Supplementary Materials for:

Supramolecular host-guest hyaluronic acid hydrogels enhance corneal wound healing through dynamic spatiotemporal effects

By Fernandes-Cunha et al.

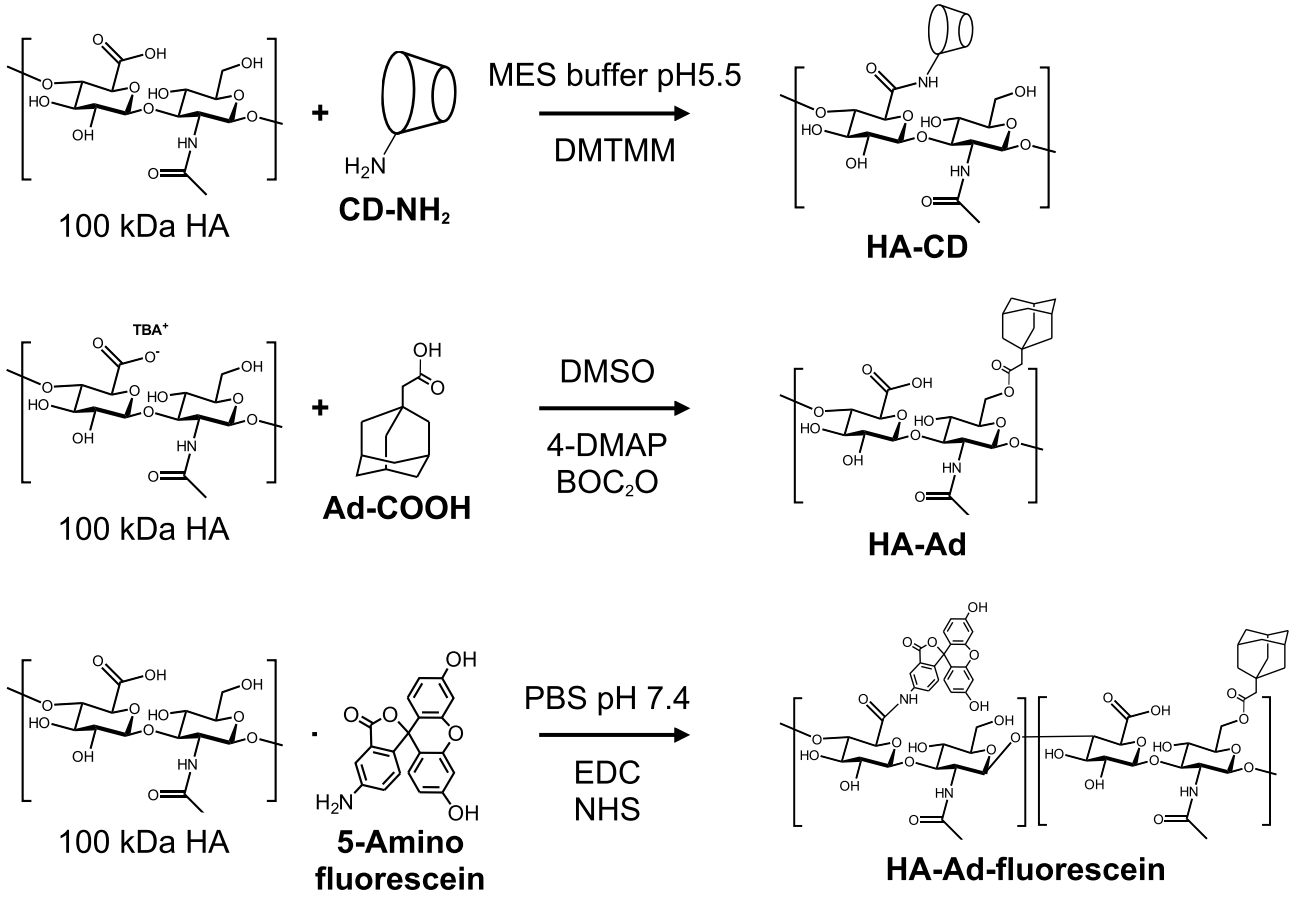

Fig. S1: Schematic illustration for the synthesis of HA-CD, HA-Ad, and HA-Ad-fluorescein macromers.

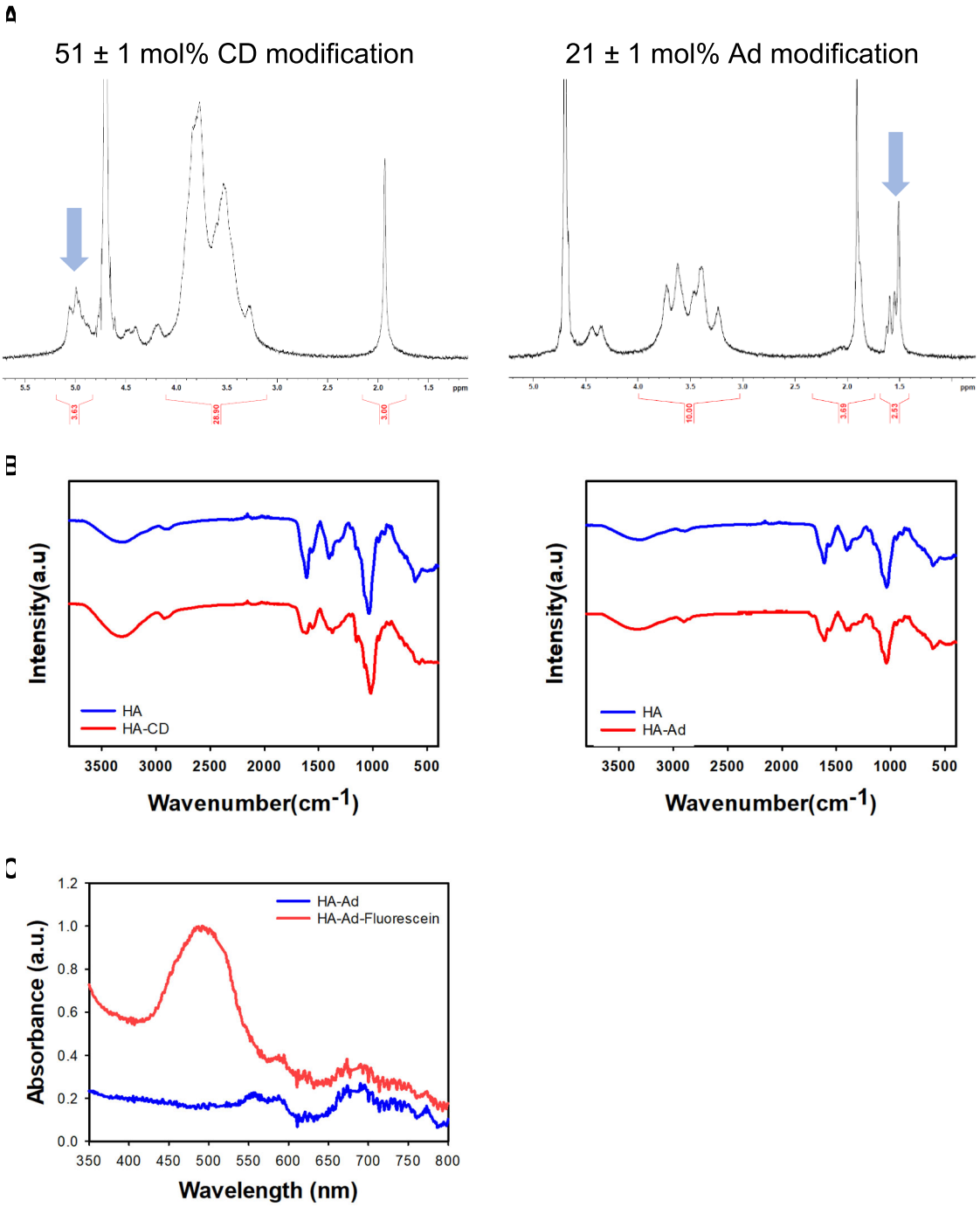

Fig. S2: Characterization of HA macromers. (**A)** 1H NMR spectrum shows 21 ± 1 mol% Ad modification and 51 ± 1 mol% CD modification on HA backbone. The arrows indicate the functionalized groups detected (**B)** FT-IR spectrum for the analysis of amide and ester linkage formation in HA-CD and HA-Ad. The arrows indicate the functionalized groups detected (**C)** The absorbance of HA-Ad-Fluorescein observed at 496 nm.

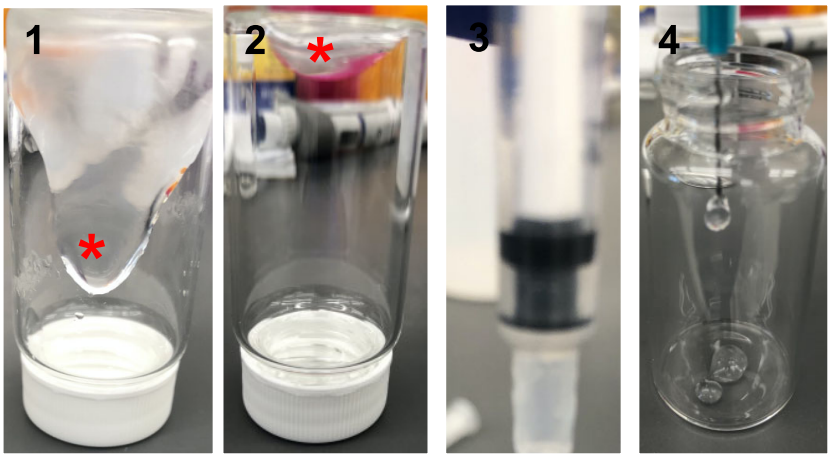

Fig. S3: (1) Viscous behavior of individual macromers (2) gel-forming solution after mixing both macromers together (3) s-HA hydrogel appearance after mixing the macromers in a syringe and (4) s-HA hydrogel appearance after passing through a needle.

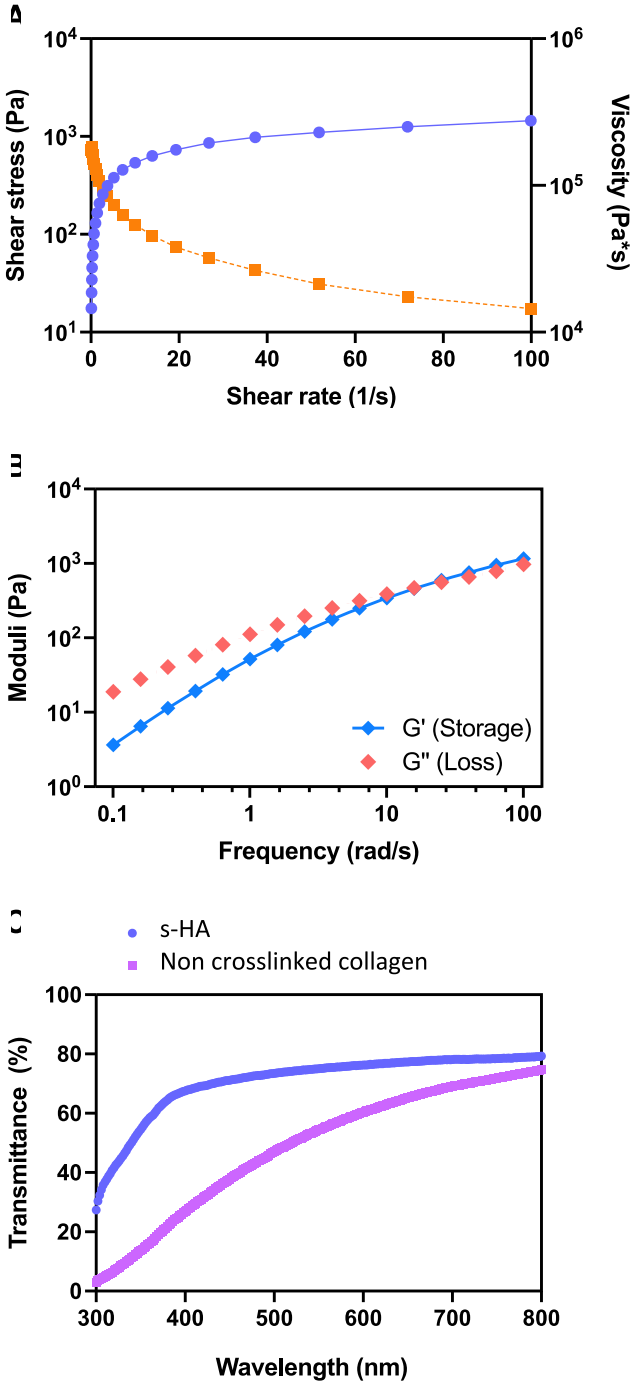

Fig. S4: (**A**) Continuous flow analysis for the gradual decrease of s-HA hydrogel viscosity with increasing applied shear stress. (**B**) Frequency sweep analysis for both storage and loss moduli with increasing applied frequency for s-HA hydrogel. (**C**) The transmittance profile of s-HA *vs* non crosslinked collagen hydrogels at day 0.

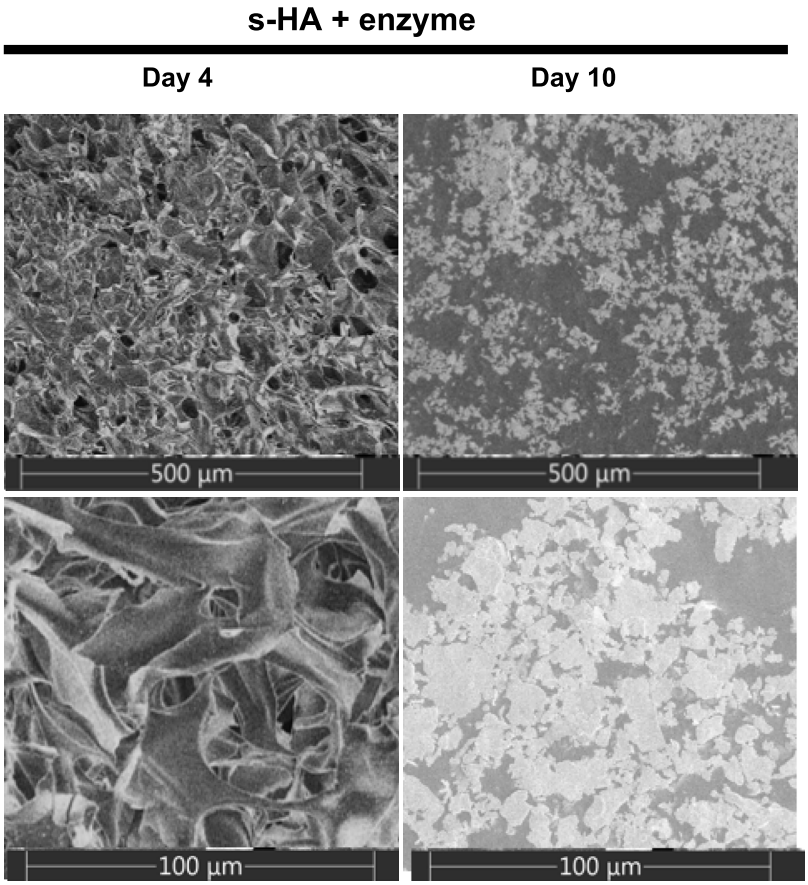

Fig. S5: SEM images of s-HA hydrogel incubated with enzyme (hyaluronidase) at day 4 and 10 show the decrease in the material porosity over time.

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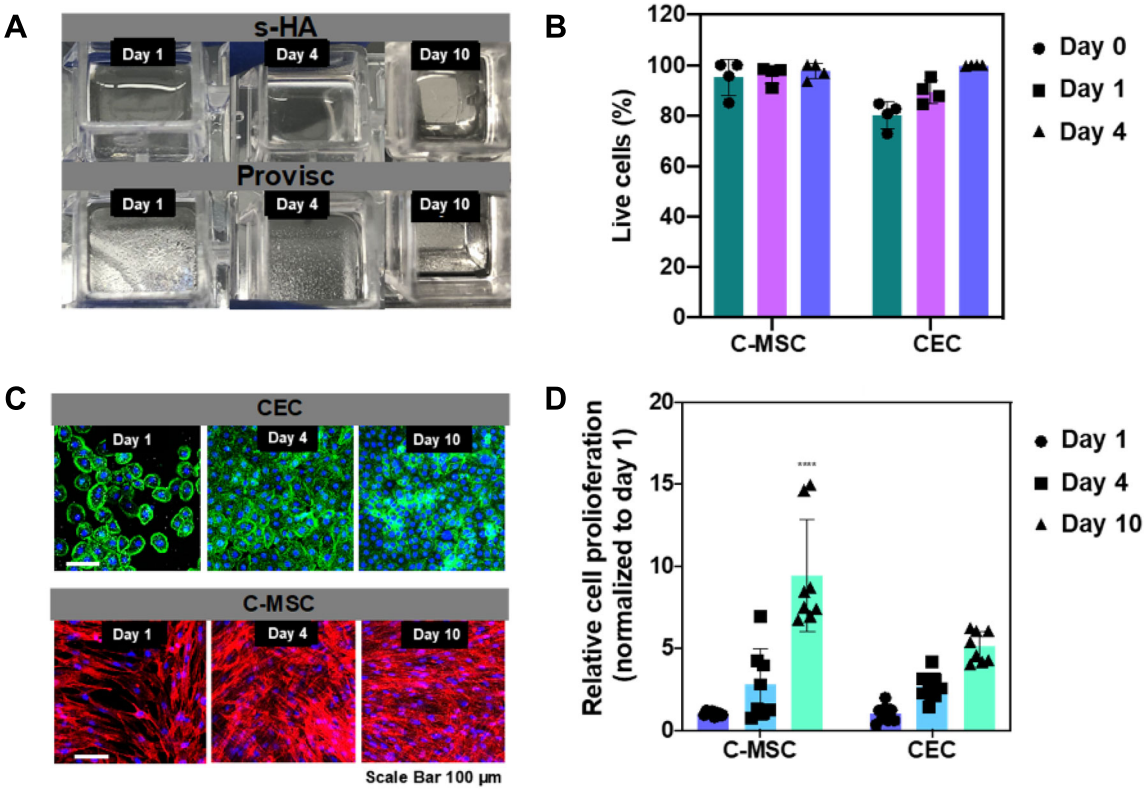

Fig. S6: (**A)** Presence of s-HA hydrogel (marked with the black star) after encapsulating cornea cells at day 1, 4 and 10 showing that *in vitro* degradation does not occur in 10 days. HA linear viscoelastic solution is not observed either at day 1, 4 and 10. (**B)** Quantification of live cells after encapsulation in s-HA at day 0, 4 and 10. (**C)** CEC stained with phalloidin 488 and c-MSC stained with phalloidin 555 after encapsulating in HA linear viscoelastic solution show cells adhered and spread on TCP and the absence of cells within the HA linear viscoelastic solution at day 1, 4 and 10. (**D)** Quantification of corneal cells on the TCP after encapsulating in s-HA hydrogel at day 1, 4 and 10. Cell proliferation significantly changed at day 10 for c-MSC **** $p < 0.0001$ *vs.* CEC day 10; data is presented as mean ± SD, Two-way ANOVA ($p < 0.005$) was used to detect statistical differences followed by Sidak's multiple comparisons test.

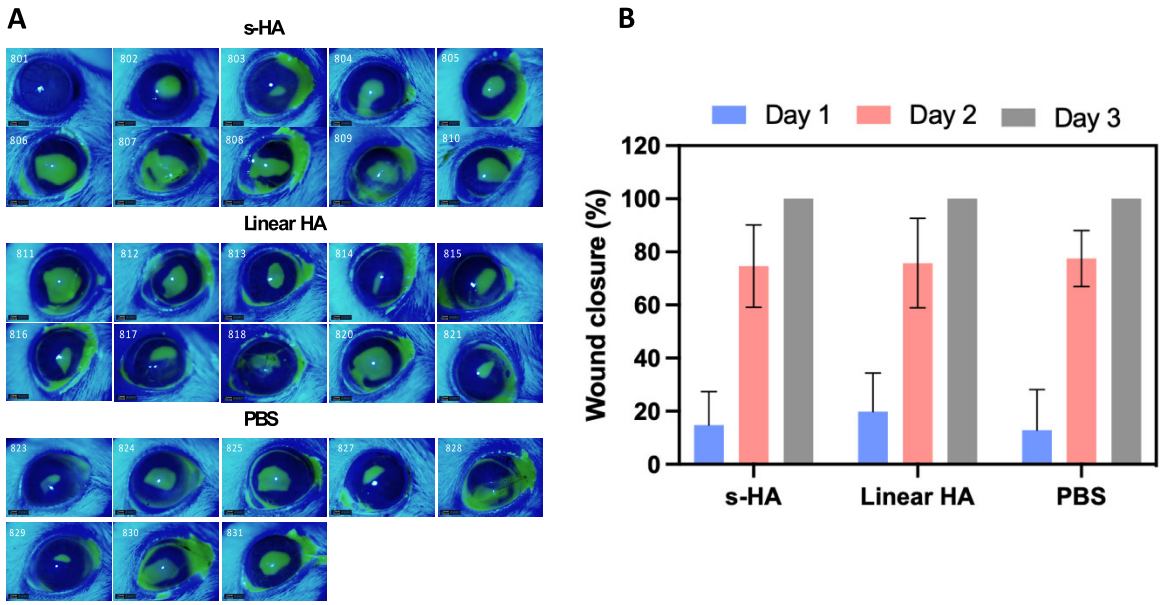

Fig. S7: (A) Fluorescein staining the wounded area on day 2 for the rat corneas treated with s-HA, linear HA and PBS. Number in the images represent the rats ear tag (**B**) Percentage wound closure observed at day 1, 2 and 3 after epithelial debridement. There was no statistically significant difference observed in wound closure between the groups.

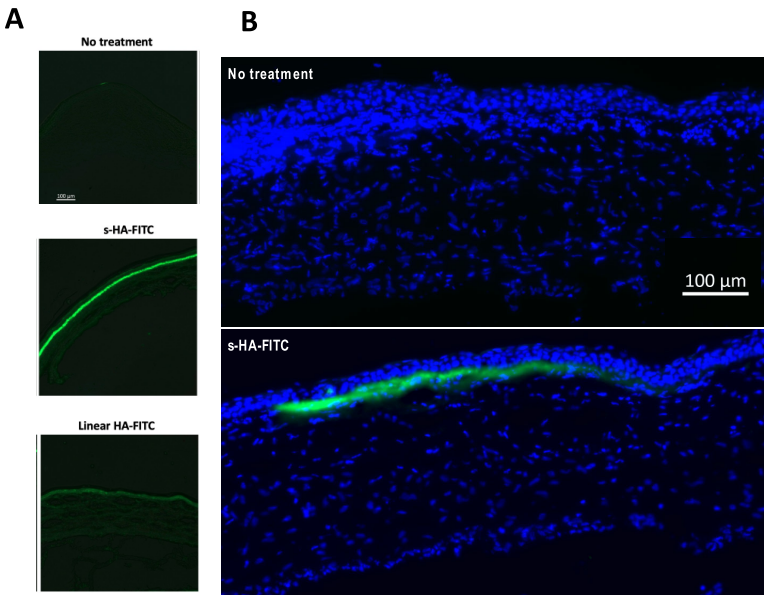

Fig. S8: (A) Presence of s-HA-FITC hydrogel at day 3 after corneal injury. The FITC fluorescence was not observed for the linear HA group or no treatment group (**B**) Images showing the epithelium growing on the s-HA-FTIC hydrogel.