Supplementary Information

Fibrotic-like collagen matrices as 3D in vitro models for investigating the impact of pathological extracellular matrix on skeletal muscle cell behavior

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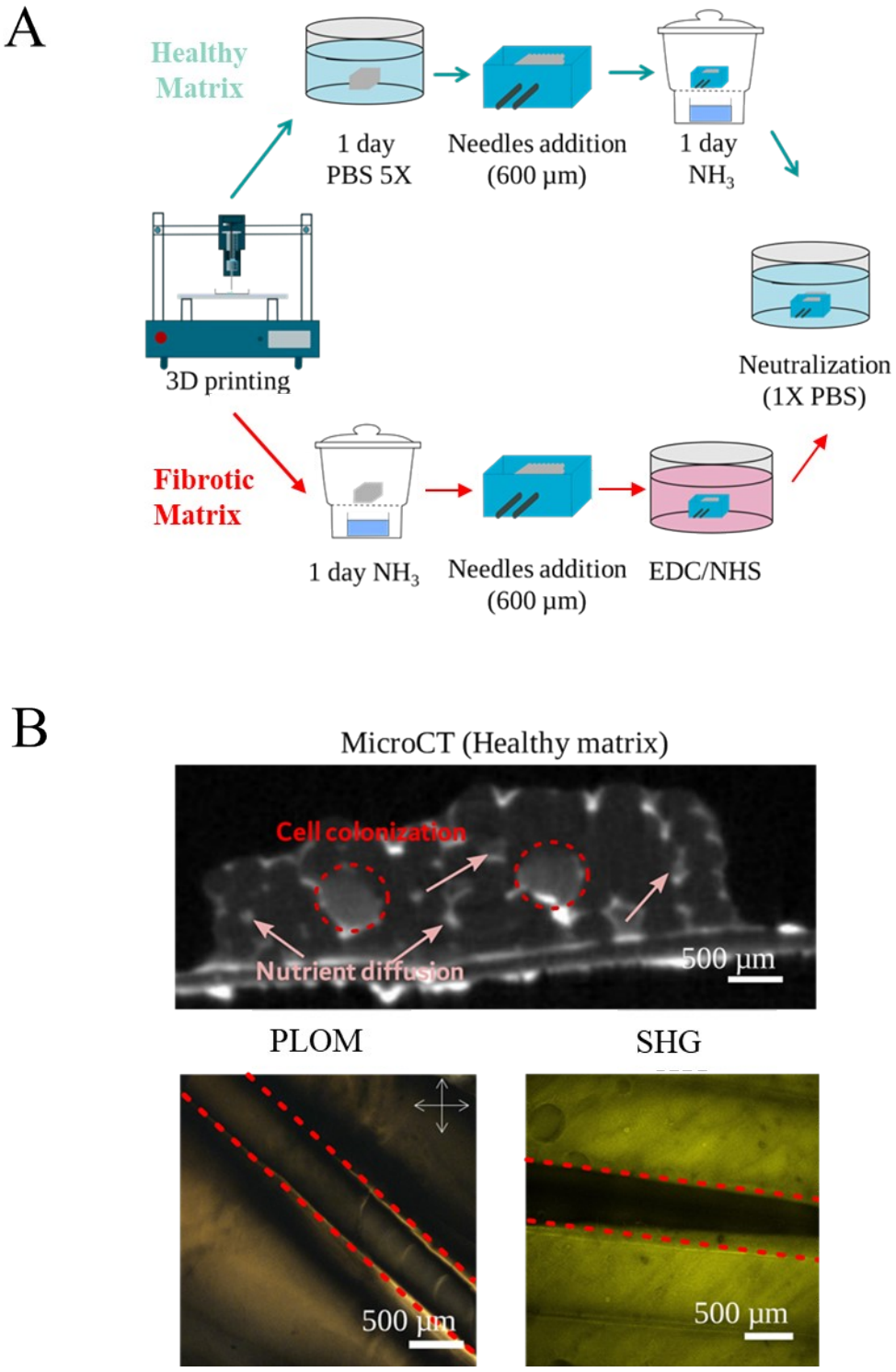
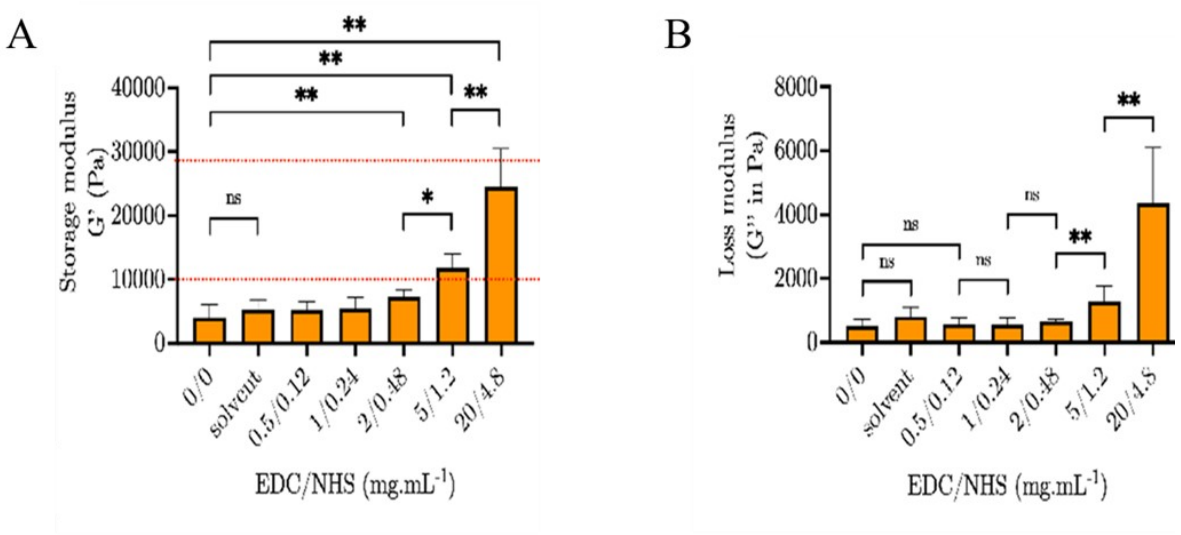
EDC	**NHS**
0 $mg \cdot mL^{-1}$	0 $mg \cdot mL^{-1}$
0.5 $mg \cdot mL^{-1}$ (3.2 mM)	0.12 $mg \cdot mL^{-1}$ (1 mM)
1 $mg \cdot mL^{-1}$ (6.4 mM)	0.24 $mg \cdot mL^{-1}$ (1.5 mM)
2 $mg \cdot mL^{-1}$ (12.8 mM)	0.48 $mg \cdot mL^{-1}$ (3.1 mM)
5 $mg \cdot mL^{-1}$ (32.2 mM)	1.2 $mg \cdot mL^{-1}$ (7.7 mM)
20 $mg \cdot mL^{-1}$ (129 mM)	4.8 $mg \cdot mL^{-1}$ (30.1 mM)

Supplementary Information 1: Cross-linker concentrations tested to rigidify dense collagen matrices.



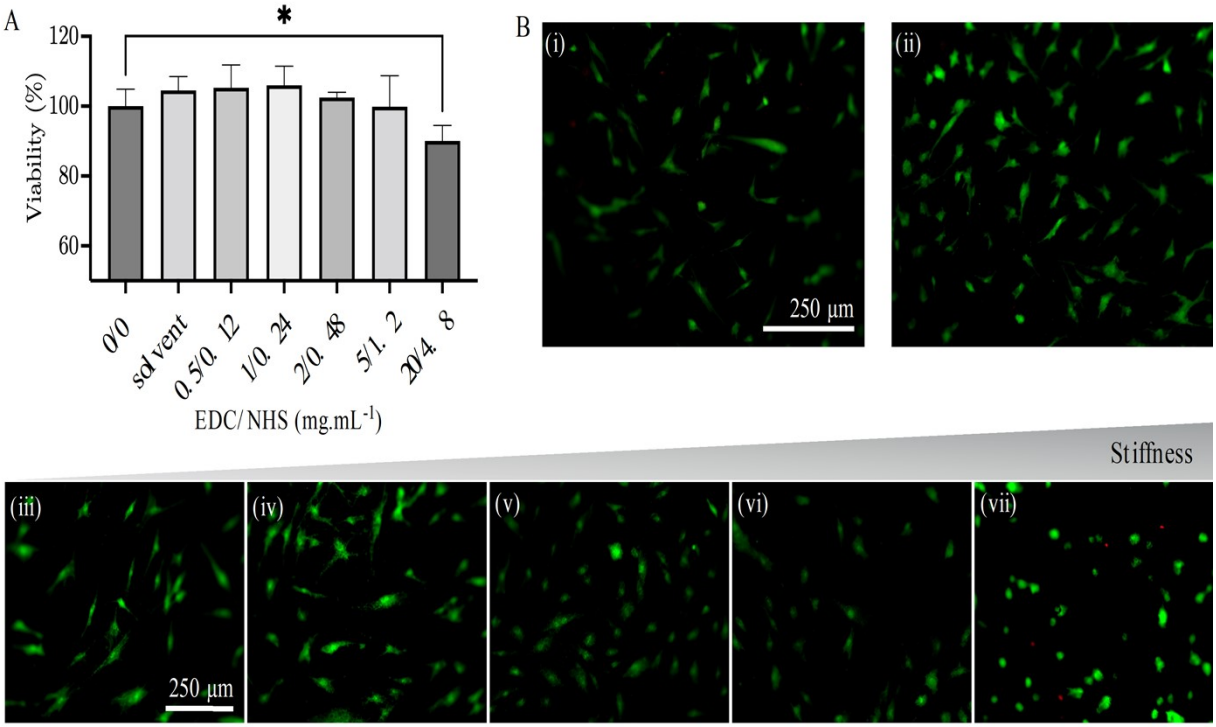
Supplementary Information 2: (A) Generation of macroporosity dedicated to cell colonization by introduction of needles (B) macroposoity observed by X-Ray microtomography (MicroCT), Polarized Light Microscopy (PLOM) and second harmonic generation microscopy (SHG).



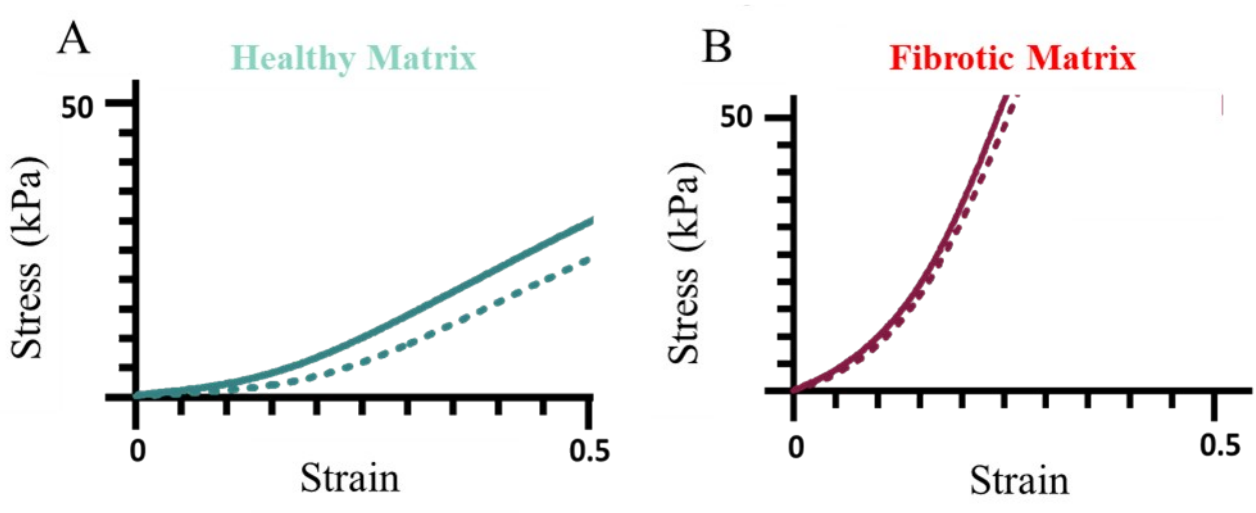
C

Matrix	EDC/NHS (mg.mL^{-1})	tan δ
Healthy Matrix	0/0	0.11
Fibrotic Matrix	5/1.2	0.1

Supplementary Information 3: Rheological properties of casted collagen matrices after cross-linking with EDC/NHS. (A) storage modulus, (B) Loss modulus and (C) tan delta according to the EDC/NHS ratio used (n=4); *: $p \leq 0.05$.



Supplementary Information 4: EDC/NHS cytotoxicity on normal human dermal fibroblasts. (A): Alamar blue assay (4 hours) to assess cell metabolism. (B): Live (green)/Dead (red) staining for the different concentrations of cross-linkers. Collagen hydrogel (i) without cross-linker, (ii) with Ethanol 75%, (iii) EDC/NHS=0.5/0.12 mg.mL-1, (iv) EDC/NHS=1/0.24 mg.mL-1, (v) EDC/NHS=2/0.48 mg.mL-1, (vi) EDC/NHS=5/1.2 mg.mL-1, (vii) EDC/NHS=20/4.8 mg.mL-1. *: $p<0.05$.



Supplementary Information 5: Mechanical properties of 3D printed healthy (A) and pathological matrices (B) assessed by tensile tests. Matrices were longitudinally stretched (plain lines) or perpendicularly stretched (dotted lines).

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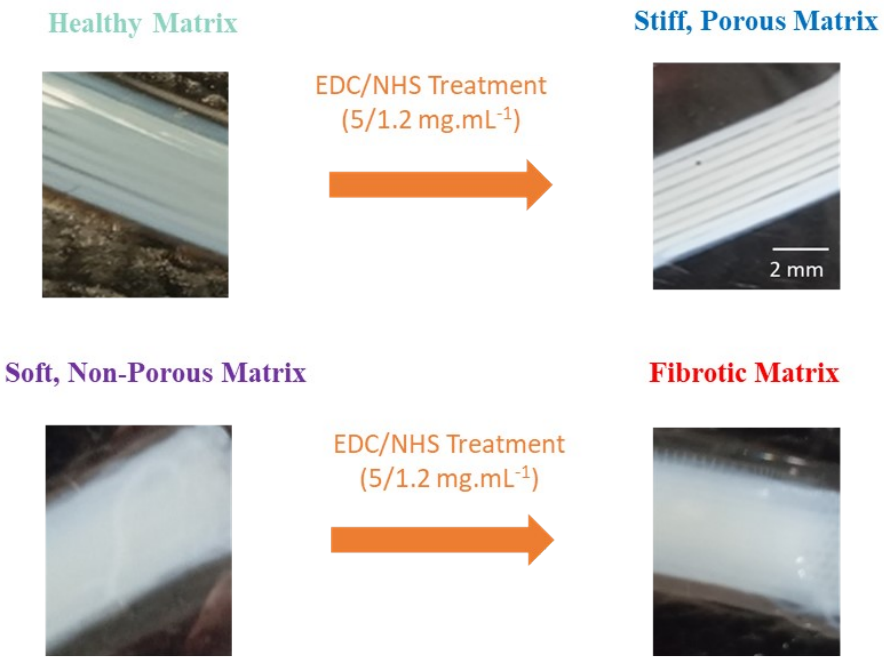
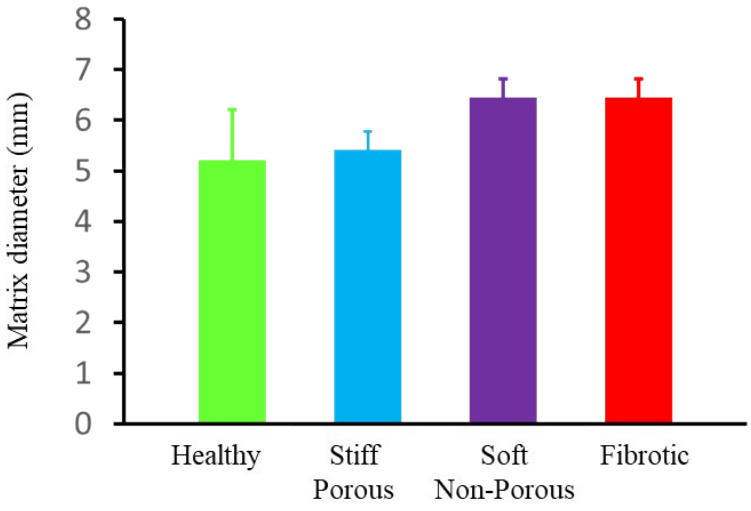
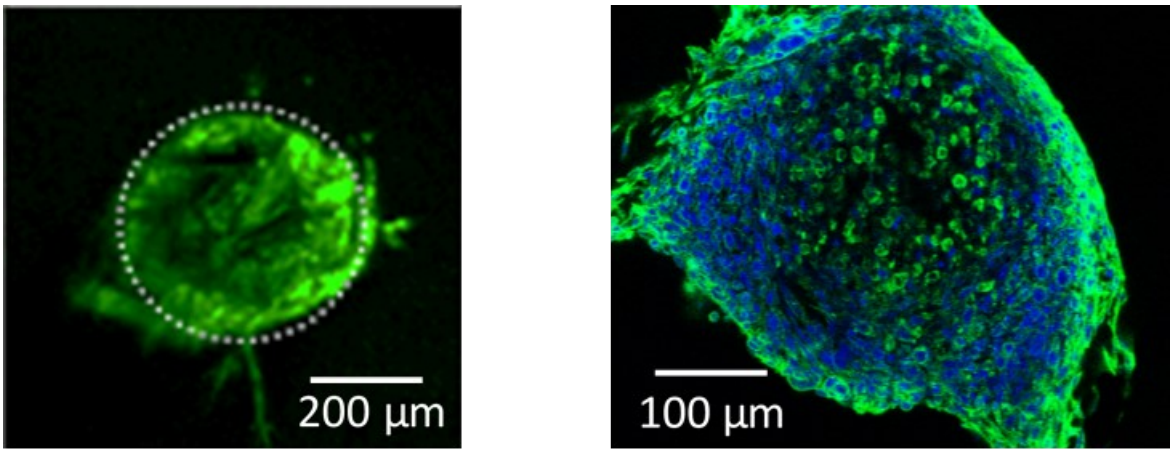
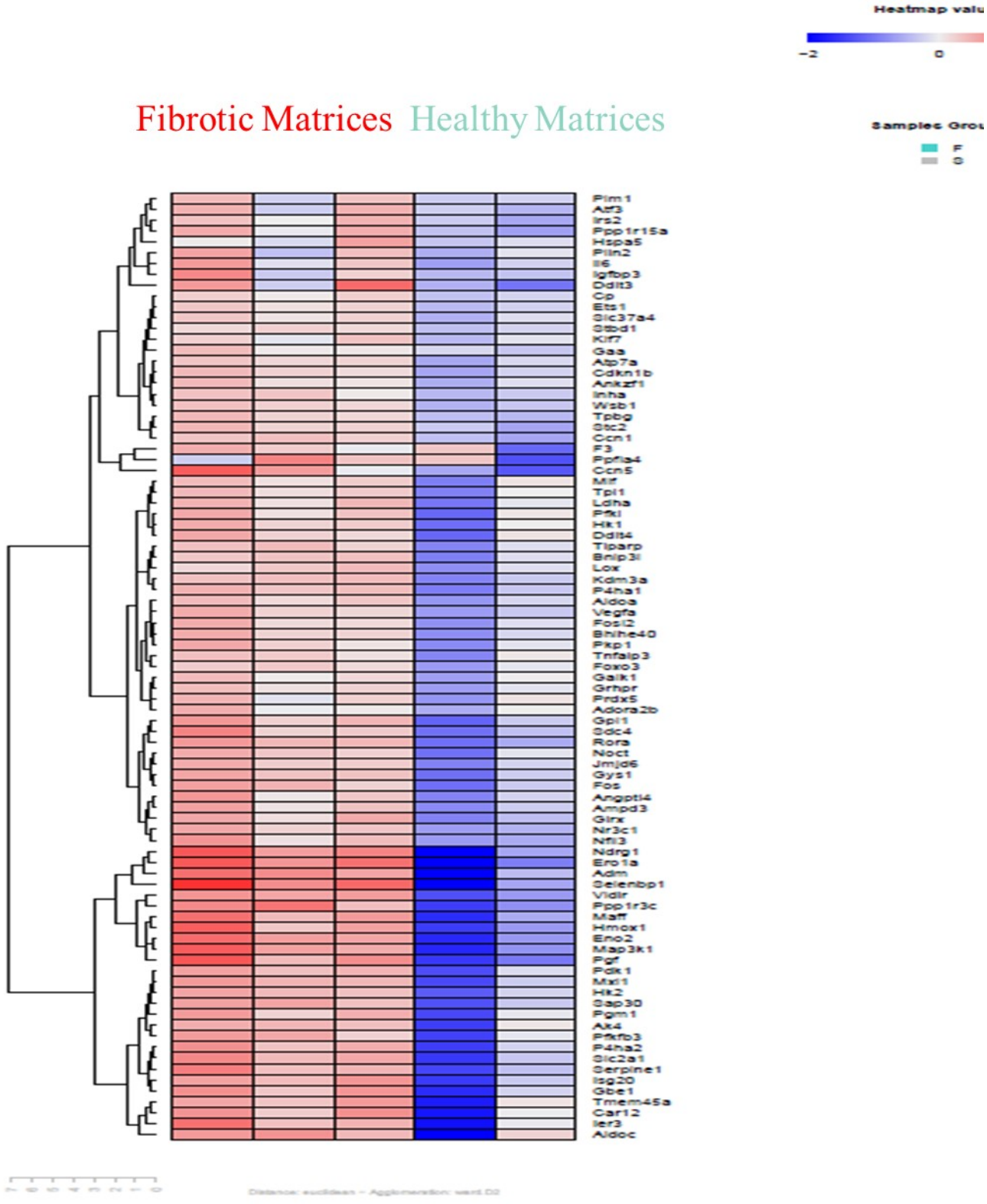




Supplementary Information 6: Effect of the cross-linking with EDC/NHS on the structure of 3D printed collagen matrices. Evaluation of a potential shrinking/swelling.by measure of the matrix diameter before and after cross-linking.



Supplementary Information 7: Transversal view of macropores dedicated to cell colonization after 5 days in culture. Nuclei labelled in blue (DAPI) and cytoskeleton in green (Alexa Fluor 488 Phalloidin).



Supporting Information 8: Hierarchical clustering of genes in response to hypoxia. Comparison Fibrotic matrices versus healthy matrices.

Glycolysis

Gene Symbol	Gene Name	Regulation	Fold-Change
Hk2	hexokinase 2	up	2,18
Hk1	hexokinase 1	up	1,75
Pkm	pyruvate kinase, muscle	up	1,70
Gpi1	glucose-6-phosphate isomerase 1	up	2,24
Pcx	pyruvate carboxylase	up	2,21
Ldha	lactate dehydrogenase A	up	1,79

VEGF Signalling

Gene Symbol	Gene Name	Regulation	Fold-Change
Vegfd	vascular endothelial growth factor D	up	3,72
Vegfa	vascular endothelial growth factor A	up	1,79
Vegfb	vascular endothelial growth factor B	up	1,66

ROS metabolic process

Gene Symbol	Gene Name	Regulation	Fold-Change
Gpx3	glutathione peroxidase 3	up	5,04
Gstp1	glutathione S-transferase, pi 1	up	1,73
Nos2	nitric oxide synthase 2, inducible	up	2,81
Sod2	superoxide dismutase 2, mitochondrial	up	1,81
Nox4	NADPH oxidase 4	up	1,70

Supporting Information 9: Comparison fibrotic matrices vs healthy matrices. Regulation of genes involved in glycolysis, VEGF signaling and ROS metabolic process.

A

Inflammation

Link to Pathway	Pathway Description	Nb Genes in Pathway	Nb Regulated Genes (Up / Down)	P-Value (All)
mmu04668	TNF signaling pathway	110	24 (16/8)	2,21E-07
WP387	IL-6 signaling Pathway	99	11 (9/2)	6,75E-02
WP37	IL-1 Signaling Pathway	37	6 (6/0)	3,50E-02

Supplementary Information 10: Pathways analysis of genes involved in inflammation.

A

ECM-Receptor Interactions

Gene symbol	Gene name	Regulation	Fold change
Agrn	agrin	down	1,75
Cd44	CD44 antigen	down	1,65
Col4a1	collagen, type IV, alpha 1	down	1,94
Col4a2	collagen, type IV, alpha 2	down	2,05
Col4a5	collagen, type IV, alpha 5	down	1,97
Col6a1	collagen, type VI, alpha 1	down	1,80
Col6a2	collagen, type VI, alpha 2	down	2,04
Col6a3	collagen, type VI, alpha 3	down	2,10
Hmmr	hyaluronan mediated motility receptor (RHAMM)	down	8,10
Itgb3	integrin beta 3	down	1,71
Itgb7	integrin beta 7	up	1,89
Spp1	secreted phosphoprotein 1	down	1,53
Sdc4	syndecan 4	up	2,28
Itga10	integrin, alpha 10	down	4,77
Thbs2	thrombospondin 2	down	1,65
Thbs3	thrombospondin 3	down	2,31
Tnc	tenascin C	down	4,61
Cdh15	cadherin 15	down	1,53
Has1	hyaluronan synthase 1	down	2,14
Has2	hyaluronan synthase 2	down	1,90

B

Metalloproteinases

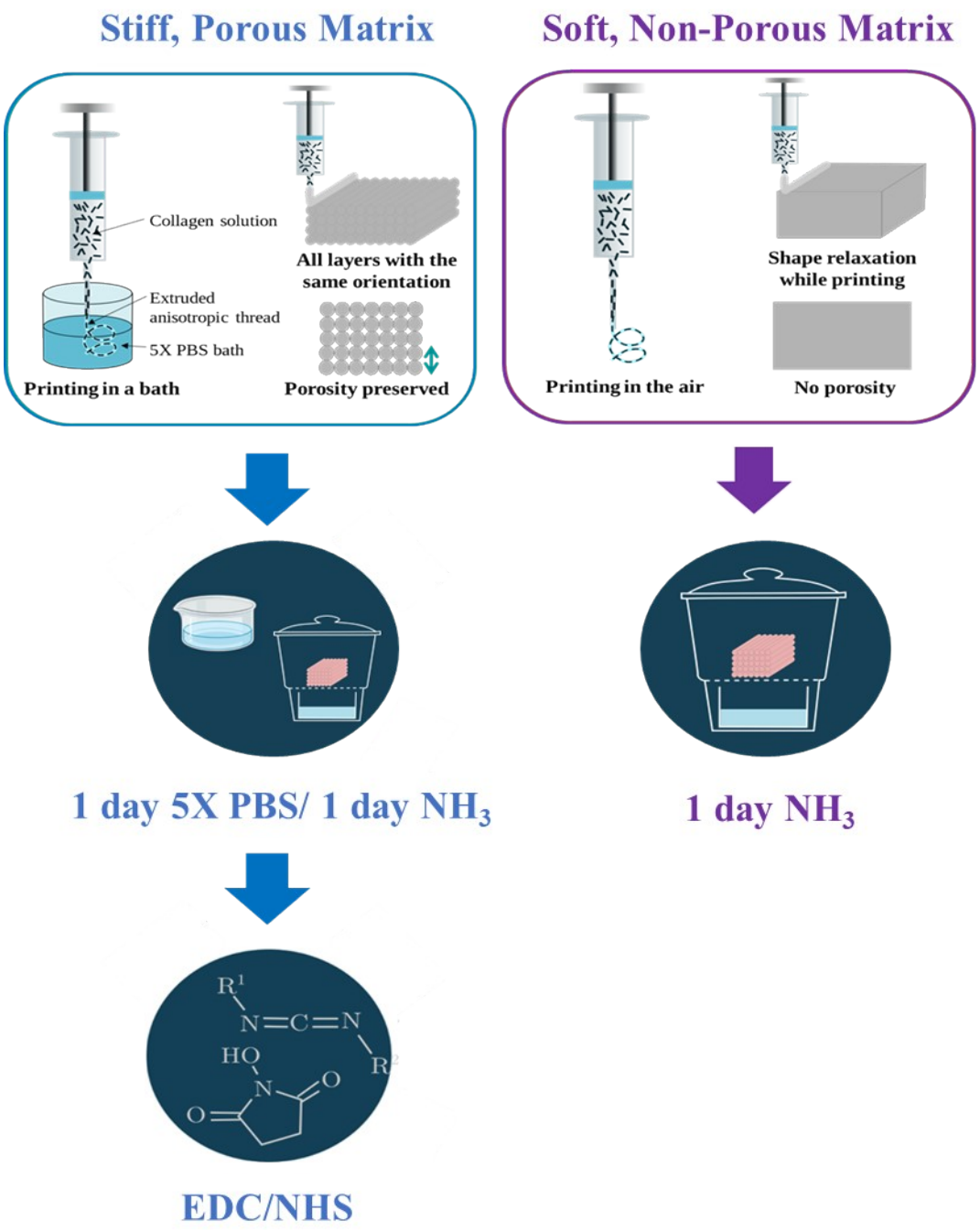
Gene symbol	Gene name	Regulation	Fold change
Mmp9	matrix metallopeptidase 9	down	5,86
Mmp14	matrix metallopeptidase 14 (membrane-inserted)	down	1,55
Timp1	tissue inhibitor of metalloproteinase 1	down	1,80
Mmp16	matrix metallopeptidase 16	up	2,73

C

Collagen maturation and Crosslinking

Gene symbol	Gene name	Regulation	Fold change
Plod1	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	up	2,20
Plod2	procollagen lysine, 2-oxoglutarate 5-dioxygenase 2	up	2,02
P4ha1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha 1 polypeptide	up	2,05
P4ha2	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha II polypeptide	up	2,44
P4hb	prolyl 4-hydroxylase, beta polypeptide	up	2,74
Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1	up	2,69
Loxl4	lysyl oxidase-like 4	up	1,82
Lox	lysyl oxidase	up	1,69

Supplementary Information 11: Modulation of genes involved in ECM synthesis, maturation and remodeling. (A) Genes involved in cell/ECM interactions, (B) Metalloproteinases and (C) collagen maturation and cross-linking.



Supplementary Information 12: Fabrication of stiff, porous and soft, non-porous matrices by 3D printing. Stiff, porous matrices were printed in 5X PBS, gelled in 5X PBS for one day and in ammonia vapors for another day. After several rinses to decrease the pH down to 7, matrices were cross-linked using EDC/NHS. Soft, non-porous matrices were printed in air and gelled in ammonia vapors for one day.

Pathways

Link to REACTOME Pathway	Pathway Description (REACTOME)	Nb Genes in Pathway	Nb Regulated Genes (Up / Down)	P-Value (All)
R-MMU-1592389	Activation of Matrix Metalloproteinases	36	6 (1/5)	1,04E-03
R-MMU-8948216	Collagen chain trimerization	43	11 (7/4)	9,97E-08
R-MMU-1442490	Collagen degradation	68	13 (7/6)	2,81E-07
R-MMU-1650814	Collagen biosynthesis and modifying enzymes	66	14 (10/4)	2,44E-08
R-MMU-2022090	Assembly of collagen fibrils and other multimeric str	70	16 (10/6)	7,56E-10
R-MMU-1474290	Collagen formation	98	20 (13/7)	5,22E-11

Matrix metallopeptidases

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Extracellular matrix organization	Mmp11	matrix metallopeptidase 11	down	1,88	1,28E-04
Extracellular matrix organization	Mmp13	matrix metallopeptidase 13	down	4,56	3,49E-03
Extracellular matrix organization	Mmp2	matrix metallopeptidase 2	down	1,59	1,52E-02
Extracellular matrix organization	Mmp9	matrix metallopeptidase 9	down	3,41	1,80E-05

Adhesion Receptors

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Extracellular matrix organization	Itga11	integrin alpha 11	up	1,87	1,83E-03
Extracellular matrix organization	Sdc1	syndecan 1	down	1,63	2,90E-05
Extracellular matrix organization	Itga10	integrin, alpha 10	down	3,60	6,88E-06

Colllagens

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Extracellular matrix organization	Col18a1	collagen, type XVIII, alpha 1	up	1,57	8,63E-03
Extracellular matrix organization	Col5a1	collagen, type V, alpha 1	up	1,55	2,02E-02
Extracellular matrix organization	Col8a1	collagen, type VIII, alpha 1	up	2,51	2,03E-16
Extracellular matrix organization	Col1a1	collagen, type I, alpha 1	up	1,97	7,58E-04
Extracellular matrix organization	Col1a2	collagen, type I, alpha 2	up	1,77	1,96E-05

Collagen crosslinking

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Collagen formation	Lox	lysyl oxidase	up	1,70	3,49E-02
Collagen formation	Loxl4	lysyl oxidase-like 4	up	1,71	2,23E-02
Collagen formation	Pcolce2	procollagen C-endopeptidase enhancer	up	1,70	8,62E-03

ECM polymers

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Degradation of the ECM	Dcn	decorin	down	2,94	1,35E-10
Laminin interactions	Lama1	laminin, alpha 1	up	3,40	5,27E-07
Laminin interactions	Lamb3	laminin, beta 3	up	1,84	1,31E-02
extracellular structure organization	Has1	hyaluronan synthase 1	down	1,81	2,95E-05

Supplementary Information 13: Comparison stiff porous matrices vs healthy matrices. List of relevant pathways regulated (Reactome Analysis).

Pathways

Link to REACTOME or GO Pathway	Pathway Description	Nb Genes in Pathway	Nb Regulated Genes (Up / Down)	P-Value (All)
GO:0140253	cell-cell fusion	59	4 (0/4)	5,95E-02
R-MMU-1474244	Extracellular matrix organization	283	19 (7/12)	9,84E-05
R-MMU-1442490	Collagen degradation	68	8 (1/7)	2,83E-04
R-MMU-8948216	Collagen chain trimerization	43	7 (1/6)	8,55E-05
R-MMU-1474290	Collagen formation	98	10 (3/7)	1,68E-04
R-MMU-216083	Integrin cell surface interactions	77	7 (2/5)	3,08E-03
GO:0001525	angiogenesis	488	23 (15/8)	2,85E-03
GO:0031012	extracellular matrix	441	28 (8/20)	6,86E-06

List of genes

Pathway	Gene Symbol	Gene Name	Regulation	Fold Change	Statistic
Extracellular matrix organization	Emilin1	elastin microfibril interfacer 1	down	1,57	4,43E-03
Extracellular matrix organization	Col13a1	collagen, type XIII, alpha 1	down	2,23	7,16E-03
Extracellular matrix organization	Col15a1	collagen, type XV, alpha 1	down	3,16	1,58E-03
Extracellular matrix organization	Col18a1	collagen, type XVIII, alpha 1	up	1,55	1,67E-02
Extracellular matrix organization	Col6a1	collagen, type VI, alpha 1	down	1,87	2,73E-03
Extracellular matrix organization	Col6a2	collagen, type VI, alpha 2	down	1,89	1,04E-02
Extracellular matrix organization	Itga4	integrin alpha 4	down	1,80	2,54E-02

Supplementary Information 14: Comparison soft non-porous matrices vs healthy matrices. List of relevant pathways regulated (Reactome or GO Analysis).