGAAGGGTTGGTGTTC
SOX9	275	108	**F:**TGGAAACTTCAGTGGCGCGGA **R:**AGAGCAAAAGTGGGGGCGCTT

TWIST1	250	91	**F:**AGCAGGGCCGGAGACCTAGATGTCA **R:**ACGGGCCTGTCTCGCTTTCTCT
ACAN	175	99	**F:**GGTACCAGTGCACAGAGGGGTT **R:**TGCAGGTGATCTGAGGCTCCTC
TREK1	250	99	**F:**CAATTCGACGGAGCTGGATG **R:**CTTCTGTGCGTGGTGAGATG
LOXL2	250	88.7	**F:** TGACGACTTCTCCATCCACG **R:** GTGTGCTTGCAGTCAGTGAC
TRPV4	250	90	**F:**TCCACCCTATATGAGTCCTCGG **R:**TAGGTGCCGTAGTCAAACAGT

Immunofluorescence staining

Table S2. Antibodies used in immunofluorescence staining

Reagent	Designation	Source	Dilution
Primary antibody	Mouse anti-TREK1	(F-6): sc-398449, Santa Cruz Biotechnology	1:250
Primary antibody	Rabbit anti-TRPV4	ab191580, Abcam	1:250
Primary antibody	Mouse anti-ACAN	MS X HU AGGRECAN (969D4D11), Lifetechnologies Thermo Fisher	1:250
Primary antibody	Mouse anti-COLL2A	Invitrogen, MA5-12789	1:250
Phalloidin	Alexa Fluor 568 phalloidin	Invitrogen, A12380	1:400
Secondary antibody	ALEXA FLUOR 488 PHALLO	Lifetechnologies	1:250