Supporting information

Chitosan – Alginate Multilayered Films with gradients of physicochemical cues

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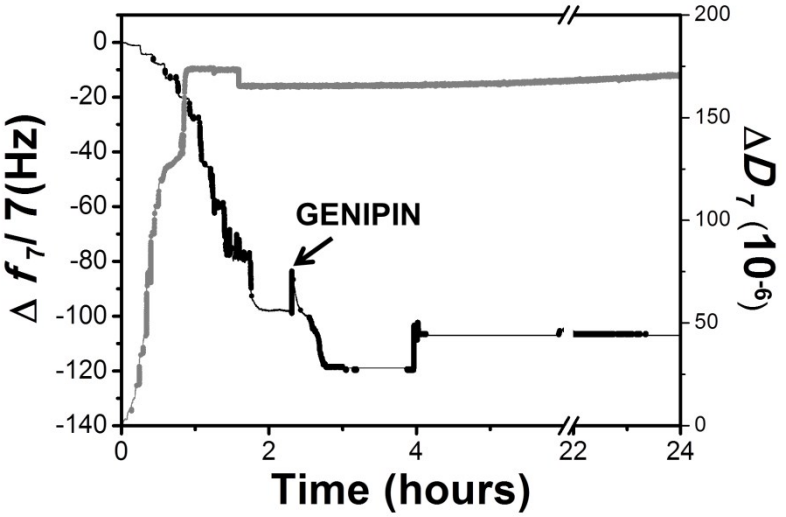

Figure S 1. *In situ* crosslinking of CHIT/ALG film followed by QCMD. Normalized frequency ($\Delta f_{7/7}$) and dissipation changes (ΔD_7) were monitored during 24 hours.

To stabilize the CHIT/ALG films genipin was used as cross-linker and the QCMD response evaluated – see Figure S1. The CHIT/ALG multilayers were built at pH 5.5 and then incubated in 5 mg.mL^{-1} genipin solution. The $\Delta f_{7/7}$ initially increases when the film is flushed with genipin, reflecting the increase of mass over the crystal. Afterwards the $\Delta f_{7/7}$ decrease which indicates that genipin maintained the stability of the film and also fomented the diffusion of water from the films. Upon cross-linking a slightly decrease of dissipation was observed, as previously reported by *Chaubaroux et al.* for the crosslinking of fibrillary ALG/collagen films [1]. However, the stiffening effect generated by genipin was confirmed with other tests.

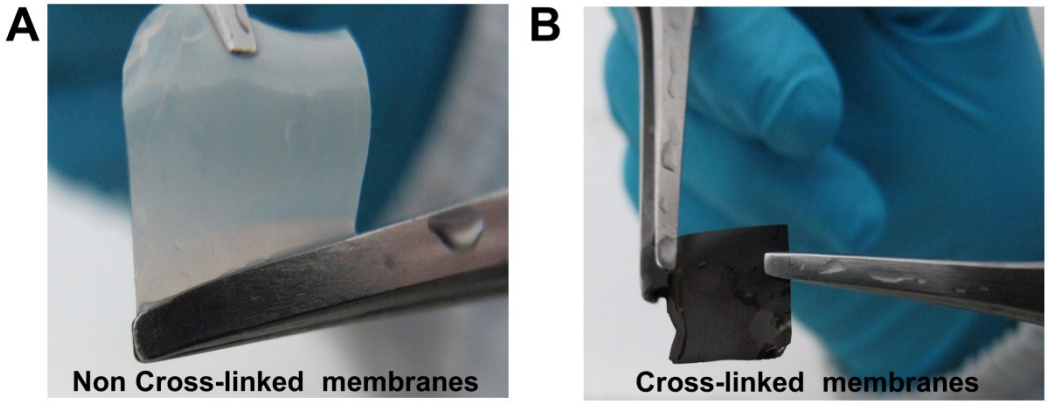

Figure S 2. Handling of freestanding CHIT/ALG membranes without (A) and after cross-linking with genipin (24 hours) (B).

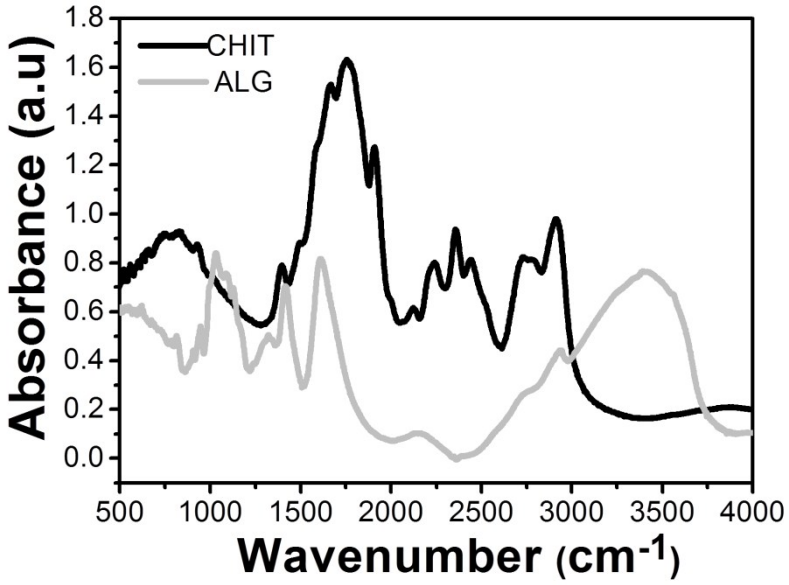

Figure S 3. FTIR measurements of pure polysaccharides: chitosan (CHIT) and Alginate (ALG).

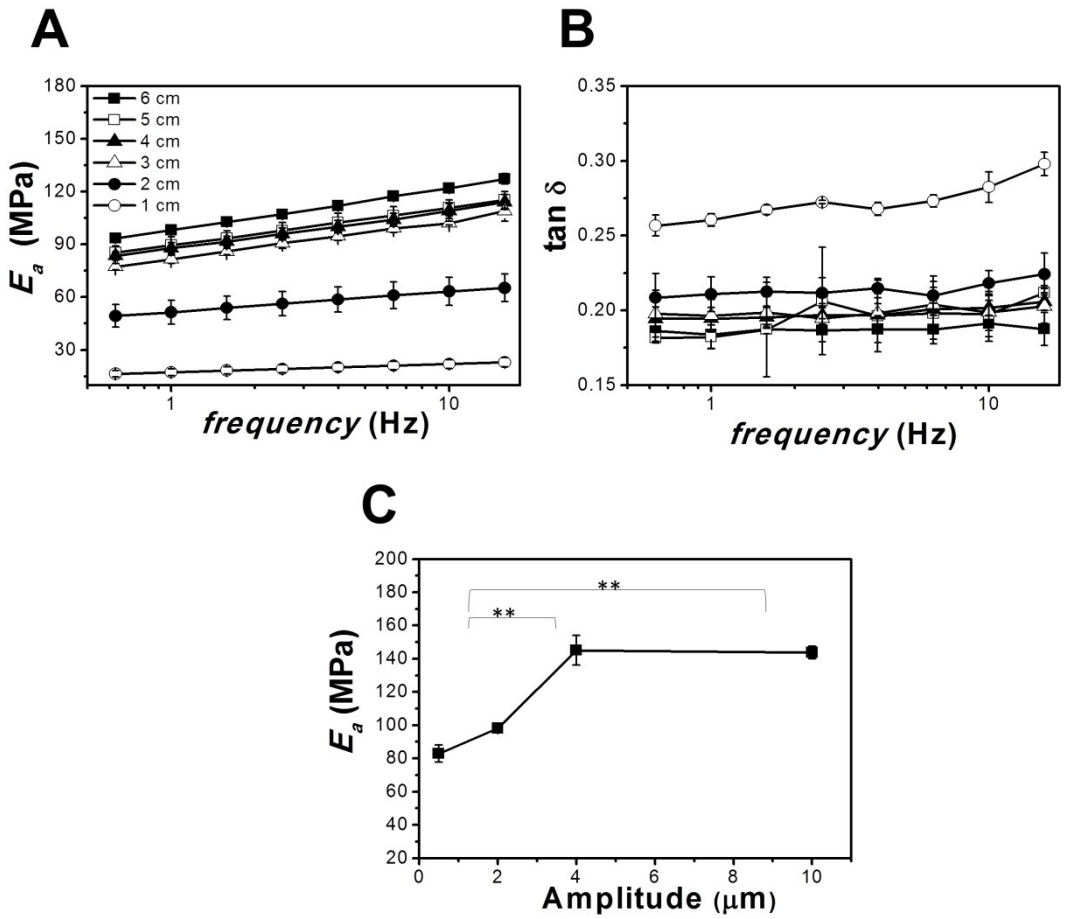

Figure S 4. Non-conventional local "micro-compression": DMA scans of gradients of stiffness obtained under immersion in acetate buffer at 37 °C. A, B: Frequency dependence of the apparent modulus (E_a) and the loss factor (tan δ) at different positions of the gradient films (displacement amplitude 2 μm). Dependence of the displacement amplitude on the E_a in the same region of stiffness (6 cm) as a function the displacement (C).Significant differences were found for (*) $p < 0.05$.

REFERENCES

1. **C. Chaubaroux, E. Vrana, C. Debry, P. Schaaf, B. Senger, J.-C. Voegel, Y. Haikel, C. Ringwald, J. Hemmerlé, P. Lavalle and F. Boulmedais, *Biomacromolecules*, 2012, 13, 2128-2135.**