Enhancing Biocompatibility of *D*-Oligopeptide Hydrogels by Negative Charges

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Supporting Information

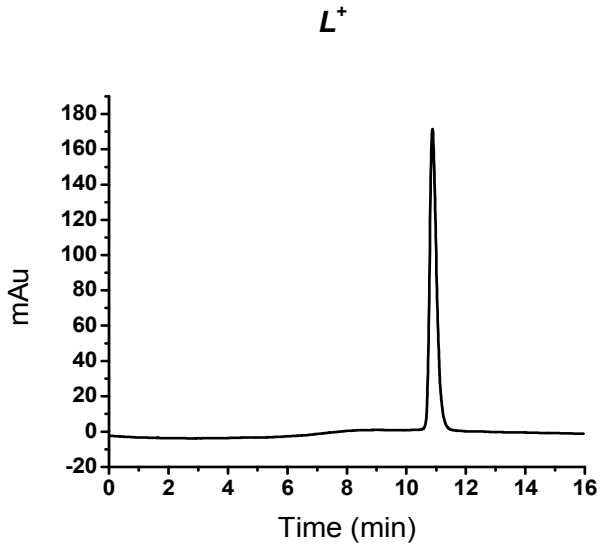

Figure S1. Analytical reversed-phase HPLC chromatogram of L^+ acquired with HP1100 chromatograph system (Agilent Technologies). Column: Zorbax 300SB-C18 (4.6 × 250 mm i.d.). Elution profiles were monitored at 280nm. Eluents: solvent A: 0.1% trifluoroacetic acid (TFA) in water, pH 2.0; solvent B: 0.1% TFA in methanol, pH 2.0. Chromatograph run conditions for all the peptides: flow rate: 1ml/min; gradient: 2% B/min; temperature: ambient.

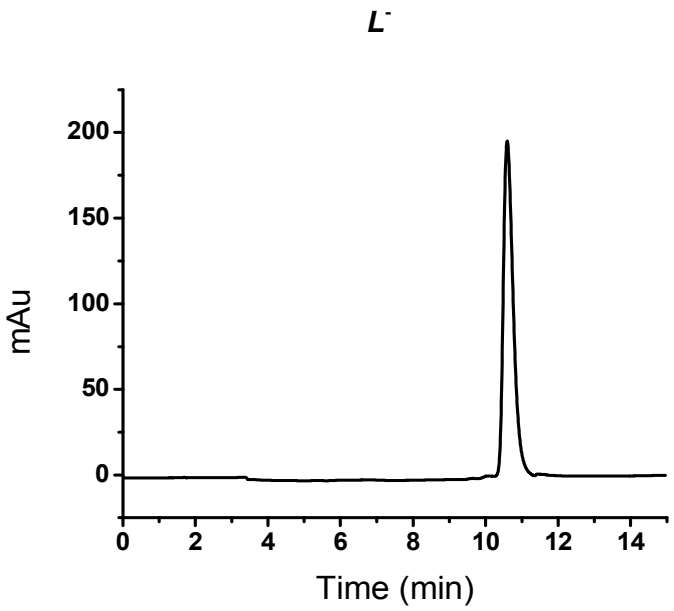

Figure S2. Analytical reversed-phase HPLC chromatogram of $\boldsymbol{L}^-$ acquired with HP1100 chromatograph system (Agilent Technologies). Column: Zorbax 300SB-C18 (4.6 × 250 mm i.d.). Elution profiles were monitored at 280nm. Eluents: solvent A: 20 mM NH4HCO3 in water, pH 7.0; solvent B: 20 mM NH4HCO3 in water (40%) + methanol (60%) mixture, pH 7.0. Chromatograph run conditions for all the peptides: flow rate: 1ml/min; gradient: 2% B/min; temperature: ambient.

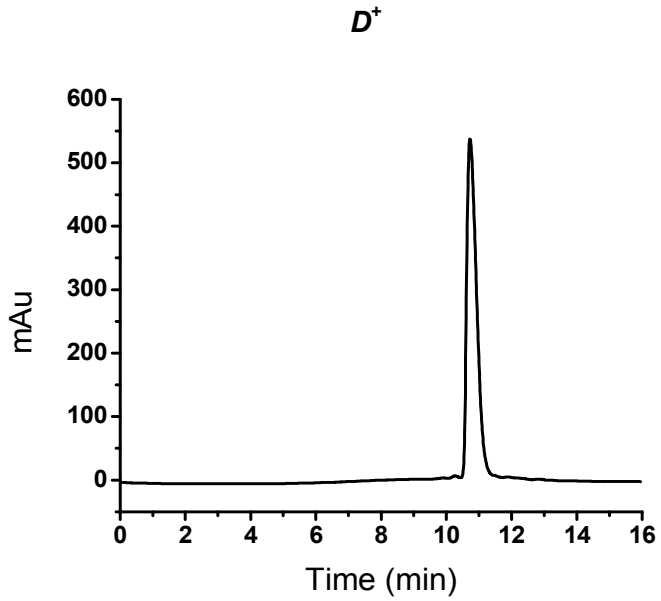

Figure S3. Analytical reversed-phase HPLC chromatogram of ***D*$^+$** acquired with HP1100 chromatograph system (Agilent Technologies). Column: Zorbax 300SB-C18 (4.6 × 250 mm i.d.). Elution profiles were monitored at 280nm. Eluents: solvent A: 0.1% trifluoroacetic acid (TFA) in water, pH 2.0; solvent B: 0.1% TFA in methanol, pH 2.0. Chromatograph run conditions for all the peptides: flow rate: 1ml/min; gradient: 2% B/min; temperature: ambient.

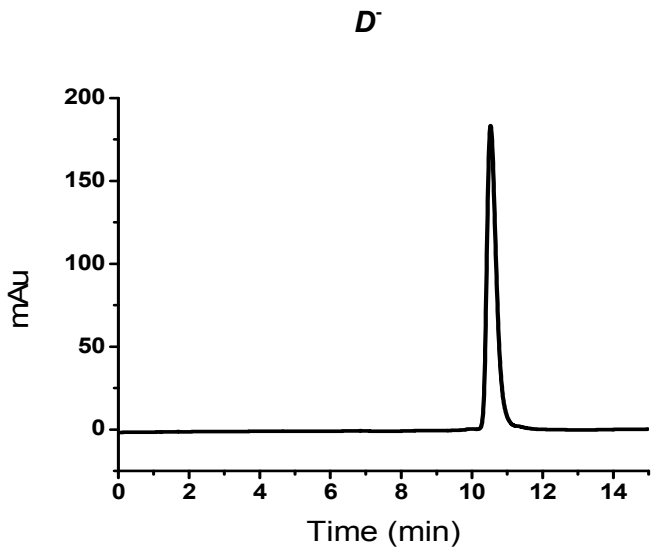

Figure S4. Analytical reversed-phase HPLC chromatogram of ***D***$^{-}$ acquired with HP1100 chromatograph system (Agilent Technologies). Column: Zorbax 300SB-C18 (4.6 × 250 mm i.d.). Elution profiles were monitored at 280nm. Eluents: solvent A: 20 mM NH4HCO3 in water, pH 7.0; solvent B: 20 mM NH4HCO3 in water (40%) + methanol (60%) mixture, pH 7.0. Chromatograph run conditions for all the peptides: flow rate: 1ml/min; gradient: 2% B/min; temperature: ambient.

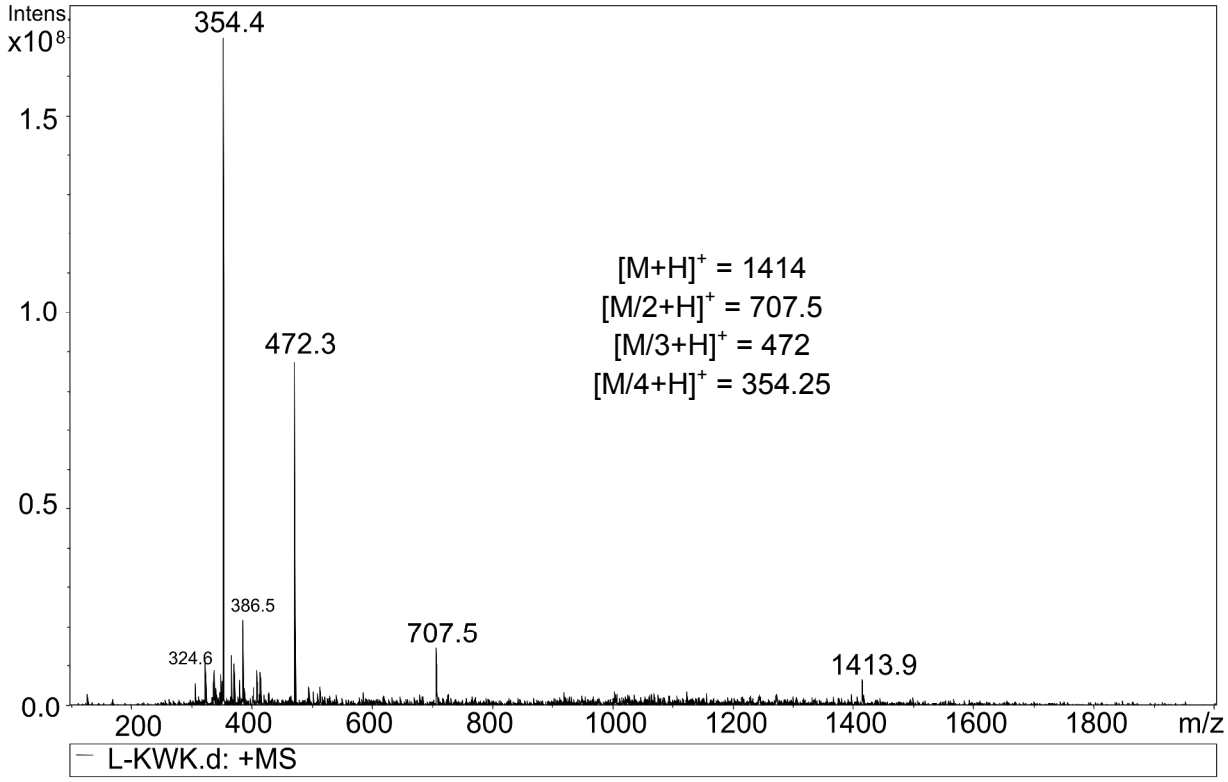

Figure S5. ***L*****$^+$** Mass spectrum acquired with an Amazon X Ion Trap Mass Spectrometer (Bruker) in positive ion mode. Flow rate of 3 μL/min, 10 psi nebulizer pressure, 4 L/min dry gas flow and 250˚C gas temperature.

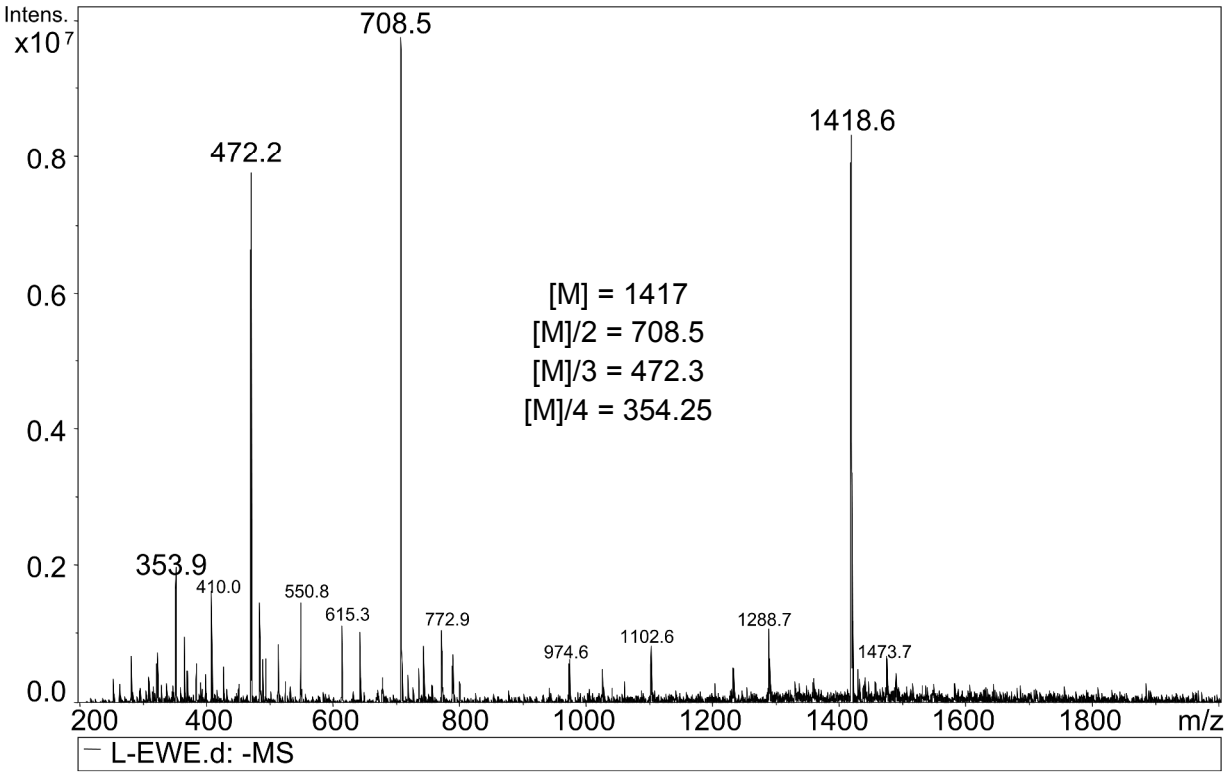

Figure S6. ***L***$^{-}$ mass spectrum acquired with an Amazon X Ion Trap Mass Spectrometer (Bruker) in negative ion mode. Flow rate of 3 µL/min, 10 psi nebulizer pressure, 4 L/min dry gas flow and 250˚C gas temperature.

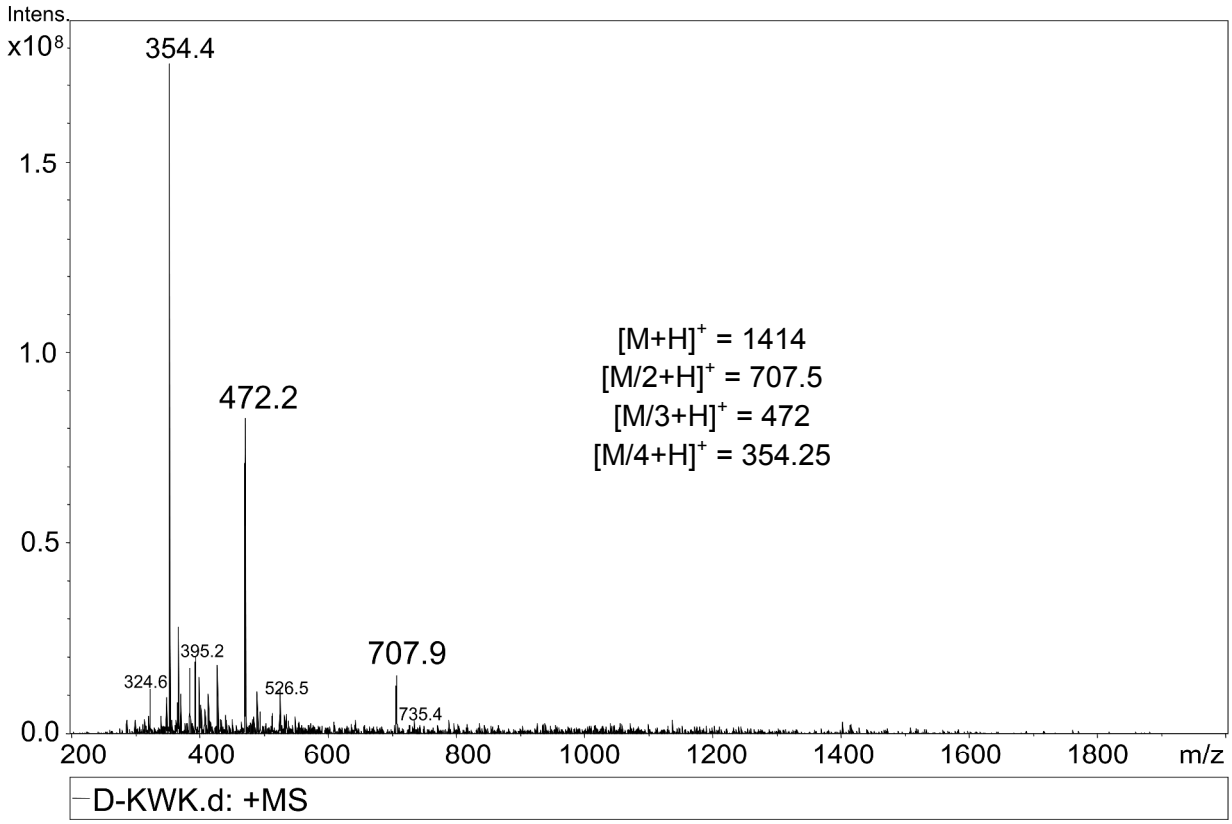

Figure S7. ***D*****$^+$** mass spectrum acquired with an Amazon X Ion Trap Mass Spectrometer (Bruker) in positive ion mode. Flow rate of 3 µL/min, 10 psi nebulizer pressure, 4 L/min dry gas flow and 250˚C gas temperature.

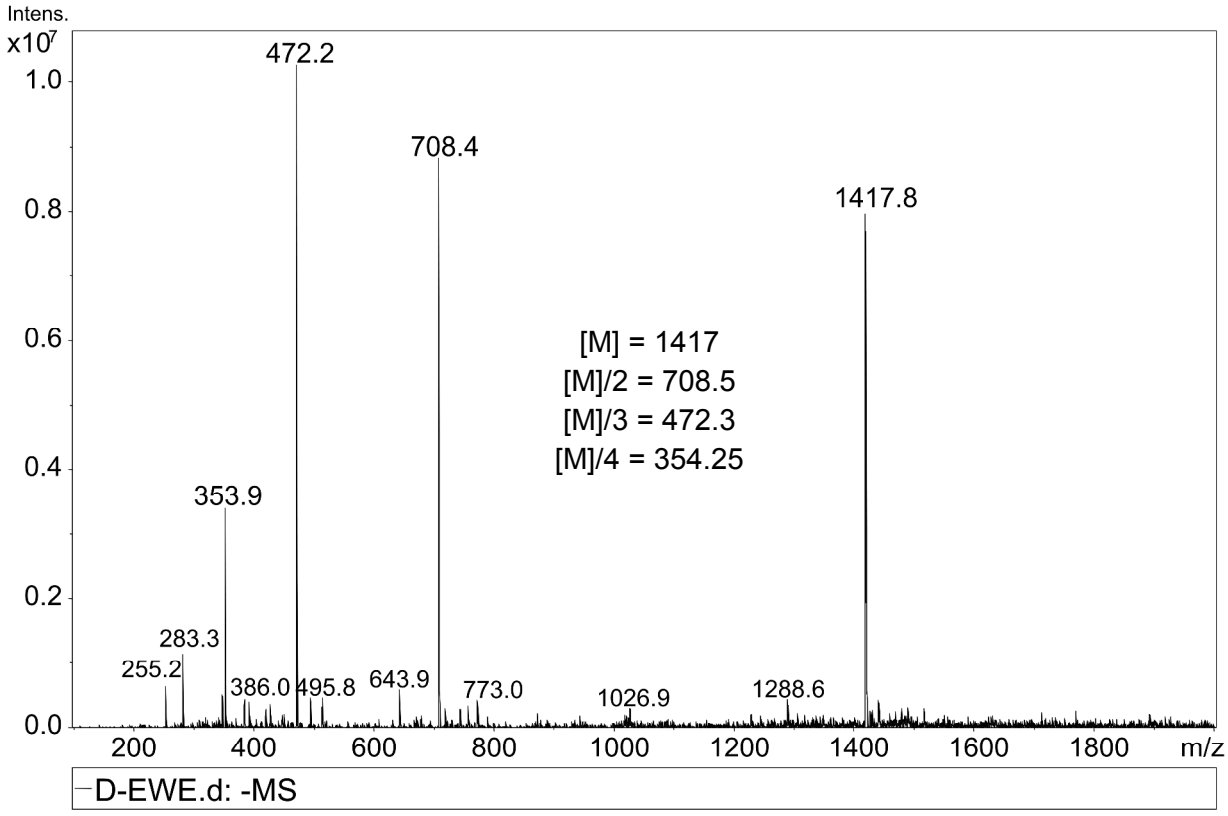

Figure S8. ***D***$^{-}$ mass spectrum acquired with an Amazon X Ion Trap Mass Spectrometer (Bruker) in negative ion mode. Flow rate of 3 µL/min, 10 psi nebulizer pressure, 4 L/min dry gas flow and 250˚C gas temperature.

Table S1. Results from a series of paired t-tests to determine significant differences in cell behavior on different hydrogel types. Table (A) shows viability and table (B) shows cell number. For acceptance, $p < 0.01$.

A. Viability (n = 54)

Hypothesis	p value	Result
Single Peptides		
$L^- > L^+$	0.2	rejected
$L^+ > D^+$	0.5	rejected
$L^- > D^-$	0.4	rejected
$D^- > D^+$	0.1	rejected
$D^- > L^+$	0.2	rejected
$L^- > D^+$	0.2	rejected
Neutral Gels		
$(LL)^0 > (LD)^0$	2.0E-06	accepted
$(LL)^0 > (DL)^0$	1.0E-05	accepted
$(DL)^0 > (LD)^0$	0.5	rejected
$(LD)^0 > (DD)^0$	0.1	rejected
$(DL)^0 > (DD)^0$	0.06	rejected
$(LL)^0 > (DD)^0$	1.0E-07	accepted
$(DD)^0 > (LLDD)^0$	4.0E-06	accepted
$(LD)^0 > (LLDD)^0$	1.0E-06	accepted
$(DL)^0 > (LLDD)^0$	8.0E-07	accepted
Charged Gels		
$(DD)^0 > (DD)^+$	0.003	accepted
$(DD)^- > (DD)^0$	0.009	accepted
$(LL)^+ > (DD)^+$	5.0E-07	accepted
$(LL)^- > (DD)^-$	0.003	accepted
$(LL)^0 > (LL)^+$	0.1	rejected
$(LL)^0 > (LL)^-$	0.08	rejected
$(LL)^- > (LL)^+$	0.4	rejected

B. Cell Number (n = 9)

Hypothesis	Day	p value	Result
Single Peptides			
$L^- > L^+$	1	0.2	rejected
$L^+ > D^+$	1	0.1	rejected
$L^- > D^-$	1	0.1	rejected
$D^- > D^+$	1	0.06	rejected
$D^- > L^+$	1	0.2	rejected
$L^- > D^+$	1	0.02	rejected
$L^- > L^+$	3	0.2	rejected
$L^+ > D^+$	3	0.5	rejected
$L^- > D^-$	3	0.5	rejected
$D^- > D^+$	3	0.3	rejected
$D^- > L^+$	3	0.3	rejected
$L^- > D^+$	3	0.2	rejected
$L^- > L^+$	7	0.4	rejected
$L^+ > D^+$	7	0.4	rejected
$L^- > D^-$	7	0.4	rejected
$D^- > D^+$	7	0.4	rejected
$D^- > L^+$	7	0.5	rejected
$L^- > D^+$	7	0.2	rejected
Neutral Gels			
$(LL)^0 > (LD)^0$	1	0.3	rejected
$(LL)^0 > (DL)^0$	1	0.3	rejected
$(DL)^0 > (LD)^0$	1	0.3	rejected
$(LD)^0 > (DD)^0$	1	3.0E-05	accepted
$(DL)^0 > (DD)^0$	1	3.0E-05	accepted
$(LL)^0 > (DD)^0$	1	7.0E-04	accepted
$(LL)^0 > (LLDD)^0$	1	0.004	accepted
$(LD)^0 > (LLDD)^0$	1	7.0E-04	accepted
$(DL)^0 > (LLDD)^0$	1	0.002	accepted
$(LLDD)^0 > (DD)^0$	1	0.2	rejected
$(LL)^0 > (LD)^0$	3	0.2	rejected
$(LL)^0 > (DL)^0$	3	0.2	rejected
$(DL)^0 > (LD)^0$	3	0.3	rejected
$(LD)^0 > (DD)^0$	3	0.4	rejected
$(DL)^0 > (DD)^0$	3	0.3	rejected

$(LL)^0 > (DD)^0$	3	0.03	rejected
$(LL)^0 > (LLDD)^0$	3	2.0E-04	accepted
$(LD)^0 > (LLDD)^0$	3	4.0E-04	accepted
$(DL)^0 > (LLDD)^0$	3	0.002	accepted
$(DD)^0 > (LLDD)^0$	3	0.012	rejected
$(LL)^0 > (LD)^0$	7	0.003	accepted
$(LL)^0 > (DL)^0$	7	6.0E-04	accepted
$(DL)^0 > (LD)^0$	7	0.3	rejected
$(DD)^0 > (LD)^0$	7	0.1	rejected
$(DD)^0 > (DL)^0$	7	0.09	rejected
$(LL)^0 > (DD)^0$	7	0.2	rejected
$(LD)^0 > (LLDD)^0$	7	3.0E-05	accepted
$(DL)^0 > (LLDD)^0$	7	3.0E-05	accepted
$(DD)^0 > (LLDD)^0$	7	5.0E-05	accepted
Charged Gels			
$(DD)^0 > (DD)^+$	1	0.004	accepted
$(DD)^- > (DD)^0$	1	5.0E-04	accepted
$(DD)^- > (DD)^+$	1	0.002	accepted
$(LL)^+ > (DD)^+$	1	5.0E-06	accepted
$(LL)^- > (DD)^-$	1	0.0004	accepted
$(LL)^0 > (LL)^+$	1	0.014	rejected
$(LL)^- > (LL)^0$	1	0.09	rejected
$(LL)^- > (LL)^+$	1	0.03	rejected
$(DD)^0 > (DD)^+$	3	0.014	rejected
$(DD)^- > (DD)^0$	3	0.1	rejected
$(DD)^- > (DD)^+$	3	0.004	accepted
$(LL)^+ > (DD)^+$	3	0.005	accepted
$(LL)^- > (DD)^-$	3	0.09	rejected
$(LL)^0 > (LL)^+$	3	0.4	rejected
$(LL)^- > (LL)^0$	3	0.4	rejected
$(LL)^- > (LL)^+$	3	0.5	rejected
$(DD)^0 > (DD)^+$	7	0.1	rejected
$(DD)^- > (DD)^0$	7	0.2	rejected
$(DD)^- > (DD)^+$	7	0.002	accepted
$(LL)^+ > (DD)^+$	7	0.006	accepted
$(LL)^- > (DD)^-$	7	0.5	rejected
$(LL)^0 > (LL)^+$	7	0.2	rejected

$(LL)^- > (LL)^0$	7	0.5	rejected
$(LL)^- > (LL)^+$	7	0.3	rejected

WST-1 subtraction procedure

Cell+Gel Average Absorbance = Avg. Abs.(cells on gel, day X) – Avg. Abs.(gel, day X)

TCPS Average Absorbance = Avg. Abs.(cells on plate, day X) – Avg. Abs.(plate, day X)

Absorbances were normalized by Avg. TCPS, day 7, i.e.,

Cell+Gel Average Absorbance/TCPS Average Absorbance (day 7) $\times$ 100%