Tunable hydrogel networks by varying secondary structure of hydrophilic peptoids provide viable 3D cell culture platforms for hMSCs

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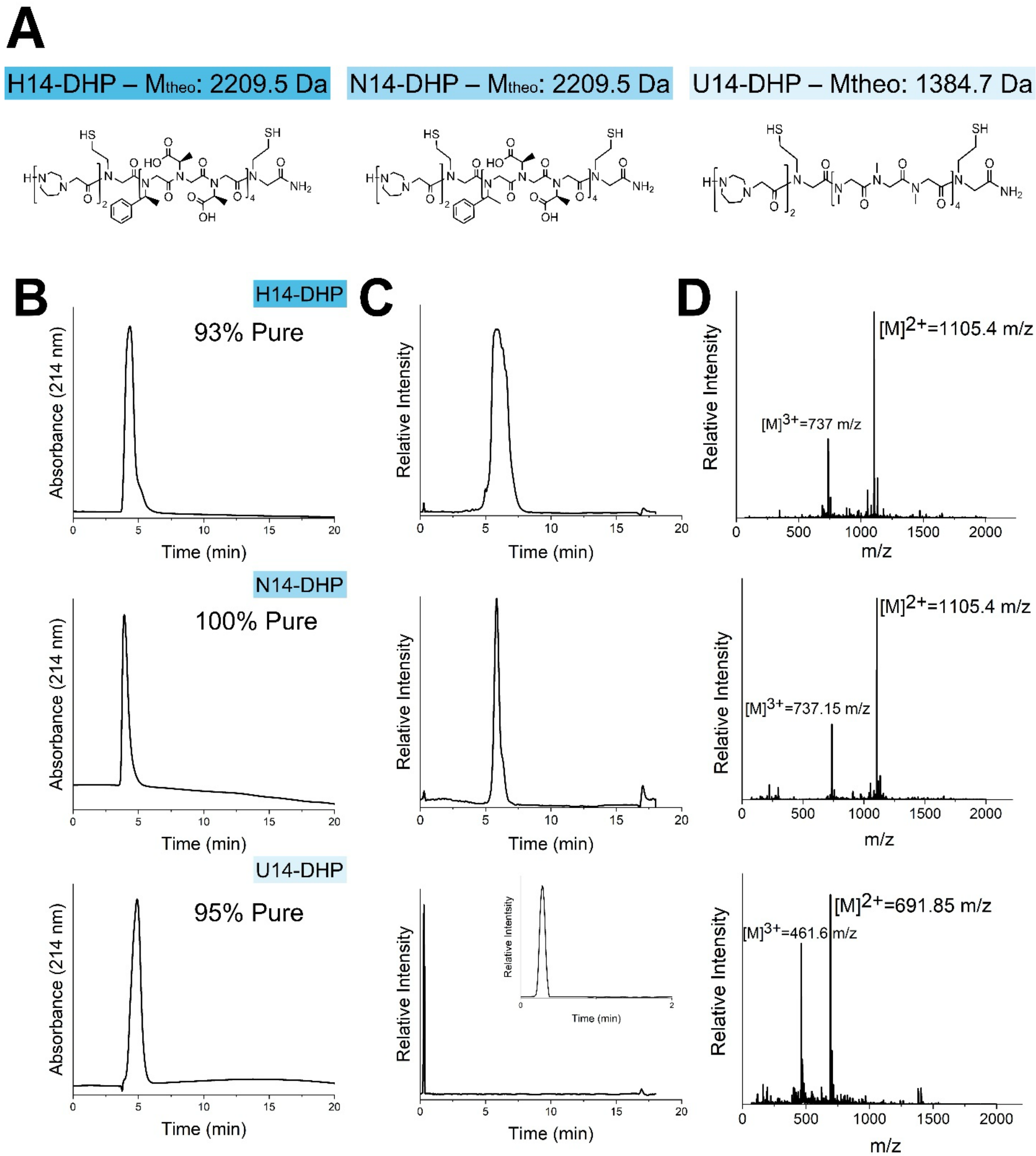

Figure S1. A) Structure of each peptoid and their theoretical masses. All hydrophilic peptoids were confirmed to be highly pure and monodisperse by B) analytical HPLC (left) monitored at 214 nm, and C) mass spectrometry of positive mode ionization (0-2000 m/z). D) ES-API mass spectrum (right) was obtained by integrating over the whole retention time of the mass chromatogram.

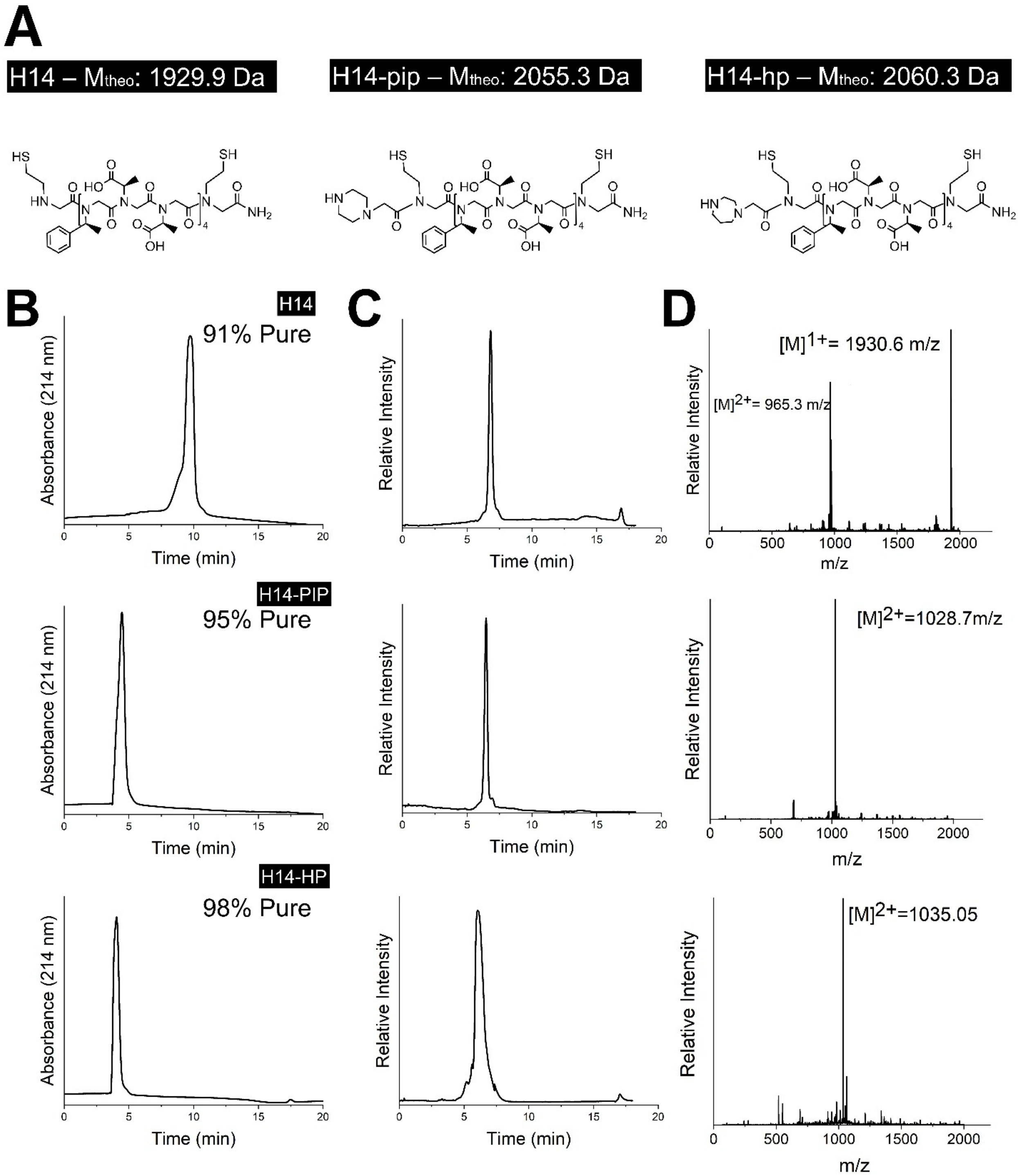

Figure S2. A) Structure of each peptoid and their theoretical masses. All hydrophilic peptoids were confirmed to be highly pure and monodisperse by B) analytical HPLC (left) monitored at 214 nm, and C) mass spectrometry of positive mode ionization (0-2000 m/z). D) ES-API mass spectrum (right) was obtained by integrating over the whole retention time of the mass chromatogram.

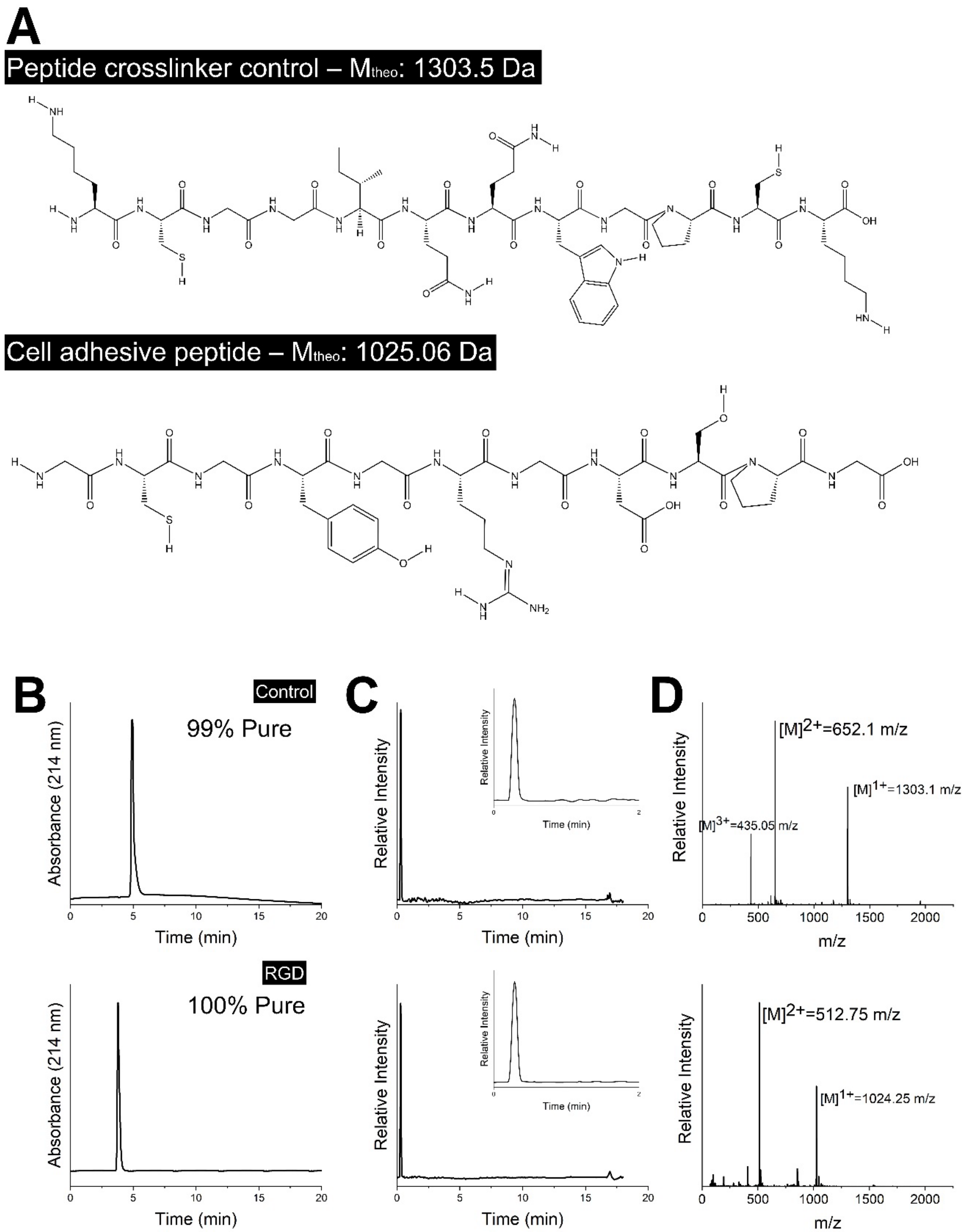

Figure S3. A) Structure of each peptide and their theoretical masses. Both peptides were confirmed to be highly pure and monodisperse by B) analytical HPLC (left) monitored at 214 nm, and C) mass spectrometry (center) of positive mode ionization (0-2000 m/z). D) ES-API mass spectrum (right) was obtained by integrating over the whole retention time of the mass chromatogram.

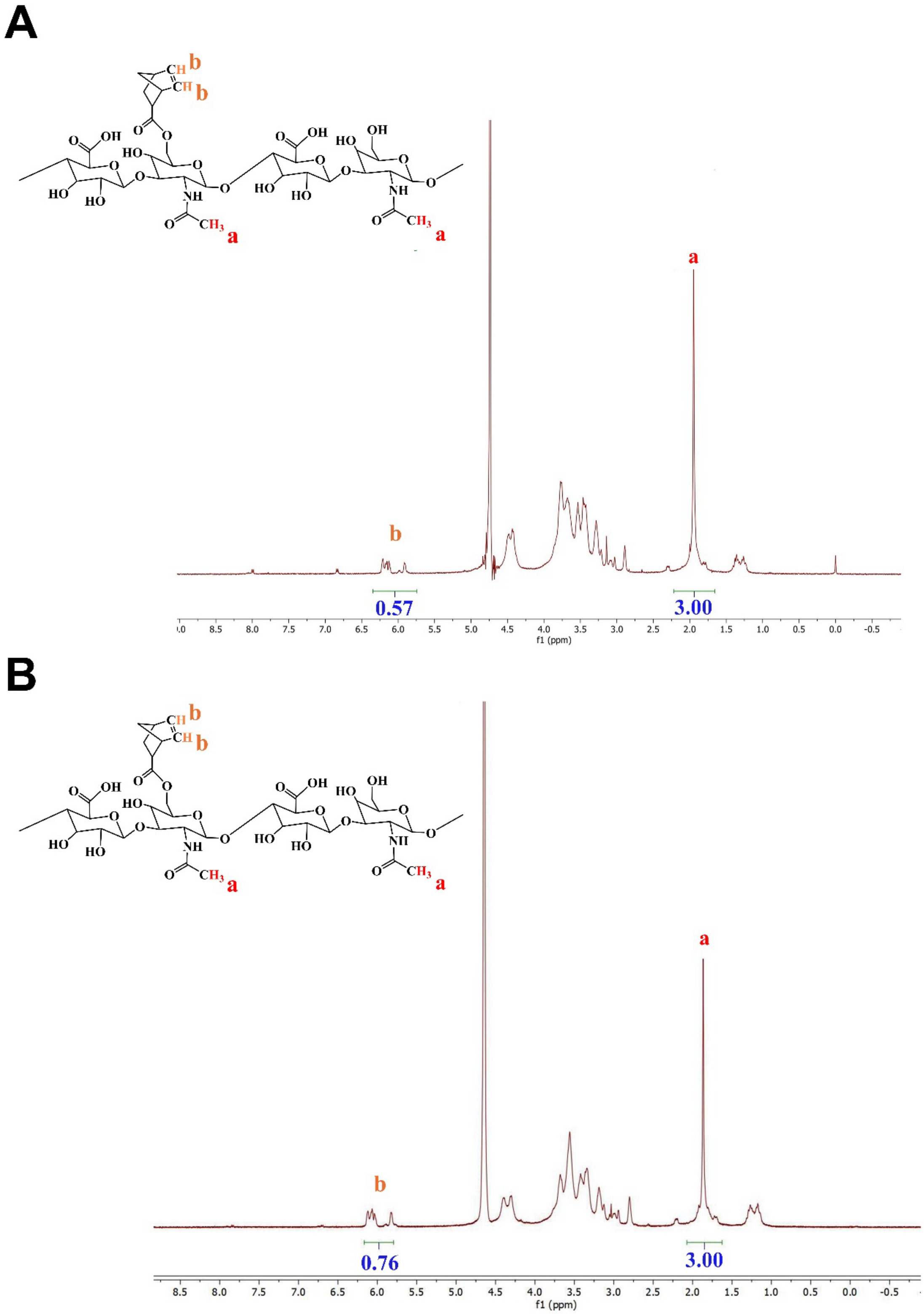

Figure S4. Norbornene-functionalized hyaluronic acid (NorHA) was synthesized to an approximate functionalization of: A) ~28% as confirmed by ^{1}H NMR for the first batch and B) ~38% as confirmed by ^{1}H NMR for the second batch.

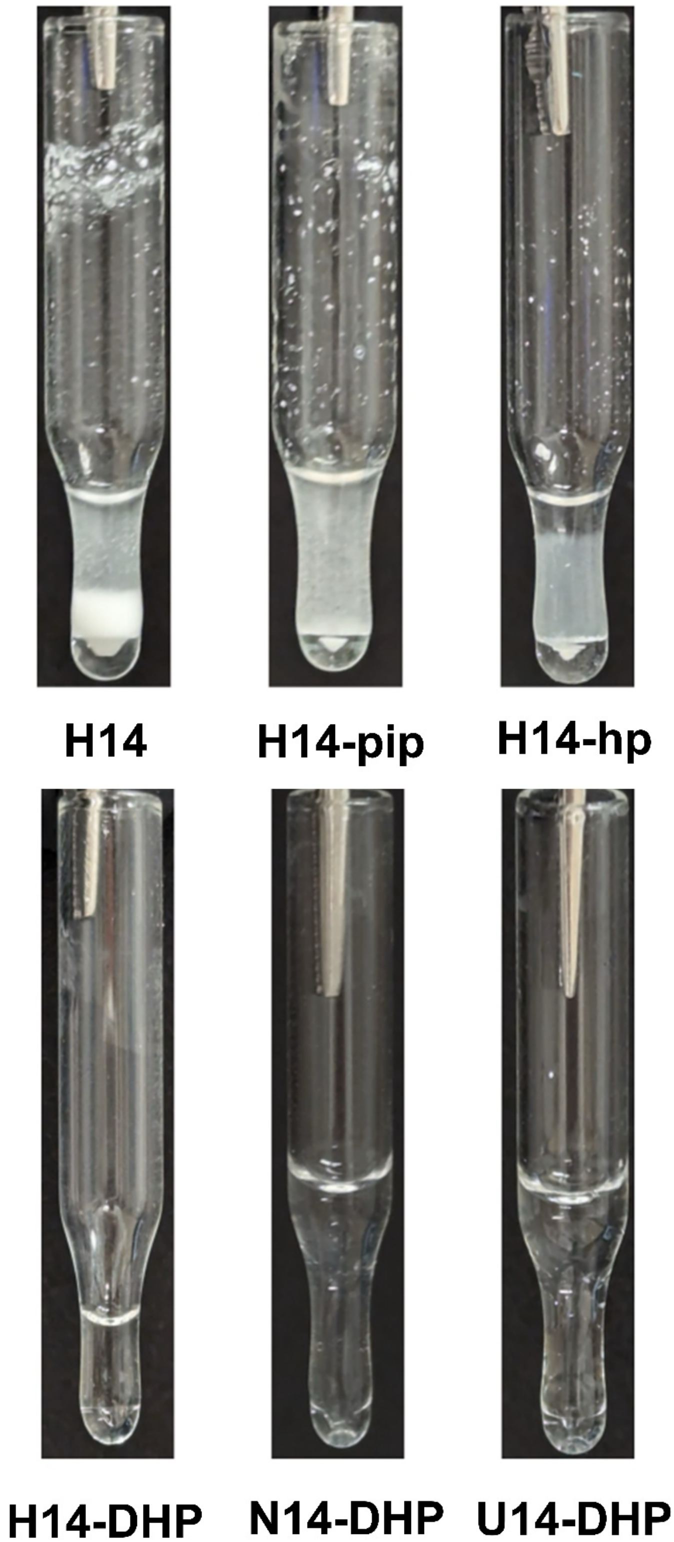

Figure S5. PBS solubility side views of each synthesized peptoid at 15 mM.

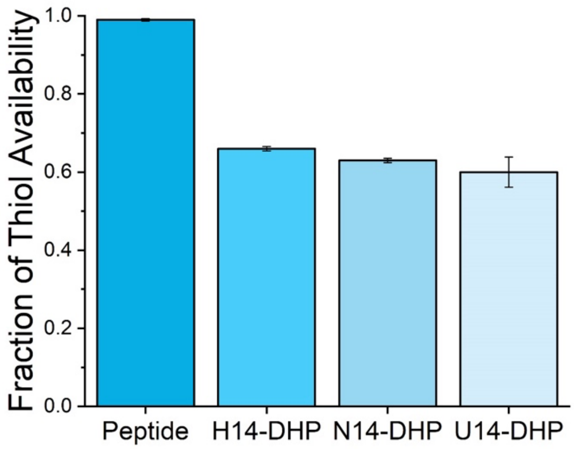

Figure S6. Free thiol content for the peptide control and all hydrophilic peptoids, as measured by Ellman's assay.

Table S1. Hydrogel formulation details.

Hydrogel Condition	mg/mL of HA	Concentration of norbornenes (mM)	LAP photoinitiator wt %	Cell adhesive peptide (mM)	Ellman's adjustment %	Concentration of thiols in hydrogel (mM)	Concentration of crosslinker in hydrogel (mM)
Control (Peptide)	30	20.16	0.05	2	99	20.16	10.18
H14-DHP	30	20.16	0.05	2	66	20.16	15.27
N14-DHP	30	20.16	0.05	2	60	20.16	16.79
U14-DHP	30	20.16	0.05	2	60	20.16	16.79

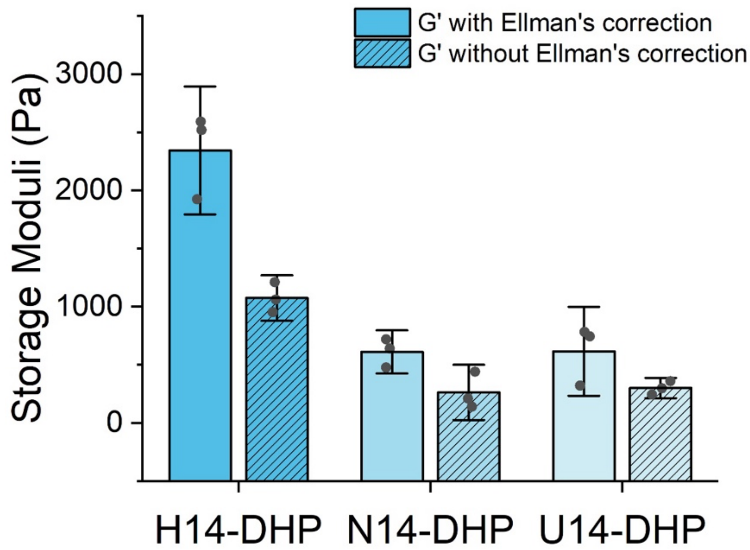

Figure S7. Storage moduli of all hydrogel conditions showing that when crosslinker amount is adjusted using Ellman's correction, elastically effective linkages are added, causing an increase in storage moduli compared to when there is not Ellman's correction.

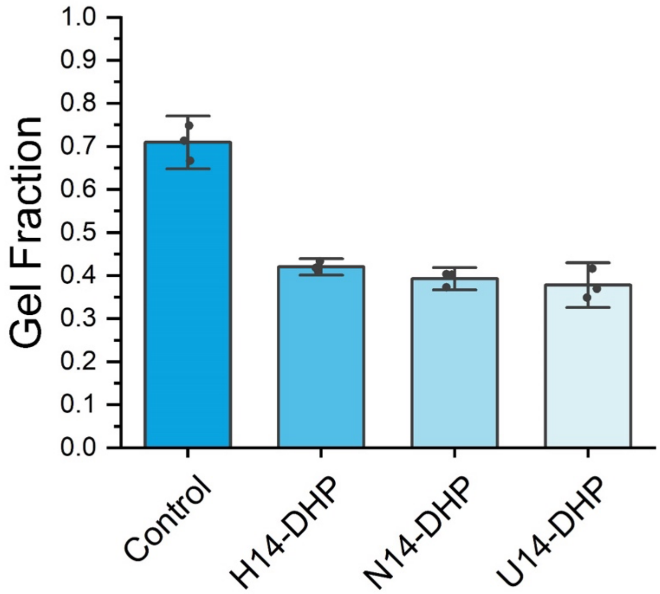

Figure S8. Gel fraction results indicating that a similar degree of crosslinking is obtained for the peptoid-crosslinked hydrogels whereas the peptide control hydrogels have a higher degree of crosslinking.

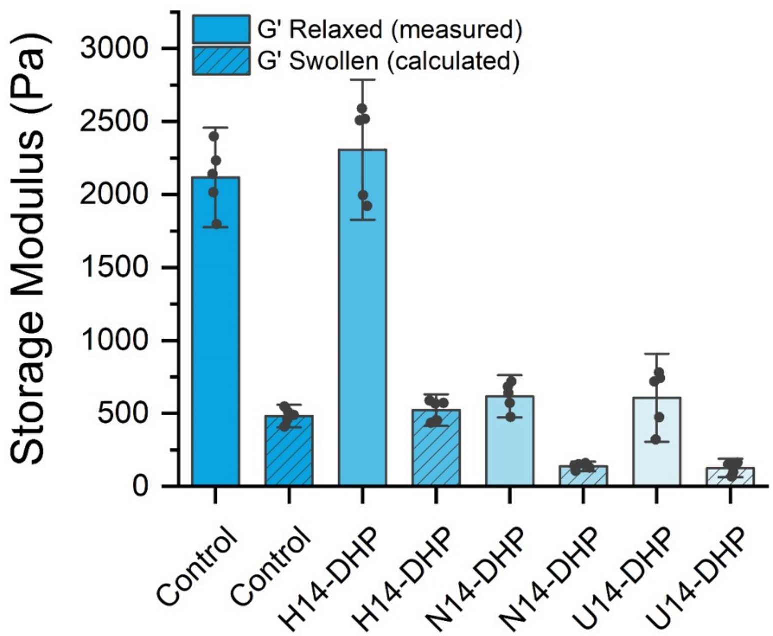

Figure S9. Calculated swollen storage modulus compared to the measured relaxed state modulus for each hydrogel condition.

Statistical Analyses to Validate the use of ANOVA:

To validate the use of ANOVA, it is essential to ensure that key assumptions—normality and homogeneity of variances—are met. The Shapiro-Wilk test is commonly used to assess the normality of residuals within each group. It tests the null hypothesis that the data are drawn from a normal distribution. The test returns a W statistic, where values closer to 1 suggest normality, and a p-value, where a value greater than the chosen significance level (typically $\alpha = 0.05$) indicates no significant deviation from normality, thus supporting the assumption. In parallel, Levene's test evaluates the homogeneity of variances across groups by testing the null hypothesis that all group variances are equal. It computes an F statistic by comparing the absolute deviations of group observations from their group means (or medians), and provides a corresponding p-value; a value greater than α suggests that variances are not significantly different between groups, justifying the use of ANOVA. When both tests yield non-significant p-values, it supports the validity of ANOVA assumptions and justifies proceeding with the analysis.

The Kruskal–Wallis test is a non-parametric alternative to ANOVA used when the assumptions of normality and homogeneity of variances are violated. Unlike ANOVA, which compares means across groups under the assumption of normally distributed data with equal variances, the Kruskal–Wallis test compares the medians by ranking all data points across groups and analyzing the distribution of these ranks. It tests the null hypothesis that all groups come from the same distribution. The test produces an H statistic (which approximates a chi-square distribution) and a corresponding p-value. A significant p-value (typically $p < 0.05$) suggests that at least one group differs significantly from the others. This test does not require the data to be normally distributed and is less sensitive to unequal variances, making it particularly useful when either the Shapiro-Wilk test indicates non-normality or Levene's test shows significant heterogeneity of variances. Thus, the Kruskal–Wallis test serves as a robust alternative to ANOVA when parametric assumptions are not met.

Transmittance Data:

Shapiro-Wilk Test Results

Group	W-statistic	p-value
H14	0.80671137571	0.13062232732772827
H14-pip	0.77185916900	0.04895104467868805
H14-hp	0.90050262212	0.38710227608680725
H14-DHP	0.81815469264	0.15861836075782776
N14-DHP	0.97126692533	0.6746912002563477

Levene's Test Results

- Statistic: 0.700
- p-value: 0.634

Kruskal–Wallis Results

- Statistic: 15.34
- p-value: 0.0090

Solubility Data:

Shapiro-Wilk Test Results

Group	W-statistic	p-value
H14	0.9795919060707092	0.7262256145477295
H14-pip	0.9758065938949585	0.7017236948013306
H14-hp	0.9758065938949585	0.7017236948013306
H14-DHP	1	0.999999
N14-DHP	0.8352554440498352	0.201774
U14-DHP	0.882641	0.33219894766807556

Levene's Test Results

- Statistic: 0.742
- p-value: 0.607

Plateau Modulus Data:

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.9928879141807556	0.9887659549713135
H14-DHP	0.7926778197288513	0.070536
N14-DHP	0.9522148370742798	0.7529988884925842
U14-DHP	0.8556444644927979	0.21302825212478638

Levene's Test Results

- Statistic: 0.702
- p-value: 0.565

Swelling Data:

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.8853664398193359	0.3403282165527344
H14-DHP	0.9774535298347473	0.7121354341506958
N14-DHP	0.9994819760322571	0.9565268158912659
U14-DHP	0.9998378157615662	0.9756718277931213

Levene's Test Results

- Statistic: 0.963
- p-value: 0.456

EdU Data

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.9891335964202881	0.952988
H14-DHP	0.8104616403579712	0.12234412878751755
N14-DHP	0.8547564744949341	0.24193963408470154
U14-DHP	0.7593454122543335	0.047059

Levene's Test Results

- Statistic: 0.508
- p-value: 0.684

Kruskal–Wallis Results

- Statistic: 11.54
- p-value: 0.0092

YAP Nuclear Localization Data

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.9720840454101562	0.7786327600479126
H14-DHP	0.9036558270454407	0.041219
U14-DHP	0.9453017711639404	0.27684009075164795

Levene's Test Results

- Statistic: 3.50
- p-value: 0.036

Kruskal–Wallis Results:

- **Statistic**: 37.42
- **p-value**: 7.50×10^{-9}

IDO Data (no IFN-γ)

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.9929120540618896	0.971877
H14-DHP	0.7535684108734131	0.041689
U14-DHP	0.93982	0.6532316207885742

Levene's Test Results

- Statistic: 1.07
- p-value: 0.383

Kruskal–Wallis Results

- Statistic: 8.00
- p-value: 0.0183

IDO Data (with IFN-γ)

Shapiro-Wilk Test Results

Group	W-statistic	p-value
Control	0.884792566299438	0.3386097550392
H14-DHP	0.862245082855224	0.2737730443477
U14-DHP	0.990825712680816	0.8167866468429

Levene's Test Results

- Statistic: 0.0038
- p-value: 0.996