Supplementary Materials for

Development of Bi- and Tri-Layer Nanofibrous Membranes based on the Sulfated Polysaccharide Carrageenan for Periodontal Tissue Regeneration

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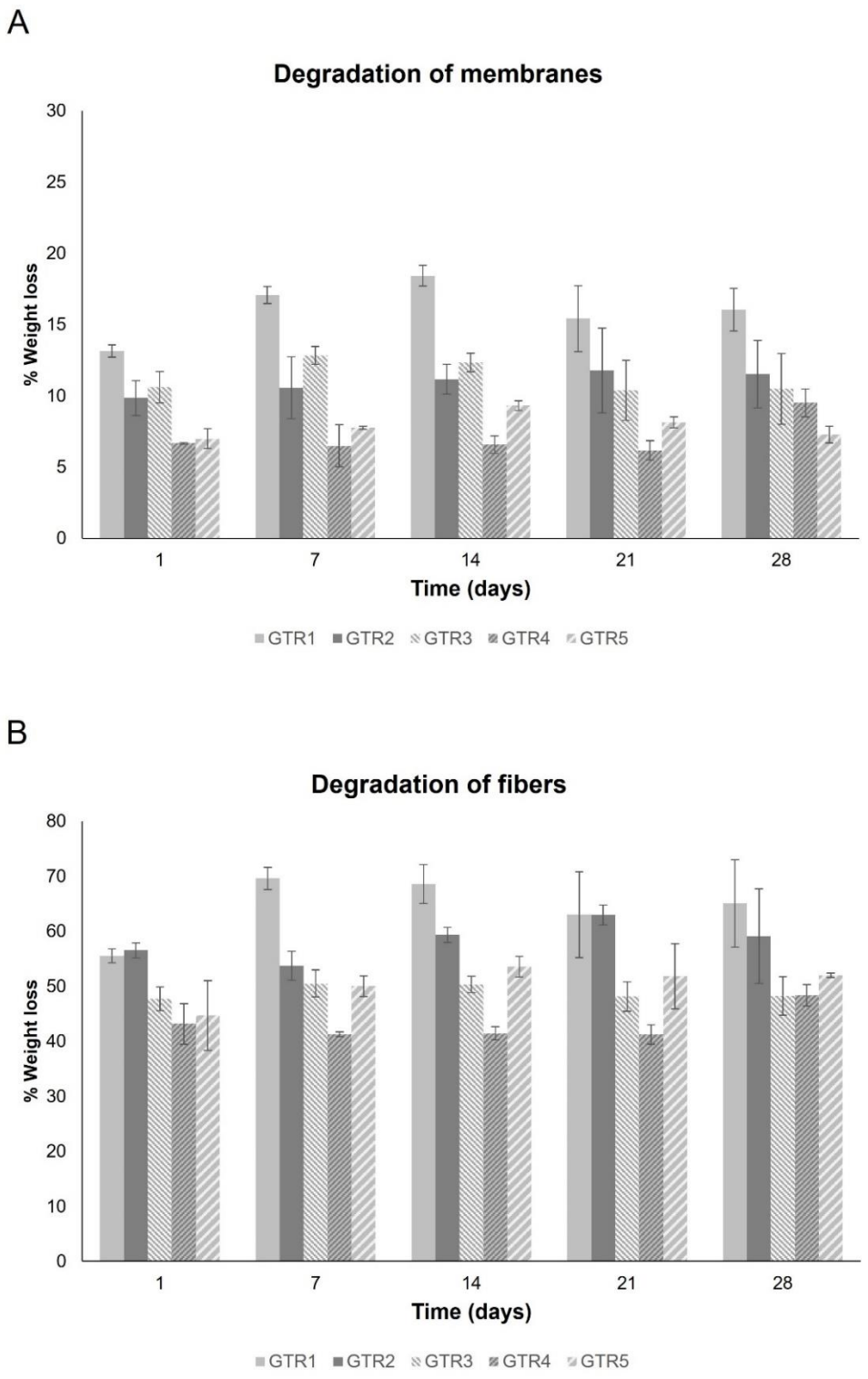

Figure S1. Degradation profiles of (**A**) GTR1, GTR2, GTR3, GTR4 and GTR5 membranes and (**B**) their fibrous layers.

Table S1. Statistical data for MTT results relating to the proliferation and growth of PDL cells. F and P values are provided for the one way ANOVA. Subsequent post hoc P values of the Dunnett t 2-sided test, refer to comparisons of GTR membrane groups with the Control group, for each time point. Statistical significance is accepted for p <0.05

time point	F value	p value	post hoc p values *vs.* CONTROL					
			GTR0	GTR1	GTR2	GRT3	GTR4	GTR5
12 H	$F_{(6,20)}$= 50.41	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18H	$F_{(6,20)}$= 13.25	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DAY 1	$F_{(6,20)}$= 63.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DAY 2	$F_{(6,20)}$= 24.88	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DAY 3	$F_{(6,20)}$= 11.66	0.000	0.000	0.000	0.001	0.000	0.000	0.018
DAY 4	$F_{(6,20)}$= 5.81	0.003	0.002	0.002	0.005	0.003	0.001	0.018
DAY 7	$F_{(6,20)}$= 30.77	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table S2. Statistical data for qRT-PCR results. F and P values are provided for the one way ANOVA. Subsequent post hoc P values of the Dunnett t 2-sided test, refer to comparisons of GTR membrane groups with the control group, for each gene and time point. Statistical significance is accepted for p <0.05.

Gene - day	F value	p value	post hoc p values *vs*. CONTROL					
			GTR0	GTR1	GTR2	GTR3	GTR4	GRT5
ALP-DAY 3	$F_{(5,35)}$= 1.902	0.125	--	--	--	--	--	--
ALP-DAY 7	$F_{(5,38)}$= 3.536	**0.012**	1.000	0.789	0.670	1.000	1.000	0.051
OCN-DAY 3	$F_{(5,40)}$= 4.776	**0.002**	0.997	**0.021**	**0.000**	**0.014**	0.529	0.541
OCN-DAY 7	$F_{(5,41)}$= 5.174	**0.001**	1.000	**0.004**	0.913	1.000	0.659	0.808
RUNX2-DAY 3	$F_{(5,37)}$= 4,14	**0.005**	0.995	**0.013**	0.995	0.07	0.995	1.0
RUNX2-DAY 7	$F_{(5,39)}$= 5.677	**0.001**	**0.025**	0.991	**0.002**	**0.001**	0.225	0.979
COL1A1-DAY 3	$F_{(5,39)}$= 7.571	**0.000**	**0.003**	0.087	**0.000**	**0.000**	**0.000**	0.935
COL1A1-DAY 7	$F_{(5,39)}$= 18.139	**0.000**	0.231	0.716	**0.025**	**0.046**	0.522	**0.000**

Table S3. Primers used for qRT-PCR analysis.

Gene	forward primers	reverse primers
gapdh	5′-TCTTCACCACCATGGAGAA-3′	5′-ACTGTGGTCATGAGCCCTT-3′
ALP	5′-GACCTCCTCGGAAGACACTC-3′	5′-TGAAGGGCTTCTTGTCTGTG-3′
OCN	5′-CGCAGCCACCGAGACACCAT-3′	5′-AGGGCAAGGGGAAGAGGAAAGAA-3′
RUNX2	5′-CCGCACGACAACCGCACCAT-3′	5′-CGCTCCGGCCCACAAATCTC-3′
COL1A1	5′-TGCTCGTGGAAATGATGGTG-3′	5′-CCTCGCTTTCCTTCCTCTCC-3′