Biomaterials Science

Electronic Supplementary Information

Dual-component Collagenous Peptide/ Reactive Oligomer Hydrogels as Potential Nerve Guidance Materials - from Characterization to Functionalization

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oPNMA- *x*				COL	base		figure	description
-*x*	**[%]**	**derivatization DEED**	**derivatization 6-AF**	**[%]**	**type**	**[%]**		
7.5	**3.5**	- -		**15**	**TEA NMPO**	**10, 8, 6, 4, 2**	**1 (S 2, S 3)**	**1:** storage modulus G´ of **cGEL**$_{monolith}$ of independent oPNMA batches and varying base concentration; **S 2:** gelation profile of **cGEL** dual component mixture; **S 3:** quantification of gelation profile S2
		-		**15**	**NMPO**	**2**	**4 (S 6)**	**4:** *in vitro* cell seeding on **cGEL**$_{disc}$ **S 6:** storage modulus of **cGEL**$_{disc}$
10	**3.5**	- x □		**7.5, 10, 12.5, 15**	**TEA NMPO NMPO**	**10, 2 2 2**	**2A**	quantification of leachable components from **cGEL**$_{disc}$
		- x		**7.5, 10, 12.5, 15**	**TEA NMPO NMPO**	**2 2 2**	**2B 3C**	storage modulus G´ of **cGEL**$_{disc}$
		- x □		**15**	**NMPO**	**6, 4, 2**	**2C**	leachables and cross-linking degree of **cGEL**$_{disc}$ and **cGEL**$_{monolith}$
		- x □		**15**	**NMPO**	**6, 4, 2**	**2D (S 4) 3D**	**2D, 3D:** storage modulus G´ of **cGEL**$_{monolith}$; **S 4:** compression test of **cGEL**$_{monolith}$
		- x		**15**	**NMPO**	**2**	**3B (S 5)**	**3B:** gelation profile of **cGEL** dual-component mixture with oPNMA derivatized for 0 h, 1 h and 4h; **S 5:** storage modulus G´ of **cGEL**$_{disc}$
		- x □		**15**	**NMPO**	**2**	**4 (S 6)**	**4:** *in vitro* cell seeding (hSGSCs) and cytotoxicity testing (L929) on **cGEL**$_{disc}$; **S 6:** storage modulus of **cGEL**$_{disc}$
			x	**15**	**NMPO**	**2**	**5C**	derivatization pattern of **cGEL**$_{conduit}$
12.5	**3.5**	- - x □		**7.5, 10, 12.5, 15**	**TEA NMPO NMPO**	**10, 2 2 2**	**2A**	quantification of leachable components from **cGEL**$_{disc}$
		- -		**7.5, 10, 12.5, 15**	**TEA NMPO**	**2**	**2B**	storage modulus G´ of **cGEL**$_{disc}$
		- x		**15**	**NMPO**	**6, 4, 2**	**2C**	leachables and cross-linking degree of **cGEL**$_{disc}$ and **cGEL**$_{monolith}$
		- x		**15**	**NMPO**	**6, 4, 2**	**2D 3D**	storage modulus G´ of **cGEL**$_{monolith}$
		x		**15**	**NMPO**	**2**	**5A, 5B (S 6)**	characterization of **cGEL**$_{conduit}$ **5A:** conduit dimensions; **5B:** leachables and water content; **S 6:** conduit dimensions after rehydration

S 1 Cross-linked hydrogel cGEL composition as used in this study. Concentrations %(w/v) are given as values during gelation; Abbreviations: oligo(PEDAS-co-NiPAAm-co-MA) (oPNMA), Collagel® (collagen hydrolysate) (COL), triethylamine (TEA), *N*-methylpiperidin-3-ol (NMPO), *N,N*-diethylethylendiamine (DEED), 6-Aminoflourescein (6-AF), human sweat gland derived stem cells (hSGSCs), L929 mouse fibroblasts (L929).

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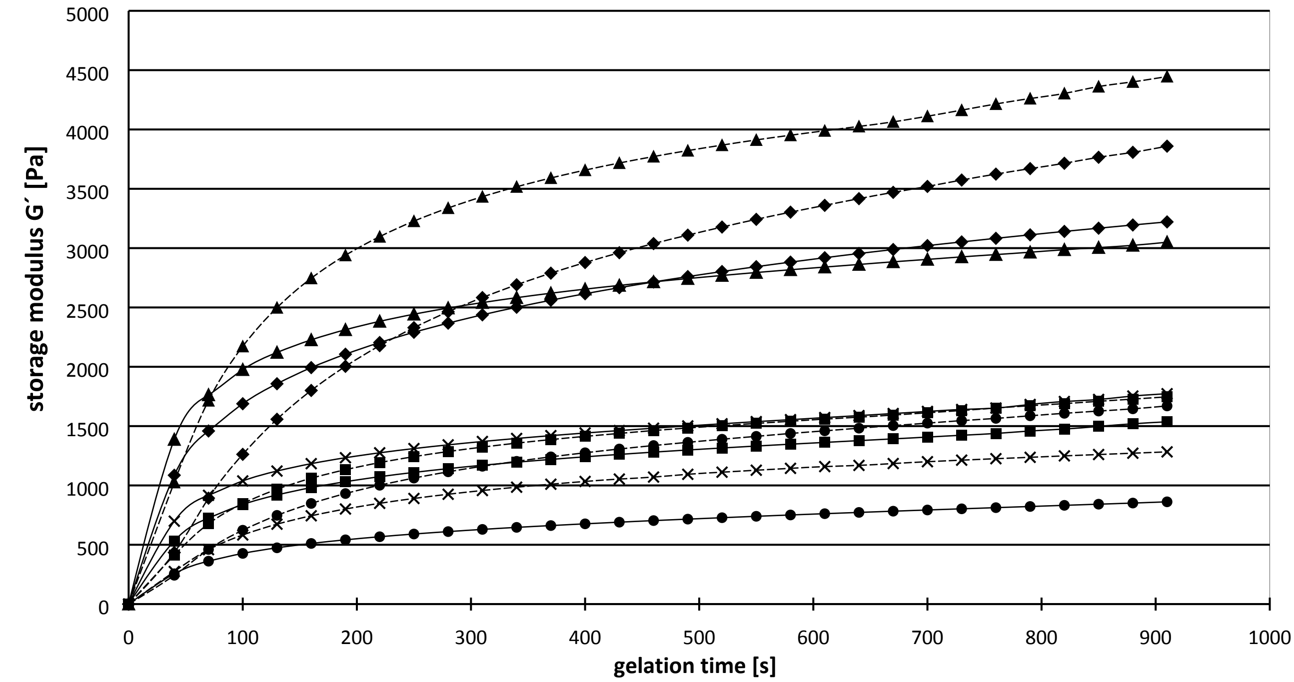
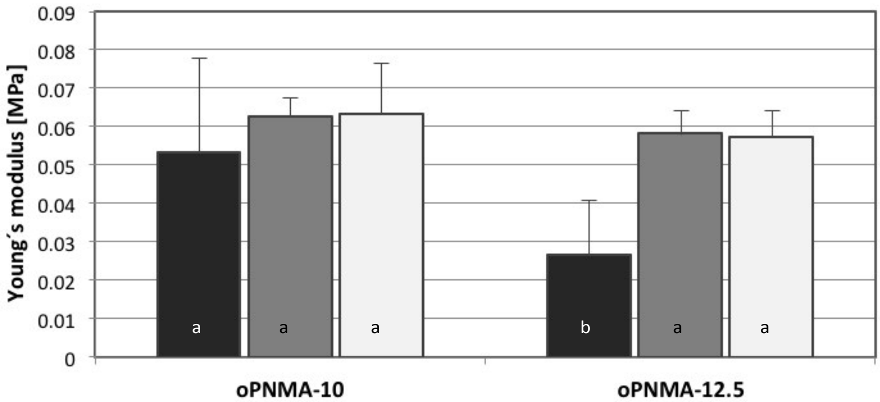
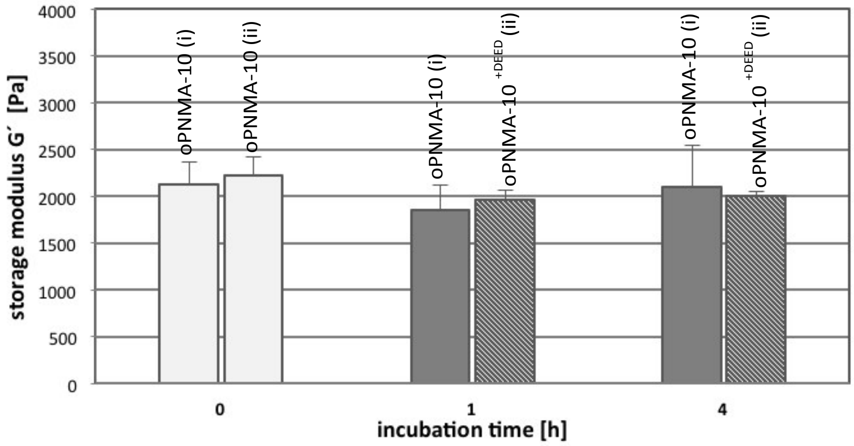


S 2 Kinetics of cGEL cross-linking. Storage moduli monitored over time for oPNMA-7.5 (3.5% in gelation mix), COL (15% in gelation mix), two base types and five base concentrations (base%). Data points are averaged (n = 5). Graphs are coded as follows: TEA (solid line), NMPO (dashed line), base%: 10% (x), 8% (□), 6% (□●), 4% (▲), 2% (◆). Base type and base% strongly affected gelation speed. Gelation decelerated at 4% and 2% NMPO. With TEA gelation was faster than with NMPO. Slowest gelation was found for 2% NMPO. Formulations with slower gelation kinetics resulted in increased storage moduli (G'_{max}) at the end of measurement.

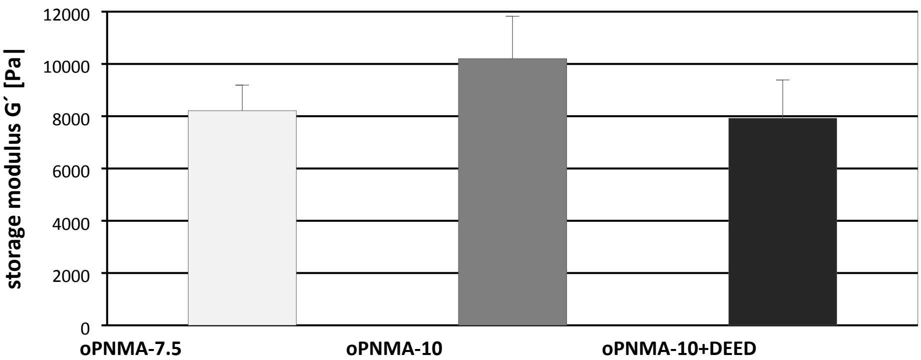
S 3 Analysis of cGEL gelatior e gelation mix). Data were
derived from the curves shov d for 10%-2% TEA (☐) and
NMPO (☐) (bars). Diamonds : standard deviation (n = 5)
and means with different lett pendent of absolute G'_{max},
the parameter $t_{0.25*G'max}$ wa: lation speed and was also
accelerated for TEA 4%. In ac ot change significantly with
reduced TEA%. Thus, TEA di elation profiles were also
extracted for NMPO. The ge Compared to NMPO 10%,
$t_{0.25*G'max}$ was significantly inc lid not directly correlate to
the gelation speed. Based on ces.



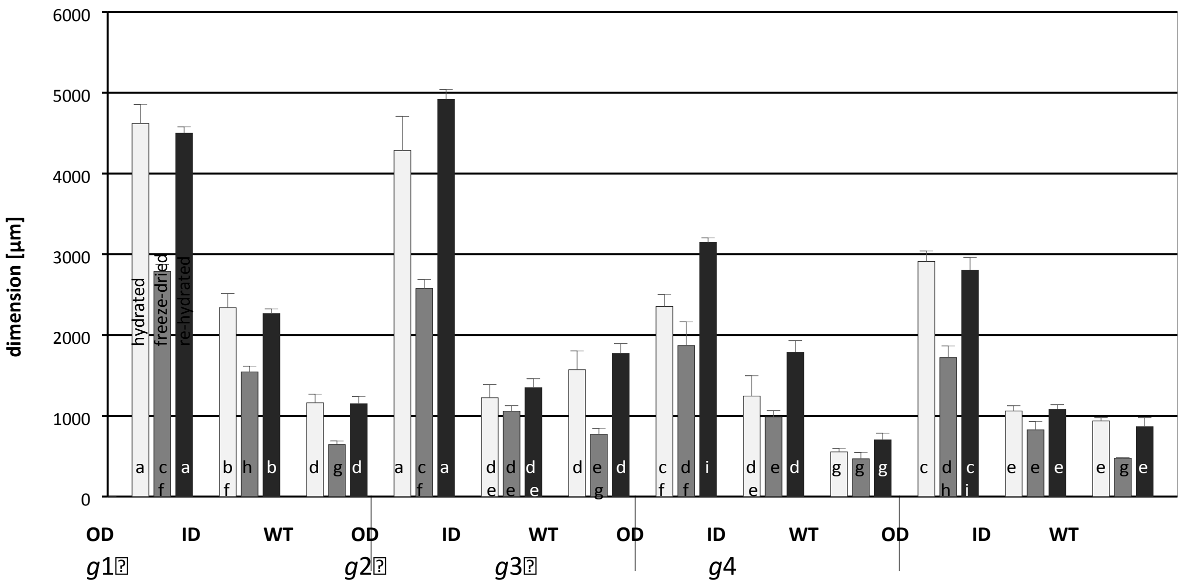
S 4 Compression test of $cGEL_{monolith}$. Young´s moduli were measured on distinct 17 mm thick $cGEL_{monolith}$ using a Sintech 5/D mechanical tester (MTS, Eden Prairie, Minnesota, USA) and 100 N load cell. Tests were carried out at a crosshead speed of 0.5 mm/min until 50% strain was reached. The force-elongation curves were converted into engineering stress-strain from which the compressive moduli were calculated. Columns represent means ± standard deviations (n = 5). Means with different letters are statistically significantly different ($p < 0.05$). The compression for cGEL cylinders from oPNMA-10 and -12.5 (3.5% in gelation mix) with COL (15% in gelation mix) and NMPO 6% (☐), 4% (☐), or 2% (☐) in gelation mix was measured. Results reflect increase of mechanical stiffness with decreased base%. Moduli were approximately one order of magnitude higher than G´ for identical formulations. Differences between oPNMA-10 and -12.5 were only statistically significant for NMPO 6%.



S 5 Storage moduli of pristine and derivatized $cGEL_{disc}$. Discs derived from COL (15% in gelation mix) and oPNMA-10 or oPNMA-10^{+DEED} (3.5% in gelation mix) with NMPO (2% in gelation mix). For measurement of moduli for pristine $cGEL_{disc}$ (☐), two oPNMA-10 solutions (*i* and *ii*) were used for fabrication. Afterwards, DEED was added to solution *ii*, derivatized to oPNMA-10^{+DEED} for 1 h and 4 h and $cGEL_{disc}{}^{+DEED}$ (striped☐) were fabricated. In parallel, solution *i* was stirred for 1 h and 4 h without DEED and control $cGEL_{disc}$ (☐) were fabricated. G´ was irrespective of functionalization and incubation time as no significant differences between pristine and derivatized $cGEL_{disc}$ were detected. Any incorporation between 1 h and 4 h can be considered without negative impact on mechanical attributes of resulting gel matrices.



S 6 Storage moduli G´ of pristine and DEED-derivatized $cGEL_{disc}$ with COL (15% in gelation mix), NMPO (2% in gelation mix) and oPNMA-7.5, -10 or -10^{+DEED} (3.5% in gelation mix) as used for cell culture experiment with hSGSCs. Data is presented as means ± standard deviations (n = 5). No statistically significant differences between groups were observed. Thus, differences in cell attachment and spreading on cGEL and $cGEL^{+DEED}$ were triggered by chemical modification and focal adhesion sites and not by differences in hydrogel stiffness.



S 7 Dimensional parameters of $cGEL_{conduit}$ fabricated from different mold assemblies. Formulations were derived from oPNMA-12.5^{+DEED} (3.5% in gelation mix), COL (15% in gelation mix) and NMPO (2% in gelation mix). Outer diameter (OD), inner diameter (ID) and wall thickness (WT) in hydrated (□), freeze-dried (□) and re-hydrated (□) state for four different conduit-molding geometries (*g*). Molds were assembled from silicone tubes and stainless steel cannula (inner diameter of silicon tubes: g_1, g_2 = 4 mm; g_3, g_4 = 2.5 mm; outer diameter of dispensing cannulas: g_1 = 1.9 mm, g_2 = 0.8 mm, g_3 = 1.65 mm, g_4 = 0.7 mm). Means with different letters are statistically significantly different ($p < 0.01$). Conduit dimensions were predefined by the mold geometry and initial dimensions were almost perfectly recovered after freeze-drying and rehydration. At the same time, conduits showed sufficient robustness during processing and predictable dimensional reconstitution after rehydration.