Supplementary Information

Tuning the Strength and Swelling of an Injectable Polysaccharide Hydrogel and the Subsequent Release of a Broad Spectrum Bacteriocin, Nisin A.

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S1 - Methods

S1.1 – Characterization of Un-Cross linked Polymers

The degree of aldehyde substitution was determined using a method reported by Zhao et al [1]. A 0.25 M solution of hydroxylamine hydrochloride was prepared in DI water. Dex-CHO_n was dissolved in the solution (0.4% w/v). 0.1 M NaOH was added in 100 µl volumes until the rate of change of pH remained constant (equilibrium). The degree of functionalization was determined using **Equation 1** below, where D.O.S is the degree of aldehyde substitution (%), V is the volume of NaOH required to reach equilibrium (L), C is the concentration of NaOH (M), n is the theoretical number of aldehyde groups per repeating unit (2), m is the mass of dextran added (0.1 g) and M is the molecular weight of a single glucose unit.

$$D.O.S = \frac{V_{NaOH}\, x\, C_{NaOH}}{n_{C=O}\, x\, \frac{m}{M}}\, x\, 100$$

Equation S1 [1]

Molecular weight, number average molecular weight, polydispersity and Mark Houwink parameters (**Equation 2**) of the functionalized dextran and alginate and the as received dextran, alginate and glycol chitosan, was determined using gel permeation chromatography (GPC) on an AKTA Pure 25 (GE Life Sciences) chromatography system and Malvern Reveal (Particular Sciences, Ireland) multi detector system. The system was calibrated with pullulan standards (1 mg ml^{-1}, 0.1_M sodium sulfate (Na_2SO_4)) and verified with dextran standards (2 mg/ml, 0.1 M Na_2SO_4). Dextran samples (2 mg ml^{-1}) were run using a 0.05 M Na_2SO_4 mobile phase, with a flow rate of 0.3 ml/min and sample volume of 100 µl with a dn/dc 0.147. Alginate samples were run with a 0.05 M Na_2SO_4 mobile phase, at a flow rate of 0.4 ml min^{-1} with a sample volume of 100 µl and a dn/dc of 0.15. Glycol chitosan samples were prepared and run using 0.1 M Na_2SO_4 prepared in 0.5 M acetic acid, with a sample volume of 100 µl and dn/dc 0.15. nn/dc values were obtained from the Malvern Reveal instrument library. All samples were filtered through 0.2 µm PES filters and run on a TSKgel SuperMultipore PW-M column (Tosoh Bioscience), at 30°C [2].

$[\eta] = K.M^{\alpha}$ – **Equation S2**

S1.2 - Scanning Electron Microscopy

To assess the morphology of the gels, Dex-Alg-GC gels, and the Dex-$GC_{6\%}$ gels were frozen for 24 hours at -80°C, after which they were freeze dried in a Telstar Lyoquest freeze drier with a condenser temperature of -75°C overnight. The dried gels were carefully sectioned using a scalpel blade and stuck to carbon tape on an SEM stub. The dried gels were gold sputtered for 1 minute and SEM's were taken on an SU70 Hitachi high resolution field emission scanning electron microscope at a voltage of 3.0 kV.

S.2 – Results

S2.1 – Characterization of Uncross-linked Polymers

Gel permeation chromatography using a multi detection system allowed for the determination of the Mw, polydispersity and the Mark Houwink (M-H) constants, α (**Equation S.2**), for the as received and functionalized polymers. The M-H equation relies on the relationship between the intrinsic viscosity, [η] and the molecular weight, where the constant alpha relates to the slope of a bi-logarithmic plot of η and Mw. This relationship has been determined to be based on the strength of the polymers intramolecular hydrodynamic interaction [3]. The parameters of the M-H equation reflect the conformation of polymer chains in solution, where a coiled structure is indicated by $\alpha < 0.5$ while stiffer polymers are indicated by $\alpha > 0.5$ [4]. Changes in Mark-Houwink plots are caused by changes in polymer structure, evident by the change in intrinsic viscosity, normally associated with a change in branching as these differences are independent of molecular weight.

The degree of aldehyde substitution was also determined for the dextran-CHO. Analysis of the uncross-linked polymers allowed for a more in depth understanding of the structure of the polymers and influence of polymer branching on the properties of the hydrogels.

Table S1. Characterization of the as received and functionalized dextrans, alginate and glycol chitosan. The Mw, Mn, M-H$^{\alpha}$ constants and polydispersity as determined by multi detector GPC.

Sample	**Mw** [kDa]	**Mw/Mn (PDI)**	**M-H$^{\alpha}$**
Dextran	231.8 ± 5.6	1.85	0.37 ± 0.16
Dex-CHO	159.9 ± 12	1.59	0.07 ± 0.17
Alginate	49.1 ± 0.24	1.44	0.96 ± 0.02
Alg-ADH	39.1 ± 3.7	1.03	0.838 ± 0.03
Glycol Chitosan	125.2 ± 2.2	1.06	0.03 ± 0.003

Dextran

The Mw of the as received dextran was determined to be 231.8 ± 5.6 kDa as shown in **Table 2**. Mark-Houwink analysis allowed for the determination of polymer architecture where a value of $\alpha < 0.5$ indicates flexible random coils. For as received dextran, the M-H$^{\alpha}$ value obtained, 0.32 ±

0.1, indicates a flexible polymer chain, a commonly observed structural characteristic of high molecular weight dextrans [5, 6]. The oxidized dextran possessed a lower molecular weight (159.5 ± 12 kDa) than the as received dextran as a result of chain cleavage during the oxidation reaction. Analysis of the Dex-CHO shows a M-H_α of 0.07 ± 0.08, indicating that the oxidized dextran appears in a coil form. The degree of aldehyde substitution of the dextran was determined to be 34% (45.5 mol%). The polydispersity index (PDI) of the as received and functionalized dextran indicate no significant difference in the molecular dispersity of the polymer, both indicative of a medium distribution.

Alginate

The as received alginate was determined to have a Mw of 49.1 ± 0.24 kDa and Mn of 34.2 ± 1.9 kDa. An M-H^α value of 0.96 ± 0.02 was obtained indicating a linearized structure. Analysis of the alginate-ADH shows a slightly reduced intrinsic viscosity, as well as a reduced M-H^α constant of 0.84 ± 0.03, indicating that after functionalization a slightly lower linear structure was evident. The higher M-H^α constant observed in the non-functionalized alginate indicates a more linearized structure. A lower PDI was observed in comparison to the dextrans, indicating higher viscosities in the alginates with less chain length variance.

Glycol Chitosan

The glycol chitosan exhibited similar properties to the functionalized alginate in terms of polymer dispersity, (1.06). The molecular weight was determined to be 125.2 ± 2.2 kDa with an Mn of 117.6 ± 1.8 kDa. Mark Houwink analysis indicated that glycol chitosan exhibits an extended linear structure.

S2.2 – Scanning Electron Microscopy

Gels that were freeze dried were cross sectioned using a surgical grade scalpel blade. The gels were observed under a voltage of 2-5 kV and images were taken. The images indicate that as GC is substituted for alginate, the gels appear to be more porous (**Fig S.1 A-C**). The Dex-$GC_{6\%}$ gel (**Fig S.1 D**) showed a layered unstructured morphology compared to the covalently crosslinked gels containing alginate-hydrazine.

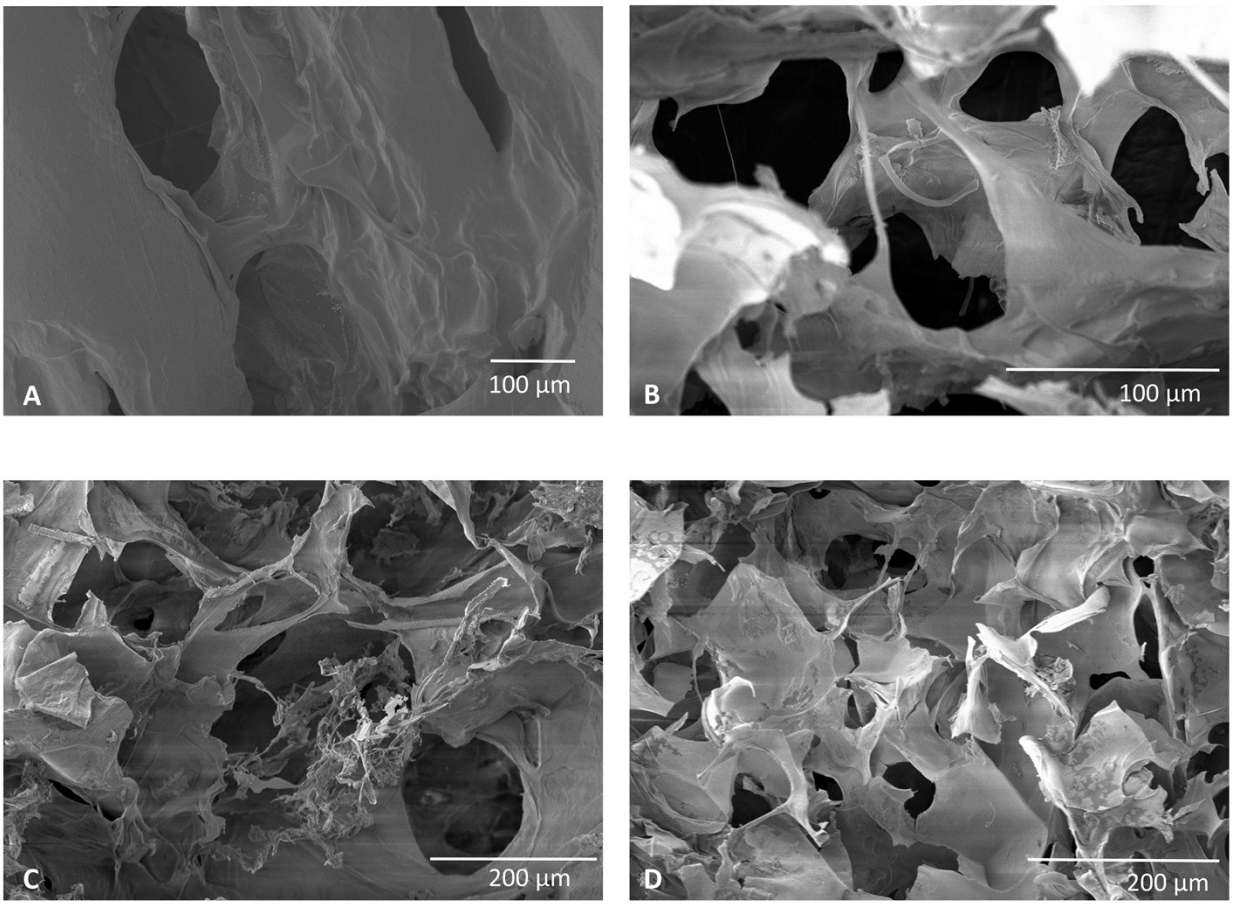

Figure S. 1 SEM images of the **A)** Dex-$Alg_{3\%}$-$GC_{0\%}$, **B)** Dex-$Alg_{1.5\%}$-$GC_{3\%}$, **C)** Dex-$Alg_{0.5\%}$-$GC_{6\%}$ and **D)** Dex-$GC_{6\%}$

References

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