Supporting Information

Decorin Regulates the Aggrecan Network Integrity and Biomechanical Functions of Cartilage Extracellular Matrix

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Table S1. List of primers used for quantitative RT-PCR (qPCR)

Gene	Forward Primer	Reverse Primer
Dcn	5'-TGAGCTTCAACAGCATCACC-3'	5'-AAGTCATTTTGCCCAACTGC-3'
Acan	5'-GACTGTGTGGTGATGATCTG-3'	5'-CTCGTAGCGATCTTTCTTCTG-3'
Col1a1	5'-TTCTCCTGGCAAAGACGGACTCAA-3'	5'-AGGAAGCTGAAGTCATAACCGCCA-3'
Col2a1	5'-GCTGGTGCACAAGGTCCTAT-3'	5'-ACCCTGCAGTCCAGTGAAAC-3'
Has2	5'-ACAGGCACCTTACCAACAGGGTGT-3'	5'-GCATGCATAGATCAAAGTTCCCACG-3'
Hapln1	5'-AAATGGGAATGGGTGGGTAG-3'	5'-TGGAGGGGAAATGAGGAAAG-3'
β-actin	5'-AGATGACCCAGATCATGTTTGAGA-3'	5'-CACAGCCTGGATGGCTACGT-3'
Gapdh	5'- TCAACAGCAACTCCCACTCTTCCA-3'	5'-ACCCTGTTGCTGTAGCCGTATTCA-3'

Table S2. Summary of biological, biochemical and structural assay outcomes of native joints as averaged by each animal

	mean ± 95% CI	*n*	mean ± 95% CI	*n*	Unit	*p*-value	*H*
	WT		*Dcn*$^{-/-}$				
sGAGs	6.3 ± 1.5	6	2.2 ± 0.6	6	%	<0.001	1
sGAGs (*iKO*)	5.5 ± 0.5	6	3.1 ± 0.5	6	%	0.002	1
collagen	21.2 ± 5.4	6	17.8 ± 5.8	6	%	0.240	0
aggrecan western	1.00 ± 0.00	6	0.61 ± 0.17	6	a.u.	0.002	1
$t_{uncalcified}$	48 ± 8	6	51 ± 6	6	μm	0.394	0
t_{total}	123 ± 10	6	130 ± 9	6	μm	0.132	0
$\rho_{cell,\ uncalcified}$	$(3.5 \pm 0.3) \times 10^{-3}$	6	$(3.5 \pm 0.6) \times 10^{-3}$	6	#/μm^2	0.818	0
$\rho_{cell,\ calcified}$	$(1.8 \pm 0.2) \times 10^{-3}$	6	$(1.8 \pm 0.4) \times 10^{-3}$	6	#/μm^2	0.699	0
SBP t_{medial}	114 ± 37	5	110 ± 34	5	μm	1.000	0
SBP $t_{lateral}$	112 ± 41	5	108 ± 15	5	μm	0.841	0
STB BV/TV_{medial}	59 ± 13	5	56 ± 11	5	%	1.000	0
STB $BV/TV_{lateral}$	43 ± 13	5	33 ± 17	5	%	0.095	0
STB Tb. N_{medial}	7.4 ± 0.9	5	7.0 ± 1.4	5	--	0.548	0
STB Tb. $N_{lateral}$	6.8 ± 1.2	5	6.7 ± 0.7	5	--	1.000	0
STB Tb. Th_{medial}	84 ± 26	5	69 ± 25	5	μm	0.548	0
STB Tb. $Th_{lateral}$	74 ± 15	5	54 ± 3	5	μm	0.008	1
Men. $OV_{ant.,\ medial}$	0.099 ± 0.056	5	0.105 ± 0.022	5	mm^3	1.000	0
Men. $OV_{ant.,\ lateral}$	0.098 ± 0.049	5	0.106 ± 0.024	5	mm^3	0.690	0
Men. $OV_{pos.,\ medial}$	0.017 ± 0.012	5	0.013 ± 0.006	5	mm^3	0.420	0
Men. $OV_{pos.,\ lateral}$	0.014 ± 0.006	5	0.016 ± 0.009	5	mm^3	1.000	0

Table S3. Summary of cartilage and meniscus biomechanical properties as averaged by each animal

	mean ± 95% CI	*n*	mean ± 95% CI	*n*	Unit	*p*-value	*H*
	WT		*Dcn*$^{-/-}$				
E_{ind} (3-day)	0.22 ± 0.08	5	0.19 ± 0.05	5	MPa	0.310	0
E_{ind} (1-week)	0.29 ± 0.11	5	0.32 ± 0.19	5	MPa	1.000	0
E_{ind} (2-week)	0.60 ± 0.06	11	0.39 ± 0.05	11	MPa	< 0.001	1
E_{ind} (1-month)	1.23 ± 0.18	5	0.57 ± 0.25	5	MPa	0.008	1
E_{ind} (3-month)	1.45 ± 0.21	12	0.46 ± 0.06	12	MPa	< 0.001	1
E_{ind} (meniscus)	5.27 ± 0.52	12	4.76 ± 1.29	8	MPa	0.335	0
E_H/E_L	7.0 ± 1.7	8	4.1 ± 0.7	8	--	0.002	1
δ_m	25.4 ± 3.6	8	11.3 ± 3.0	8	degree	< 0.001	1
k	$(6.1 \pm 1.2) \times 10^{-16}$	8	$(5.7 \pm 2.5) \times 10^{-15}$	8	$m^4/(N \cdot s)$	< 0.001	1
E_L	1.33 ± 0.49	8	0.48 ± 0.20	8	MPa	0.002	1
E_H	8.21 ± 2.55	8	1.52 ± 0.44	8	MPa	< 0.001	1
E_m	0.60 ± 0.27	8	0.25 ± 0.13	8	MPa	0.015	1
E_f	2.15 ± 0.33	8	0.27 ± 0.10	8	MPa	< 0.001	1
	WT (CS-GAG-depleted)		*Dcn*$^{-/-}$ (CS-GAG-depleted)				
E_H/E_L	2.2 ± 1.0	6	2.1 ± 0.4	6	--	0.937	0
δ_m	6.6 ± 4.0	6	2.2 ± 0.6	6	degree	0.065	0
k	$(1.2 \pm 0.6) \times 10^{-14}$	6	$(1.2 \pm 0.8) \times 10^{-14}$	6	$m^4/(N \cdot s)$	0.818	0
E_L	0.50 ± 0.21	6	0.47 ± 0.21	6	MPa	0.937	0
E_H	0.89 ± 0.18	6	0.98 ± 0.39	6	MPa	0.589	0
E_m	0.14 ± 0.05	6	0.20 ± 0.05	6	MPa	0.065	0
E_f	0.21 ± 0.03	6	0.25 ± 0.03	6	MPa	0.015	1
	Control		*Dcn*^{iKO}				
E_H/E_L	8.9 ± 1.7	6	4.7 ± 1.8	6	--	0.009	1
δ_m	29.3 ± 2.1	6	13.7 ± 2.2	6	degree	0.002	1
k	$(7.6 \pm 1.5) \times 10^{-16}$	6	$(7.4 \pm 1.0) \times 10^{-15}$	6	$m^4/(N \cdot s)$	0.002	1
E_L	1.40 ± 0.27	6	0.56 ± 0.31	6	MPa	0.002	1
E_H	12.45 ± 3.09	6	2.61 ± 1.97	6	MPa	0.002	1
E_m	0.98 ± 0.20	6	0.42 ± 0.26	6	MPa	0.002	1
E_f	1.25 ± 0.48	6	0.73 ± 0.25	6	MPa	0.022	1

Table S4. Summary of qPCR and DMMB analyses outcomes from *in vitro* chondrocyte culture

	mean ± 95% CI	*n*	mean ± 95% CI	*n*	Unit	*p*-value	*H*
	WT (no TGF-β)		*Dcn*$^{-/-}$ (no TGF-β)				
Dcn	1.00 ± 0.36	7	0.04 ± 0.02	7	relative mRNA (to WT without TGF-β)	< 0.001	1
Acan	1.00 ± 0.42	7	1.02 ± 0.54	7		1.000	0
Has2	1.00 ± 0.48	7	1.06 ± 0.53	7		0.805	0
Hapln1	1.00 ± 0.93	7	1.18 ± 0.31	7		0.318	0
Col2a1	1.00 ± 0.41	7	1.04 ± 0.40	7		0.805	0
Col1a1	1.00 ± 0.62	7	1.17 ± 0.46	7		0.710	0
media (2-day)	6.4 ± 1.7	13	5.4 ± 1.8	13	sGAGs (µg/10^6 cells)	0.305	0
media (4-day)	12.2 ± 2.1	13	11.9 ± 2.8	13		0.720	0
media (6-day)	17.6 ± 2.9	13	23.9 ± 4.3	13		0.035	1
media (8-day)	24.9 ± 4.4	13	36.4 ± 4.8	13		0.004	1
matrix	37.3 ± 4.0	13	23.2 ± 1.9	13		< 0.001	1
total	62.3 ± 5.6	13	59.6 ± 5.0	13		0.412	0
	WT (with TGF-β)		*Dcn*$^{-/-}$ (with TGF-β)				
Dcn	2.54 ± 1.00	7	0.05 ± 0.01	7	relative mRNA (to WT without TGF-β)	< 0.001	1
Acan	1.68 ± 0.49	7	1.78 ± 0.58	7		0.805	0
Has2	2.01 ± 0.54	7	2.01 ± 0.67	7		1.000	0
Hapln1	1.83 ± 1.04	7	1.87 ± 0.56	7		1.000	0
Col2a1	2.14 ± 0.49	7	1.98 ± 0.38	7		0.804	0
Col1a1	1.34 ± 0.56	7	1.16 ± 0.53	7		0.318	0
media (2-day)	10.4 ± 4.3	7	16.0 ± 4.3	7	sGAGs (µg/10^6 cells)	0.072	0
media (4-day)	18.6 ± 6.3	7	27.3 ± 7.4	7		0.053	0
media (6-day)	25.8 ± 6.2	7	40.0 ± 7.1	7		0.004	1
media (8-day)	38.5 ± 7.1	7	55.4 ± 5.9	7		0.002	1
matrix	49.6 ± 9.1	7	28.2 ± 2.2	7		0.004	1
total	88.1 ± 11.6	7	83.7 ± 7.1	7		0.710	0

Table S5. Summary of the distributions of collagen fibril nanostructure analyses

Region	Geno-type	n	Collagen fibril diameter d_{col} (nm)						Variance s^2 of d_{col} (nm^2)			
			Q_1	Q_2	Q_3	mean ± 95% CI	p-value	H	s^2	95% CI of s^2	p-value	H
Superficial Layer	WT	868	28	32	37	33.2 ± 0.5	0.297	0	58.7	[53.6 64.7]	< 0.001	1
	Dcn$^{-/-}$	928	27	33	39	33.6 ± 0.6			97.5	[89.2 107.1]		
Territorial Matrix	WT	213	52	60	67	59.7 ± 1.3	0.225	0	96.8	[81.1 118.8]	0.015	1
	Dcn$^{-/-}$	385	53	60	70	60.9 ± 1.1			130.8	[114.4 151.8]		
Interterritorial Matrix	WT	218	58	66	75	67.5 ± 1.8	0.145	0	177.0	[148.5 216.6]	< 0.001	1
	Dcn$^{-/-}$	253	57	68	82	69.7 ± 2.3			344.1	[292.3 414.7]		
Meniscus	WT	1,267	41	67	85	64.0 ± 1.4	0.783	0	628.6	[582.4 680.6]	0.250	0
	Dcn$^{-/-}$	1,183	45	67	83	64.3 ± 1.4			588.6	[543.9 639.0]		

Table S6. Summary of the distributions of molecular adhesion analyses

		With decorin protein					Without decorin protein					p-value	H
		n	Q_1	Q_2	Q_3	mean ± 95% CI	n	Q_1	Q_2	Q_3	mean ± 95% CI		
aggrecan *versus* aggrecan	F_{adh} (nN)	213	1.22	1.44	1.76	1.53 ± 0.06	201	0.89	1.09	1.32	1.13 ± 0.05	< 0.001	1
	E_{adh} (fJ)	213	0.77	0.88	0.97	0.87 ± 0.03	201	0.43	0.48	0.54	0.50 ± 0.02	< 0.001	1
aggrecan *versus* collagen fibrils	F_{adh} (nN)	270	1.30	2.51	4.14	2.92 ± 0.22	181	0.60	1.15	1.63	1.23 ± 0.12	< 0.001	1
	E_{adh} (fJ)	270	0.52	1.38	3.11	2.23 ± 0.27	181	0.36	0.91	1.38	0.93 ± 0.09	< 0.001	1

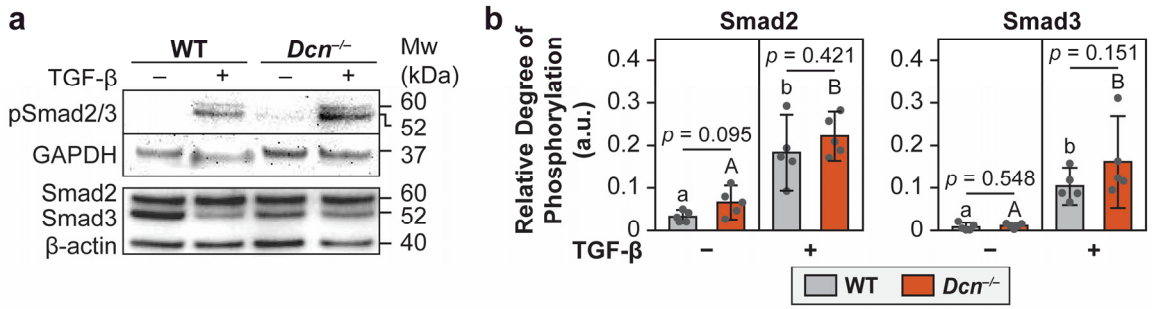

Figure S1. a) Representative western blot shows the phosphorylation of Smad2/3 under the stimulus of 10 ng/mL TGF-β1 for primary chondrocytes cultured in alginate beads in chondrogenic DMEM for 8 days, evidencing the activation of canonical TGF-β signaling in both WT and *Dcn*$^{-/-}$ chondrocytes. b) Semi-quantitative analysis shows that between WT and *Dcn*$^{-/-}$ chondrocytes shows no significant differences in the relative degree of the phosphorylation of Smad2 and Smad3 (mean ± 95% CI, n = 5 biological repeats for each group *via* Mann-Whitney U test). Different letters indicate significant differences between the untreated and TGF-β1 treated groups within each genotype *via* Mann-Whitney U test ($p < 0.01$). Each data point represents the value of one biological repeat of cells pooled from five 1-month-old mice of the same genotype.

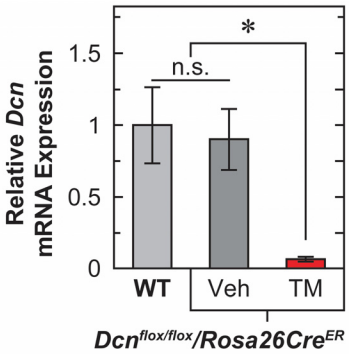

Figure S2. Confirmation of the induced knockout of *Dcn* gene in *Dcn*^{iKO} mice *via* tamoxifen administration. In 1-month-old *Dcn*$^{flox/flox}$/*Rosa26Cre*^{ER} mice, intraperitoneal (i.p.) injection of 3 mg tamoxifen (TM)/40 g body weight for 3 consecutive days reduces the expression of decorin (*Dcn*) to the baseline level (mean ± SEM, n = 5 animals, *: $p < 0.05$ *via* Kruskal-Wallis tested followed by Tukey-Kramer post-hoc multiple comparison), as tested on day 5, while injection of vehicle (Veh) does not alter the level of *Dcn* expression in comparison to the wild-type (WT) (p = 0.933).

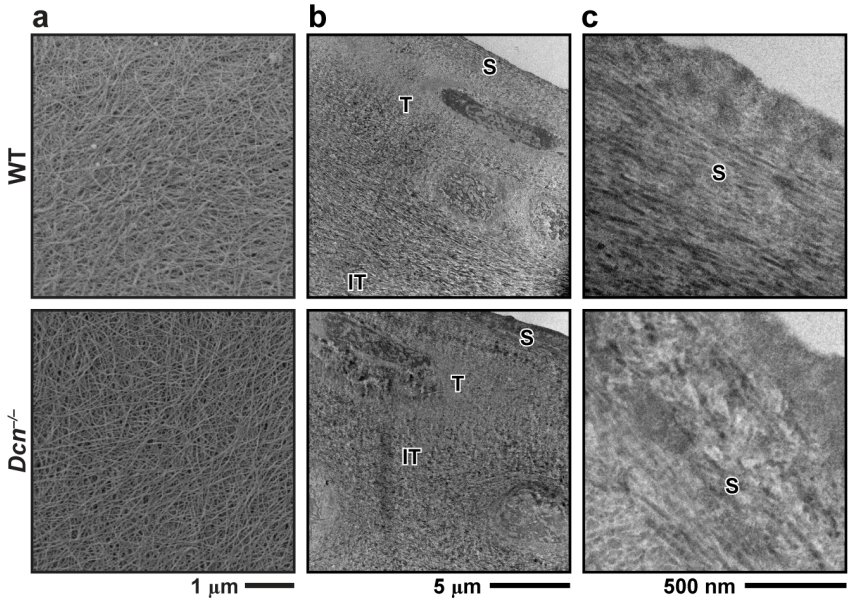

Figure S3. Scanning electron microscope (SEM) and transmission electron microscope (TEM) images of murine knee cartilage. a) Representative low resolution SEM images of cartilage surfaces from 3-month-old mice shows that cartilage surface is dominated by randomly aligned, transversely oriented collagen fibrils in both genotypes. b) Representative low resolution TEM images of cartilage sagittal sections illustrate the identification of superficial layer (S), as well as territorial (T) and interterritorial (IT) extracellular matrices of the middle/deep zone. c) Higher resolution images of the superficial layer show the presence of transversely oriented collagen fibrils in both genotypes. All the images were taken from 3-month-old WT and *Dcn*$^{-/-}$ mice.

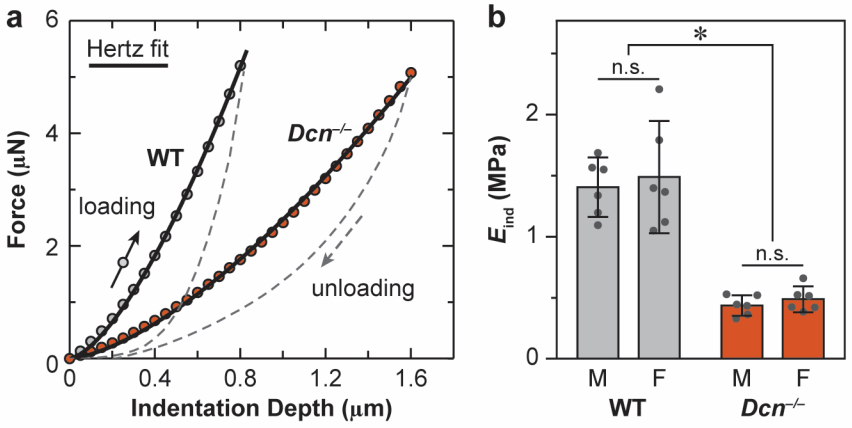

Figure S4. AFM-nanoindentation. a) Representative indentation force *versus* depth (*F-D*) curves measured on the condyle cartilage of 3-month-old WT and *Dcn*$^{-/-}$ mice, and corresponding Hertz model fit (solid line, tip radius $R \approx 12.5$ μm, nominal $k \approx 5.4$ N/m, $R^2 > 0.99$). b) Comparison of indentation modulus, E_{ind}, between cartilage of 3-month-old female and male mice (*: $p < 0.001$ between WT and *Dcn*$^{-/-}$ cartilage *via* Kruskal-Wallis test followed by Tukey-Kramer post-hoc multiple comparison). No significant animal sex-associated differences were detected ($p > 0.99$ between male (M) and female (F) mice for both genotypes).

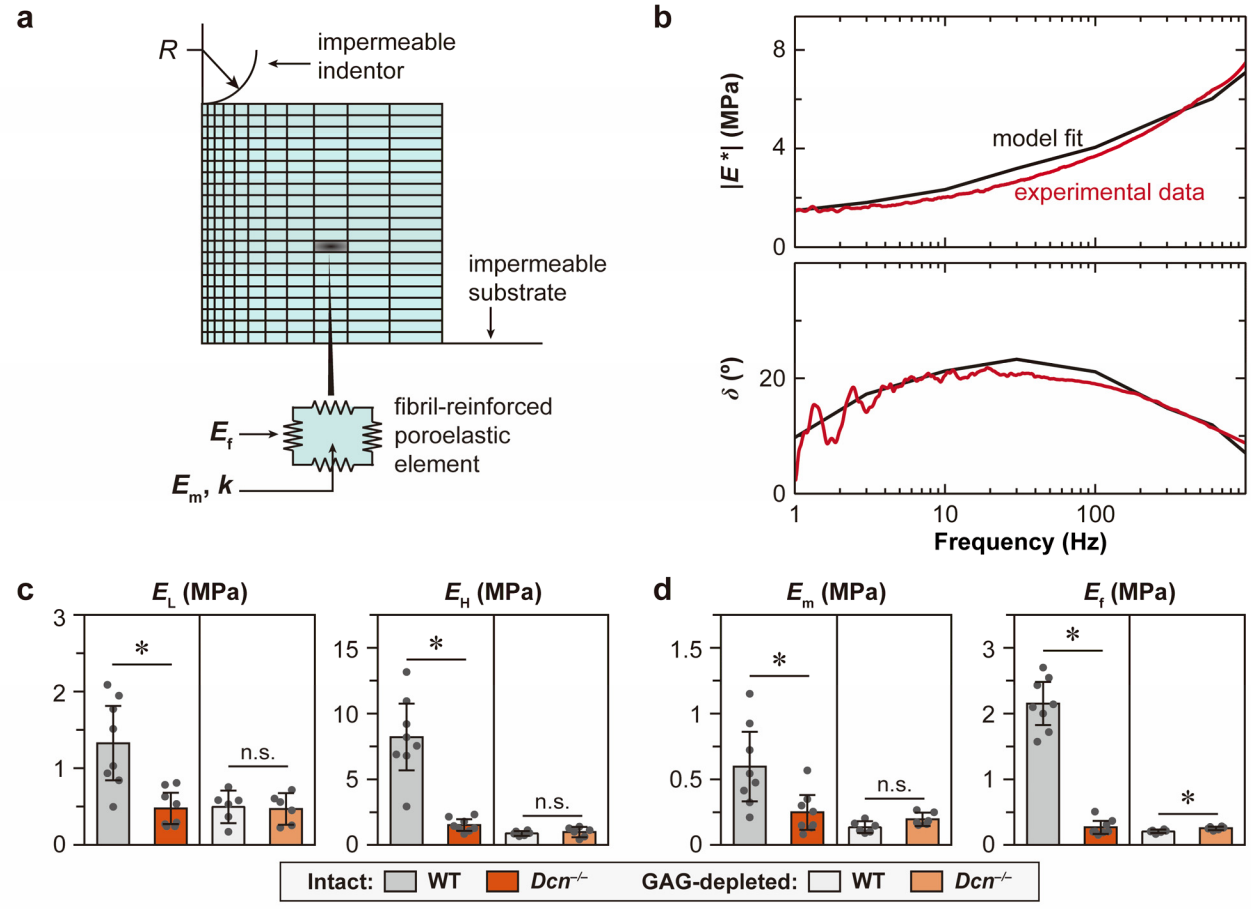

Figure S5. a) Schematics of the fibril-reinforced finite element model (FEM) for analyzing AFM-nanorheometric test outcomes. b) Representative model fit to the experimental data of dynamic modulus, $|E^*|$, and phase angle, δ, shown for one outcome measured on intact WT medial condyle cartilage at 3-month age. c-d) Additional elastic and poroelastic mechanical properties of intact and CS-GAG-depleted cartilage for both WT and *Dcn*$^{-/-}$ genotypes: c) averaged dynamic modulus at the low frequency end ($\leq$ 5 Hz), E_L, and the high frequency end (800-1,200 Hz), E_H, d) isotropic nonfibrillar matrix elastic modulus, E_m, and tensile-only Young's modulus, E_f, extracted *via* the fibril-reinforced FEM (mean ± 95% CI from n = 8 animals for intact cartilage, and n = 6 for GAG-depleted cartilage, *: $p < 0.05$ *via* Kruskal-Wallis test followed by Tukey-Kramer post-hoc multiple comparison). All the experiments were performed on 3-month-old medial condyle cartilage in 1× PBS with protease inhibitors using microspherical tips ($R \approx$ 12.5 µm, nominal $k \approx$ 16 N/m).