Supplementary Information

Nano-Enabled Dynamically Responsive Living Acellular Hydrogels

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The SI includes 6 Figures (S1-S6).

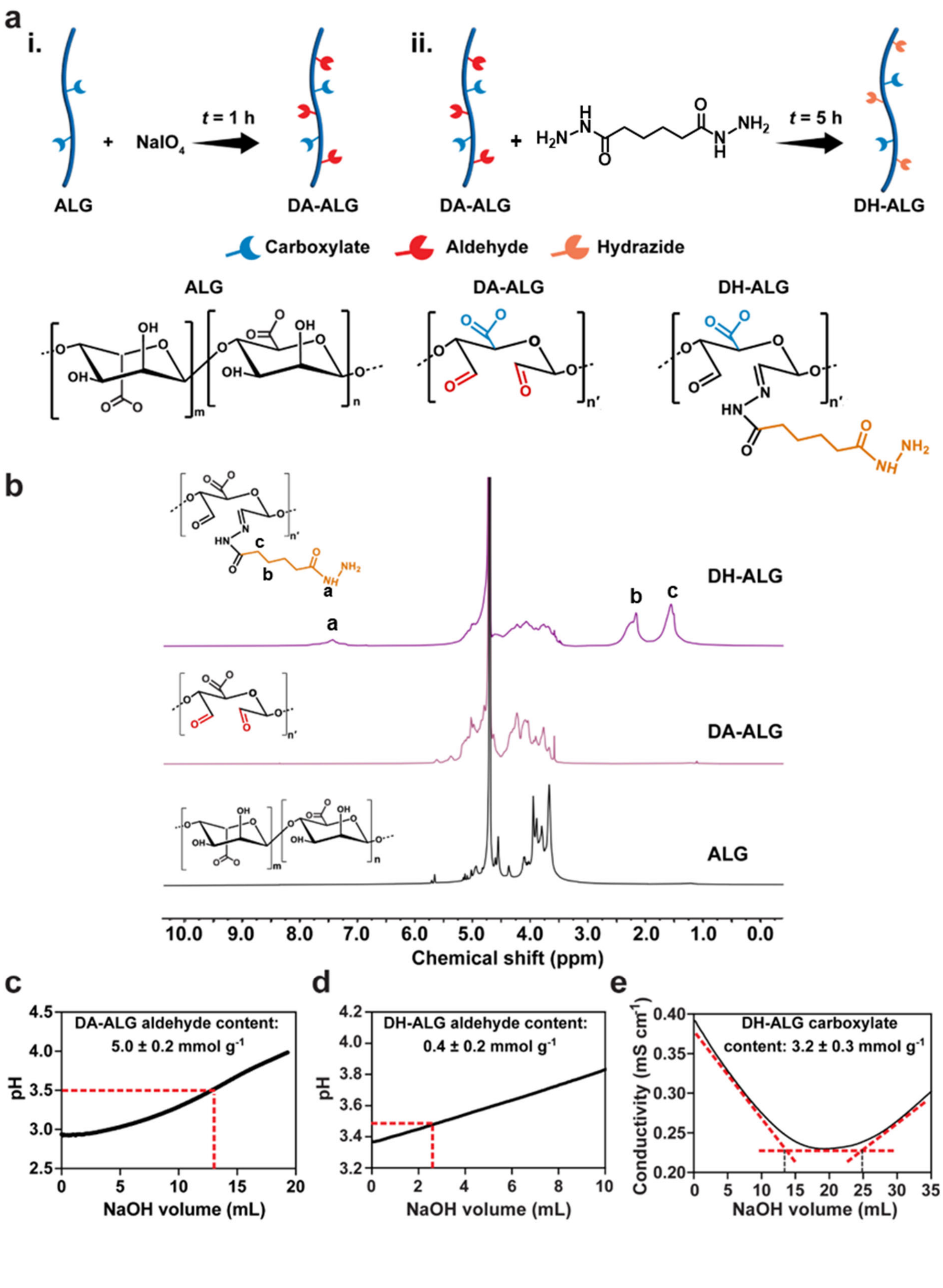
a
i.
ii.
ALG
+
NaIO4
t = 1 h
DA-ALG
DA-ALG
+
t = 5 h
DH-ALG
Carboxylate
Aldehyde
Hydrazide
ALG
DA-ALG
DH-ALG
b
DH-ALG
DA-ALG
ALG
a
b
c
Chemical shift (ppm)
c
DA-ALG aldehyde content:
5.0 ± 0.2 mmol g-1
pH
NaOH volume (mL)
d
DH-ALG aldehyde content:
0.4 ± 0.2 mmol g-1
pH
NaOH volume (mL)
e
DH-ALG carboxylate
content: 3.2 ± 0.3 mmol g-1
Conductivity (mS cm-1)
NaOH volume (mL)

Fig. S1. DH-ALG synthesis and characterizations. (a) DH-ALG synthesis via successive periodate oxidation and Schiff base reaction, along with the corresponding changes in ALG chemical structure. Note that the chemical structures are just representatives of functionalization. **(b)** ^{1}H NMR spectra of ALG, DA-ALG, and DH-ALG, confirming the successful functionalization of ALG with aldehyde and hydrazide groups. **(c)** Aldehyde group content of DA-ALG, determined by the NaOH-titration of HCl released from the reaction of aldehyde groups with $NH_2OH·HCl$. **(d)** Conductometric titration curve of DH-ALG based on the titration of weak acid (i.e., carboxylate) using NaOH, yielding a carboxylate group content of 3.2 mmol g^{-1}.

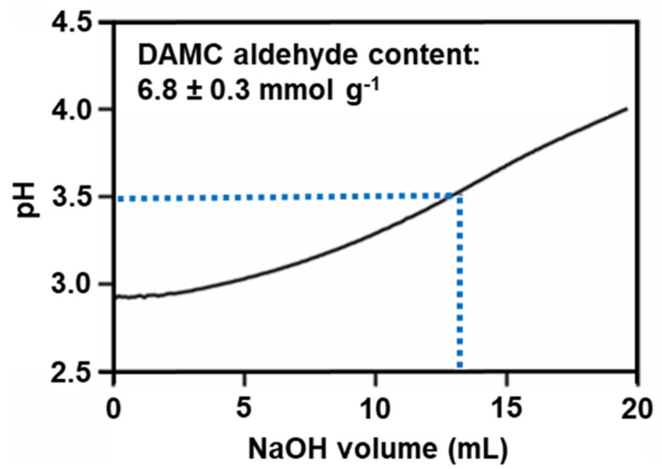

Fig. S2. Representative aldehyde titration curve of DAMC, obtained via an oxime formation method.

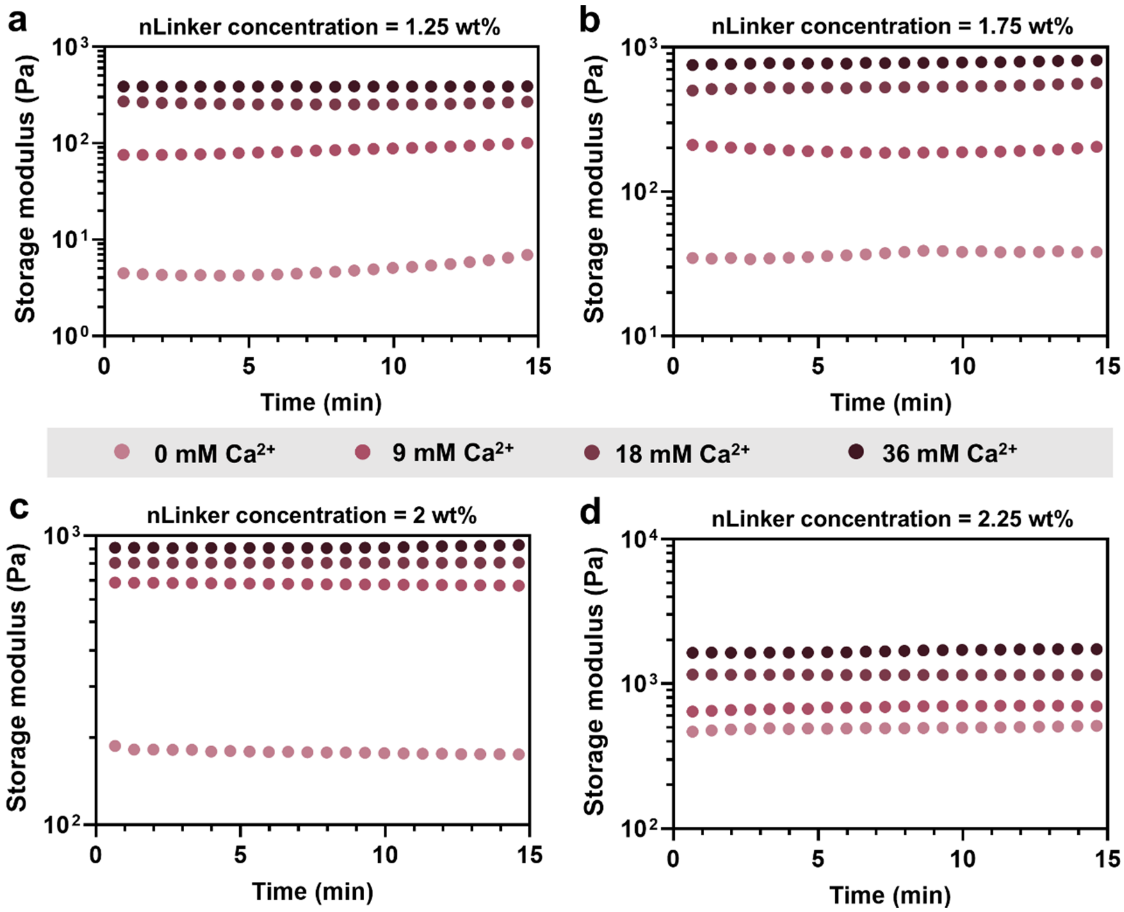

Fig. S3. Effect of time on the storage modulus of NCH, containing varying concentrations of nLinker (**a**: 1.25 wt%, **b**: 1.75 wt%, **c**: 2 wt%, **d**: 2.25 wt%) and Ca^{2+}, at constant frequency = 1 rad s^{-1} and strain = 0.1%. The tests were performed on gels ~ 5 min after preparation.

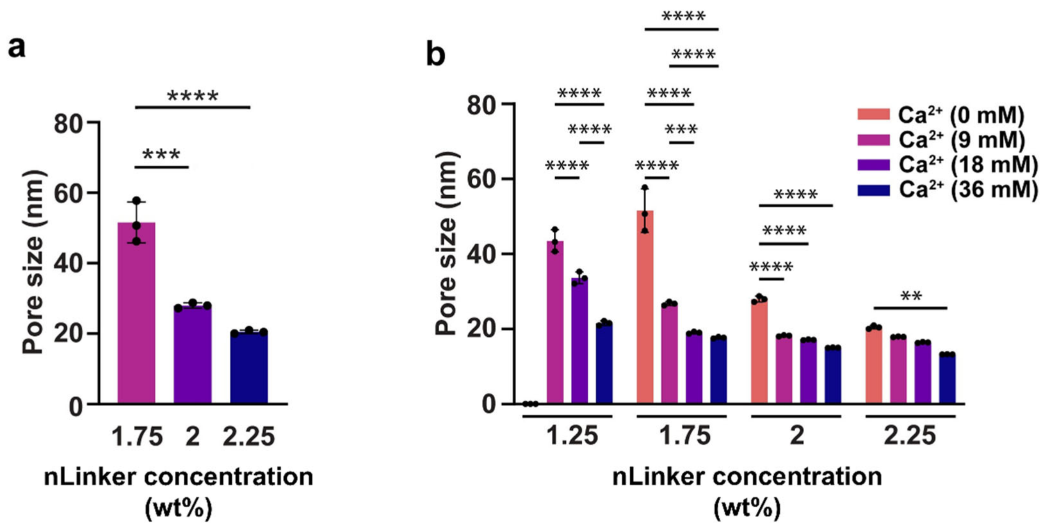

Fig. S4. Calculated LivGel pore size. Average pore size of LivGels, containing **(a)** varying nLinker concentration (Ca^{2+} concentration = 0 mM), or **(b)** varying nLinker concentrations at varying Ca^{2+} concentrations (0 - 36 mM), calculated using the storage modulus (*G′*) at constant strain = 1% and frequency = 1 rad s^{-1}. The pore size decreased by increasing the nLinker and/or Ca^{2+} concentrations. ($n = 3$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

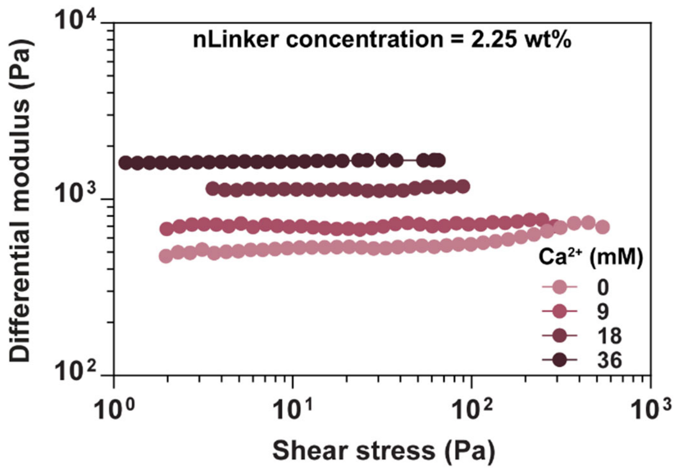

Fig. S5. Differential modulus (*K*′) versus oscillatory shear stress for the NCH, containing 2.25 wt.% of nLinker loaded with varying Ca^{2+} concentrations. The Ca^{2+}-loaded NCH were not strain-stiffening.

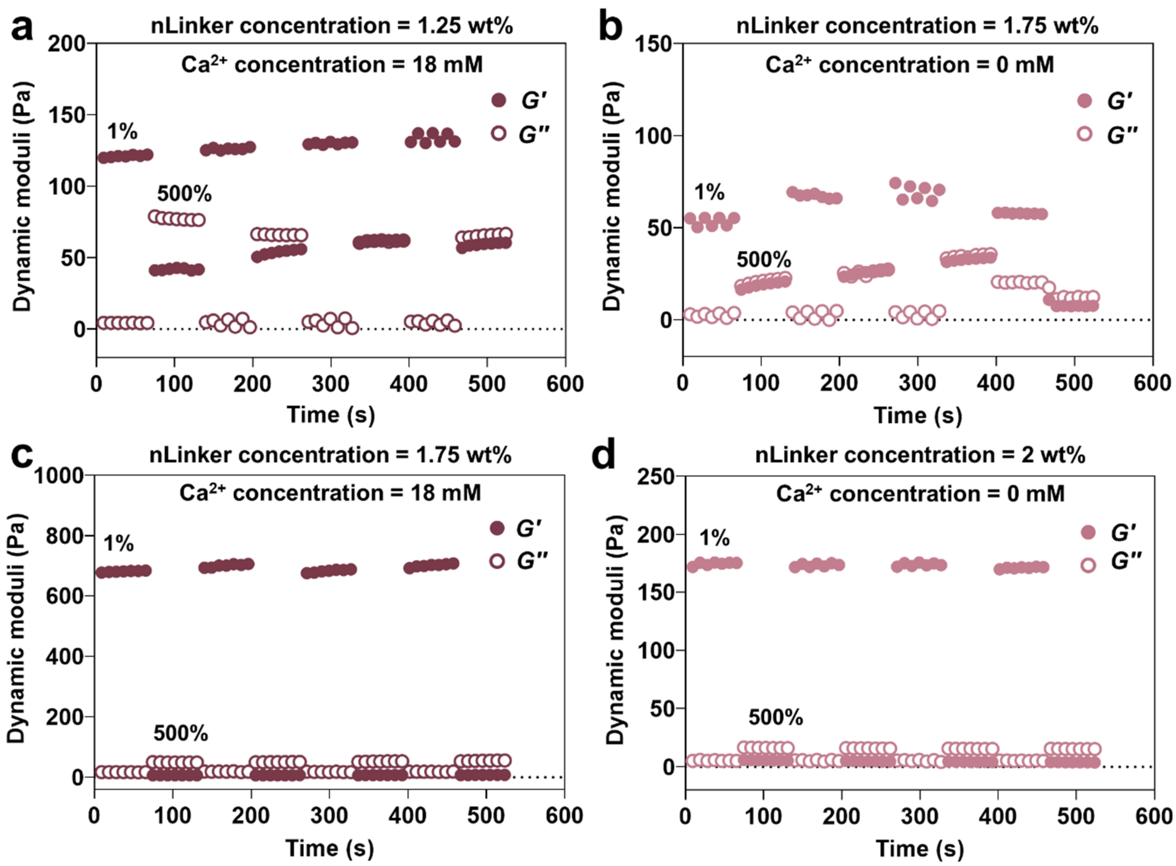

Fig. S6. Oscillatory strain amplitude tests at alternating low (1%)-high (500%) strain cycles, conducted on the LivGels, containing **(a)** 1.25 wt.% of nLinker with 18 mM of Ca^{2+}, 1.75 wt.% of nLinker with **(b)** 0 mM of Ca^{2+} or **(c)** 18 mM of Ca^{2+}, or 2 wt.% of nLinker with **(d)** 0 mM of Ca^{2+}.