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SUPPLEMENTARY INFORMATION

Polysaccharide based covalently linked multi-membrane hydrogels

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Discussion about the determination of gelation point

Rheological measurement at one frequency allows to determine the gelation point at G' = G'' only as a first approximation. However, the crossing of G´ and G´´ can be very close to the real gelation value for certain conditions and is, in a simple term, mainly deviating due to temperatures close to the glass transition, entanglements and stiffness of the polymer chains [S1]. The criterion of temperatures well above the glass temperature does of course not account for hydrogels as cross-linking is taking place in aqueous solution. Thus, maximum shifting between the real gel point and the crossover of G' and G'' can be observed with high molecular weight polymers at the highest concentration. In these cases, the initial G' values are high, maybe even higher than G'', due to entanglements, so that the cross-over of the 2 curves either deviates strongly from the gel point or does not even occur as during the whole gelation process G' > G''.

Having this in mind, we did, from the whole range of possible molecular weights of hyaluronic acid and molar ratios between cross-linker and hyaluronic acid [S2], choose hydrogel compositions with relatively low molecular weight hyaluronic acid (150 kDa) at concentrations of 2 w/v% respectively 5 w/v%, depending on type of cross-linking. Under these conditions, entanglement and viscosity increase independent of cross-linking are negligible. Hence, determination of gelation point via the crossover of G' and G'' at one frequency may be performed for these conditions with a reasonable precision. We support this by a literature report of a similar system where the authors have performed numerous rheological experiments that show a deviation of gelation point determined by crossover of G' and G'' and frequency sweeping tests according to Winter-Chambon power law of ± 5% [S3]. Therefore, we estimate the error for the gelation point as determined by the crossover of G' and G'' to be maximum ± 5% also in our study.

Most importantly, the gelation point as such is not of major importance in this study. For the multi-layer gel preparation, it was simply important to have the gelation slow enough, so that mixing of the two component system cam be performed and the core hydrogel embedded before significant gelation already takes place which would lead to inhomogeneities. Gelation should also occur fast enough, within 20-30 minutes, so that the overall process of creating a multi-layer system does not take too long. Therefore, determination of gelation time with an error of ± 5% is absolutely sufficient for the purpose of this study.

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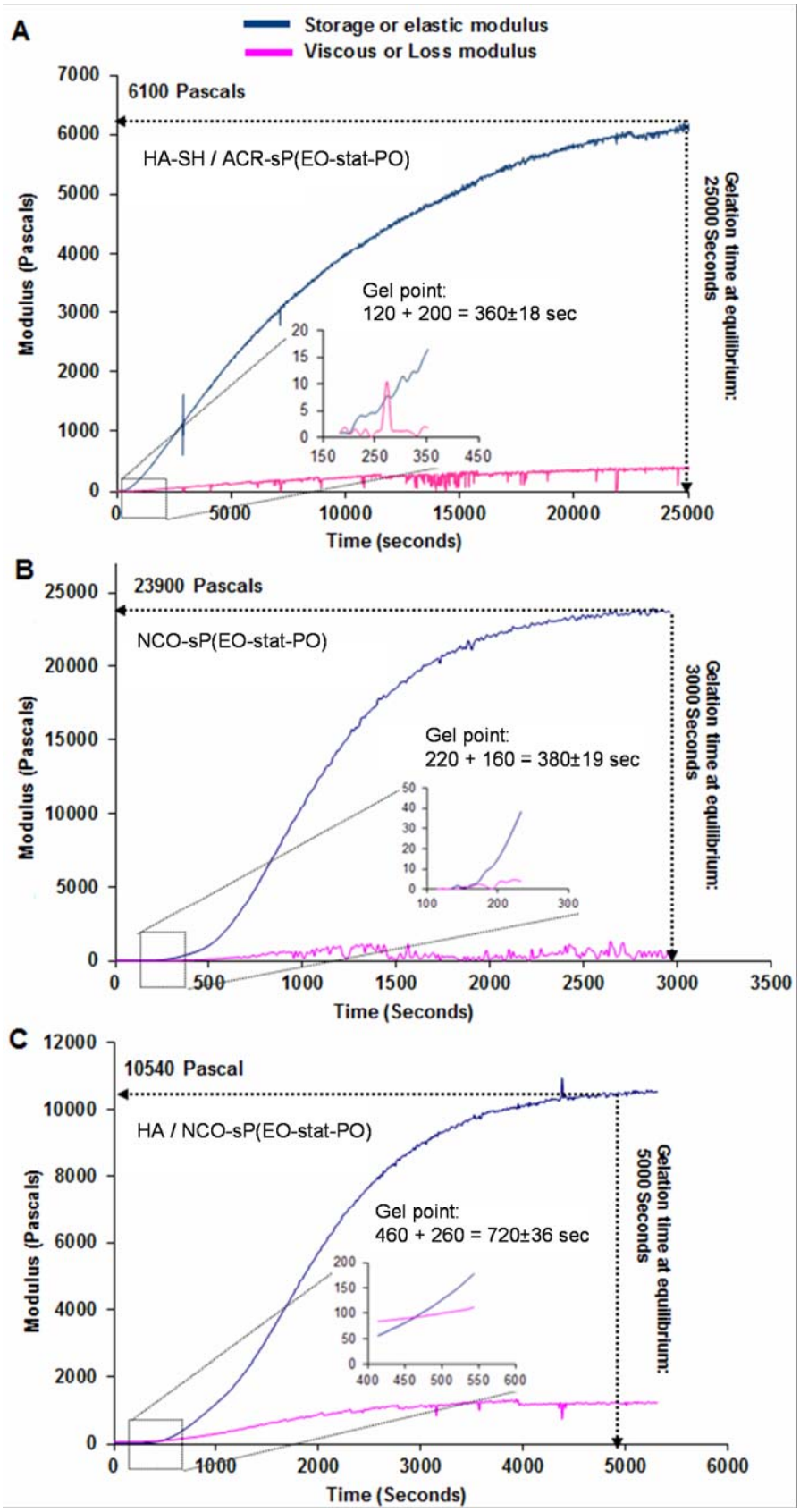

Figure S1: Evolution of G' and G'' over time for all three hydrogel systems used in this study, HA-SH / ACR-sP(EO-stat-PO) / HA-SH (A), NCO-sP(EO-stat-PO) (B) and HA / NCO-sP(EO-stat-PO) (C). Gel points are given as added time of the time between mixing of the two components and start of the measurement and the timepoint where the curves for G' (storage modulus) and G'' (loss modulus) cross.

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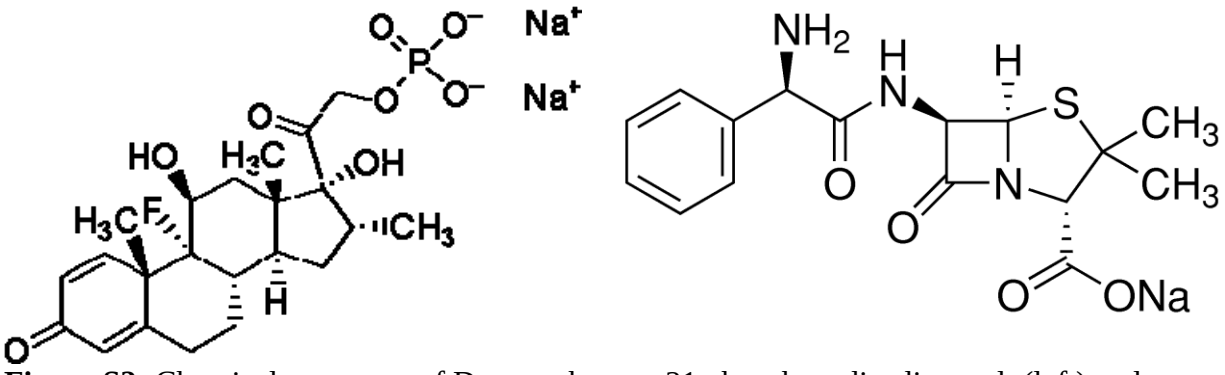

Figure S2: Chemical structures of Dexamethasone 21 phosphate disodium salt (left) and Ampicillin sodium salt (right).

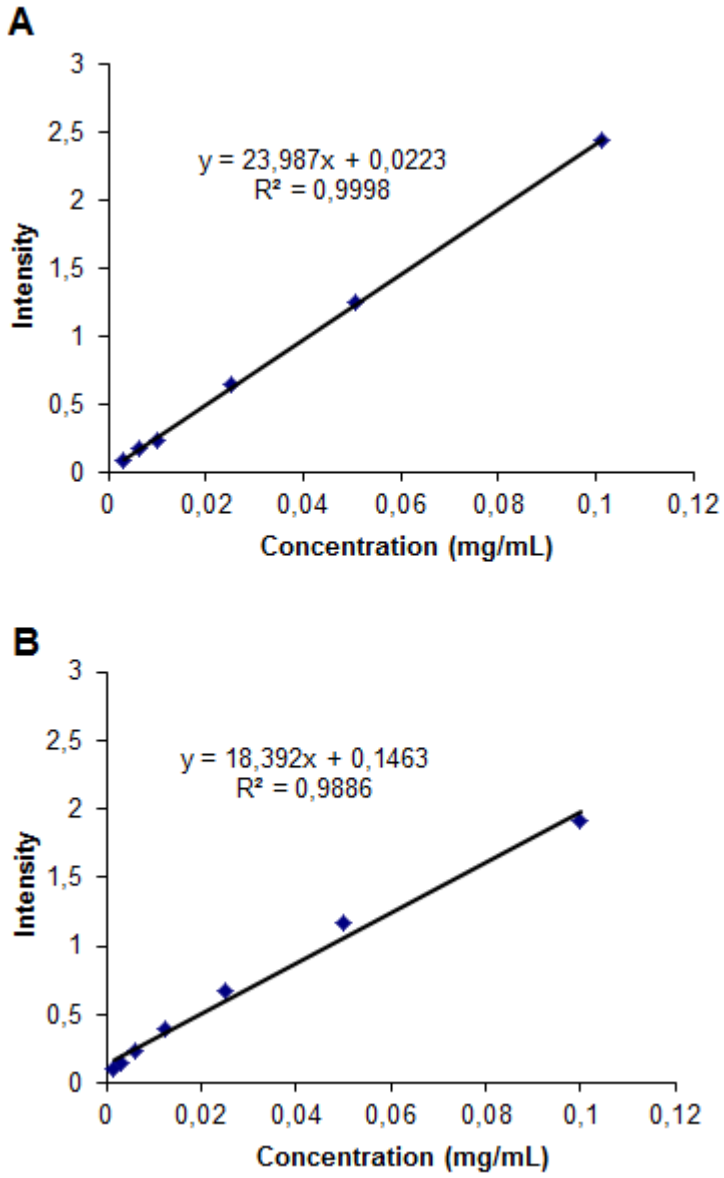

Figure S3: UV-calibration curves for the quantification of drug concentration for Dexamethasone 21 Phosphate disodium salt (A) and Ampicillin disodium salt (B).

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Interaction possibilities between drugs and hydrogels

Regarding chemical reactivity between drug and hydrogel, dexamethasone possesses 2 alcohol groups that could potentially react with isocyanate to form stable urethane bonds. However, the presence of the phosphate groups and the two sodium counter ions make the molecule extremely hydrophilic, while the isocyanate groups are placed at rather hydrophobic isophorone-rings. Moreover, Hyaluronic acid possesses a large number of alcohol groups. These two circumstances lower the reaction probability between dexamethasone 21 phosphate disodium salt and NCO-sP(EO-stat-PO), so that Dexamethasone is not chemically embedded into the hydrogel network and thus quantitatively released from NCO-sP(EO-stat-PO) gels after 3 days. This slow release however shows that the drug has multiple possibilities to physically interact with the hydrogel network through hydrogen bridges.

In contrast to Dexamethasone, initial experiments showed that Ampicillin is not compatible with NCO-sP(EO-stat-PO) for hydrogel formation. The amine group present in Ampicillin reacts with the isocyanates so that the drug is covalently embedded in the network and is not released by simple diffusion. Depending on the amount of drug, also a significant effect on cross-linking kinetics was observed. As we were not interested in chemical modification of the drugs and were aiming at drug release by diffusion, we did not include this system in the present study.

However, Ampicillin could be added to the ACR-sP(EO-stat-PO) / HA-SH system without affecting the cross-linking kinetics and without chemical reaction with the gelating system. The thiol groups present in HA-SH are much more reactive than the amine groups in Ampicillin for Michael Addition, so that the drug is not binding to ACR-sP(EO-stat-PO) and is quantitatively released by diffusion within 20 hours. This fast release shows that Ampicillin has much less possible interactions with the hydrogel network than Dexamethasone in the NCO-sP(EO-stat-PO) hydrogel, where urethane, urea and amine groups are present that offer multiple interaction possibilities for hydrogen bridge formation.

References

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[S2] A. Dhanasingh, J. Salber, M. Möller, J. Groll, *Soft Matter* 2010, ***6***, 618-629.
[S3] L. Weng, X. Chen, W. Chen, *Biomacromolecules.* 2007; ***8(4)***, 1109–1115.