Supporting Information

A stepwise funnel selection approach identifying natural polymer derived hydrogels for long-term islet delivery restoring normoglycemia in type-1 diabetes

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Synthesis of hydrogel

1. Alginate: Initially, 10 ml of 2% HV/LV alginate solution and 10 ml of 2% LV Na-alginate solution were prepared. Then, a 5 mL solution was prepared by mixing HV alginate and LV alginate in a ratio of 2:3 and properly mixed using a vortex. After that, the moulds were kept on a clean surface, and a 1% $BaCl_2$ solution was sprayed over the moulds. Subsequently, 200µl of the mixed HV-LV alginate solution was poured into the moulds, and again, 1% $BaCl_2$ solution was sprayed over the moulds. Lastly, the hydrogels were carefully retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

2. Agar: 10 ml of 2% agar solution was prepared and kept in a stirrer (600 rpm) and heated at 80 °C for half an hour. Then the temperature of the reaction mixture was slowly decreased to 40 °C. After that, 200µl of the solution was poured into the moulds and allowed to solidify for 20 minutes. Subsequently, it was dipped in 5% citric acid solution for 20 minutes to crosslink. Finally, the hydrogels were carefully retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for degradation.

3. Pectin: 10 ml of 7.5% pectin solution was prepared and kept in a stirrer (600 rpm) and heated at 50 °C until proper mixing. Then the temperature was slowly decreased to 40 °C. After that, 200 µl of the resulting solution was poured into the moulds and allowed to solidify for 20 minutes. The moulds were then dipped in 1% $BaCl_2$ solution for an hour to crosslink. Finally, the hydrogels were carefully retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

4. Cellulose: Initially, 700 mg NaOH and 1.2g urea were dissolved in 10 ml deionised water. Then, 800 mg of cellulose was added to the previous mixture, and the resultant mixture was stirred (600 rpm) at room temperature for an hour. After that, it was kept in a refrigerator at -20 °C for two hours. It was then thawed at room temperature for half an hour, and 200 µl of 20% citric acid solution was added to it and kept stirring until the viscosity of the solution increased. Finally, 200 µl of the reaction mixture was poured into the moulds and the moulds were again dipped in the 20% citric acid solution bath for 10-15 minutes. The hydrogels were carefully retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

5. Agarose: 10ml of 1% agarose solution was prepared and kept in a stirrer (600-700rpm) and heated at 50 °C for one hour. After that, the temperature was decreased to 40 °C -42 °C. Then, 200µl of the agarose solution was poured into the moulds and allowed to solidify at room temperature for 15 minutes. Subsequently, the moulds were dipped in 5% citric acid solution bath to crosslink for 20 minutes. Finally, the hydrogels were carefully retrieved from the moulds. The weight and diameter

of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

6. Chitosan: 2% chitosan was dissolved in a 20% acetic acid solution. The resulting solution was heated in a magnetic stirrer for 1.5 hr, maintaining a temperature of 40 °C - 50 °C. From that, 200 µl solution was poured into the moulds and 2 drops of 2% Glutaraldehyde solution were added gently and mixed in every mould. It was then crosslinked for 1 hour. Hydrogels were then retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

7. Gelatin: 10 ml of 10% gelatin solution was prepared and kept in a stirrer (400 rpm) and heated at 60 °C for 15 minutes. Then, from the resulting solution, 200 µl was poured into the moulds. After that, the moulds were kept in a refrigerator (4 °C- 8 °C) for half an hour upon solidification. Subsequently, the moulds were dipped in crosslinking solution consisting of glutaraldehyde (5%) and acetone. Finally, the moulds were kept at 8 °C for 1 hr to crosslink, and hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Lastly, dipped in 1X PBS for the degradation study.

8. Starch: 10 ml of 20% starch solution was prepared and kept in a stirrer (600 rpm) and heated at 90 °C for half an hour. Then, 1.8 g of $CaCl_2$ was directly added to the previous solution, and stirring was continued for another 45 minutes at 60 °C. After that, 200 µl of the resulting solution was poured into the moulds and kept for solidification for 24 hours. Subsequently, the hydrogels are retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

9. Xanthan: 10 ml of 3% xanthan solution was prepared and kept in a stirrer (600 rpm) and heated for 10 minutes, maintaining a temperature between 90 °C and 100 °C. Then, 200µl of the resulting solution was poured into the moulds. Subsequently, two drops of 5% glutaraldehyde solution were added to the moulds, and it was allowed to solidify for 24 hours. After that, the hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

10. Guar-gum: 2% guar gum solution was prepared in deionised water and kept in a stirrer (600 rpm) and heated for 30 minutes at 50 °C. Then 200 µl of the resulting solution was poured into the moulds. After that, 2-3 drops of 25% glutaraldehyde solution were added to the moulds and properly mixed. It was then allowed to solidify for 3 hours. Subsequently, the hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

11. Chitin: 10 ml of chitin (4%) solution was prepared, and 800 mg NaOH was added to it. The resulting mixture was kept in a stirrer for 5 minutes. It was then kept in a refrigerator at -20°C for 4 hours. Finally, it was thawed and stirred at room temperature. Subsequently, epichlorohydrin (7%) solution was added to the mixture. It was then stirred (600 rpm) for 30 minutes at room temperature. Finally, it was heated at 60 °C to get a gel-like appearance and cast into moulds. Lastly, hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

12. Lignin: At first, 5 mL of NaOH (4%) solution was prepared, and into that, 1g of lignin was added. The resulting mixture was stirred at 600 rpm for 20 minutes at room temperature. Then, 7% epichlorohydrin was added to the reaction mixture and mixed properly. Finally, the reaction mixture was heated at 60 °C for 2-3 hours till a gel-like appearance came. Subsequently, 200 µl of the reaction mixture was poured into the moulds and allowed to solidify. Lastly, the hydrogels were

retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

13. Xylan: 5 ml of xylan (5%) solution was prepared, and it was stirred (400 rpm) for 1 hour, maintaining a temperature of 60 °C. Then, 200 µl of glutaraldehyde (5%) solution and 200 µl of citric acid (5%) solution were added to the mixture. Subsequently, the mixture was stirred (400 rpm) and heated at 60 °C for 2 hours. Finally, 200 µl of the reaction mixture was poured into the moulds and kept for an hour. No hydrogel formed.

14. Pullulan: 3 ml of pullulan (20 %) solution was prepared, and it was stirred (400 rpm) for 20 minutes at 37 °C. Then, 100 mg of citric acid was added to the previous reaction mixture. Subsequently, it was stirred (400 rpm) for another 20 minutes at room temperature. Finally, 200 µl of the reaction mixture was poured into the moulds. Lastly, the hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

15. Glycogen: 3 ml of glycogen (10%) solution was prepared and stirred (400 rpm) for 1 hour at 37 °C. 200µl of epichlorohydrin solution was added to the mixture and stirred for another 20 minutes. Then it was kept in a refrigerator at -20^{o}C for another 1 hour and thawed at room temperature. Subsequently, it was again stirred (400 rpm) for 2 hours at 60 °C. After that, 200 µl of the reaction mixture was poured into the moulds. Lastly, the hydrogels were retrieved from the moulds. The weight and diameter of the hydrogels were measured and viewed under bright and dark field (2X). Finally, dipped in 1X PBS for the degradation study.

16. Collagen: 5 ml of collagen (10%) solution was prepared and kept in an ice bath. To that 1 ml 10X PBS solution was added. After that, a few drops of 0.1(M) NaOH solution were added to the previous reaction mixture to maintain pH 7.4 and kept in the refrigerator at -20 °C for 1 hour. It was then thawed at room temperature, and 200 µl of glutaraldehyde (5%) solution was added to it. Finally, the reaction mixture was stirred (400 rpm) for another hour. After that, 200 µl of the reaction mixture was poured into the moulds. No hydrogels formed.

17. Gum Rosin: 10 ml of gum rosin (8%) solution was prepared and stirred (600-700 rpm) for 2 hours at 50 °C. Then the temperature was slowly decreased to 40 °C. Then, 1 ml of 2 % $BaCl_2$ solution was added to the mixture. Subsequently, the mixture was stirred (400 rpm) and heated at 60 °C for 2 hours. Finally, 200 µl of the reaction mixture was poured into the moulds and kept for an hour. No hydrogel formed.

18. Gum Ghatti: 10 ml of gum-ghatti (10%) solution was prepared, and it was stirred (600 rpm) for 30 minutes, maintaining a temperature of 60 ^{o}C. Then, 250 µl of citric acid (8%) solution was added to the mixture. Subsequently, the mixture was stirred (400 rpm) and heated at 70 ^{o}C for 1 hour. Finally, 200 µl of the reaction mixture was poured into the moulds and kept for an hour. No hydrogel formed.

19. Gum Acacia: 5 ml of gum-acacia (20%) solution was prepared, and it was stirred (600 rpm) for 20 minutes, maintaining a temperature of 50 °C. Then, 150µl of citric acid (10%) solution was added to the mixture. Subsequently, the mixture was stirred (400 rpm) and heated at 60 °C for 2 hours. Finally, 200µl of the reaction mixture was poured into the moulds and kept for an hour. No hydrogel formed.

20. Gum tragacanth: 10 ml of gum-tragacanth (8%) solution was prepared, and it was stirred (600 rpm) for 20 minutes, maintaining a temperature of 50 °C. Then, 150 µl of citric acid (10%) solution was added to the mixture. Subsequently, the mixture was stirred (400 rpm) and heated at 60 °C for 2 hours. Finally, 200 µl of the reaction mixture was poured into the moulds and kept for an hour. No hydrogel formed.

Mechanical properties of the hydrogel-

After selecting the top four leads from the degradation study, we assessed the compressibility/ mechanical stability of the top leads. All the fabricated hydrogel patches used in this study were circular in shape, with a diameter of 1-0.9 cm and a height of 0.2-0.25 cm. The mechanical testing of the lead hydrogel constructs was performed with a CT3 Brookfield Texture Analyser (TexturePro CTV1.6), and the probe that was used for putting strain on the sample was probe TA 44 (circular). The compression was set at 40-50 % compression/strain of the total height of the hydrogel patch, and the test speed was set at 0.50mm/s.

Rheological analysis of hydrogel-

Rheological analysis of all the hydrogel samples was done using Anton Paar MCR702 (multidrive) rheometer model. An oscillatory mode was used to measure storage modulus (*G*') and loss modulus (*G*") as a function of percentage strain. Angular frequencies was set between 0.01 and 100 rad/s [1] at a constant temperature of 28 °C. The hydrogel (diameter 25 mm and thickness 5 mm) were transferred onto the Peltier or rheometer plate against a parallel plate, and its surface was covered by solvent to prevent evaporation. The storage modulus (*G*') and loss modulus (*G*") of the hydrogel were recorded.

SEM imaging of hydrogel –

All the hydrogel patch samples were prepared and frozen at 20 °C. The obtained samples were immediately transferred to a lyophilizer for 24 h (-60 $^{\circ}$C and -756 mmHg). The cross-sections of the hydrogel samples were then coated with gold with the help of a sputter coater, and the surface morphology and structure of the hydrogels were examined on a scanning electron microscope.

Contact angle determination of the hydrogel sample-

The contact angles of the hydrogel samples were determined to assess the wettability of the materials. A thin polymeric layer was created on a grease-free glass slide, and the fabricated surface was characterised for the degree of wettability (hydrophilic/hydrophobic) by taking the static contact angle measurement using a Theta Lite optical tensiometer (Biolin Scientific, Finland), and the analysis was performed using OneAttension software (256 fps). Contact angle of a water droplet over the sample surface, fixed to lie flat on a horizontal plane. A fixed volume of 3µl sessile deionised water was dispensed on the sample substrate, and then the contact angle between the line tangent to the liquid droplet and the substrate surface was measured.

DPPH assay for the antioxidant property of hydrogel-

The DPPH assay was widely used to check the antioxidant capacity of a sample. DPPH (2,2-diphenyl-1-picrylhydrazyl), a purple-colored dye, becomes colourless while reacting with antioxidants. All the different hydrogel samples were prepared accordingly and kept in a 24-well plate. The DPPH working reagent was prepared by dissolving 0.78 mg of DPPH in 10 mL of 99.5% ethanol. 1 mL of the freshly prepared DPPH reagent was added to each well and incubated at RT in the dark for 30 min. Following 30 min incubation, the absorbance of each sample was recorded at 517 nm using a bio-spectrophotometer, keeping the DPPH solution as a control[17]. To quantify the scavenging activity the given formula is used:

$$Percentage\ of\ scavenging\ activity = \left(\frac{Ac - As}{Ac}\right) \times 100$$

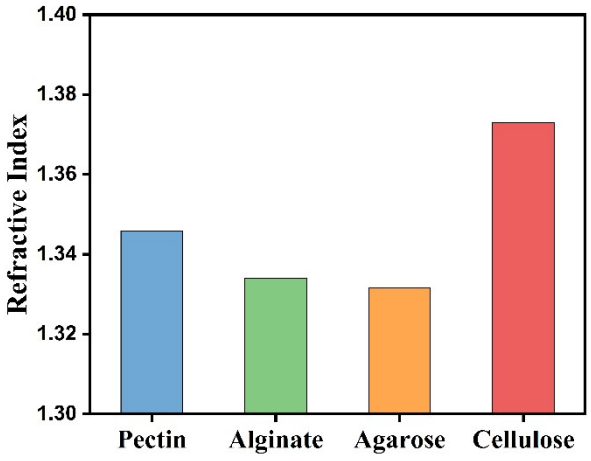
[Ac- absorbance of the control, As- absorbance of the sample]

Supporting Table 1: List of 20 different natural polymer with respective crosslinkers.

Sl No.	Polymer (%)	Crosslinker (%)	Type and time of crosslinking
1	Agarose (1%)	Citric acid (5%)	Chemical crosslinking (20 min)
2	Agar (2%)	Citric acid (5%)	Chemical crosslinking (20 min
3	Alginate (2%)	Barium chloride (1%)	Ionic crosslinking (30min)
4	Gaur gum (2%)	Glutaraldehyde (10%)	Chemical crosslinking (3 hr)
5	Chitosan (2%)	Glutaraldehyde (2%)	Chemical crosslinking (1 hr)
6	Xanthan gum (3%)	Glutaraldehyde (3%)	Chemical crosslinking (24 hr)
7	Chitin (4%)	Epichlorohydrin (7%)	Chemical crosslinking (30 min)
8	Xylan (5%)	Glutaraldehyde (5%)	Chemical crosslinking (2 hr)
9	Pectin (7.5%)	Barium chloride (1%)	Ionic crosslinking (1 hr)
10	Gum rosin (8%)	Citric acid (5%)	Chemical crosslinking (2 hr)
11	Gum tragacanth (8%)	Citric acid (10%)	Chemical crosslinking (2 hr)
12	Cellulose (8%)	Citric acid (20%)	Chemical crosslinking (15 min)
13	Glycogen (10%)	Epichlorohydrin (7%)	Chemical crosslinking (2 hr)
14	Gelatin (10%)	Glutaraldehyde (5%)	Chemical crosslinking (1 hr)
15	Gum ghatti (10%)	Citric acid (8%)	Chemical crosslinking (1 hr)
16	Collagen (10%)	Glutaraldehyde (5%)	Chemical crosslinking (1 hr)
17	Gum acacia (10%)	Citric acid (20%)	Chemical crosslinking (2 hr)
18	Pullulan (20%)	Citric acid (3%)	Chemical crosslinking (20 min)
19	Lignin (20%)	Epichlorohydrin (7%)	Chemical crosslinking (3 hr)
20	Starch (20%)	Citric acid (18%)	Chemical crosslinking (24 hr)

Refractive index measurement of hydrogel-

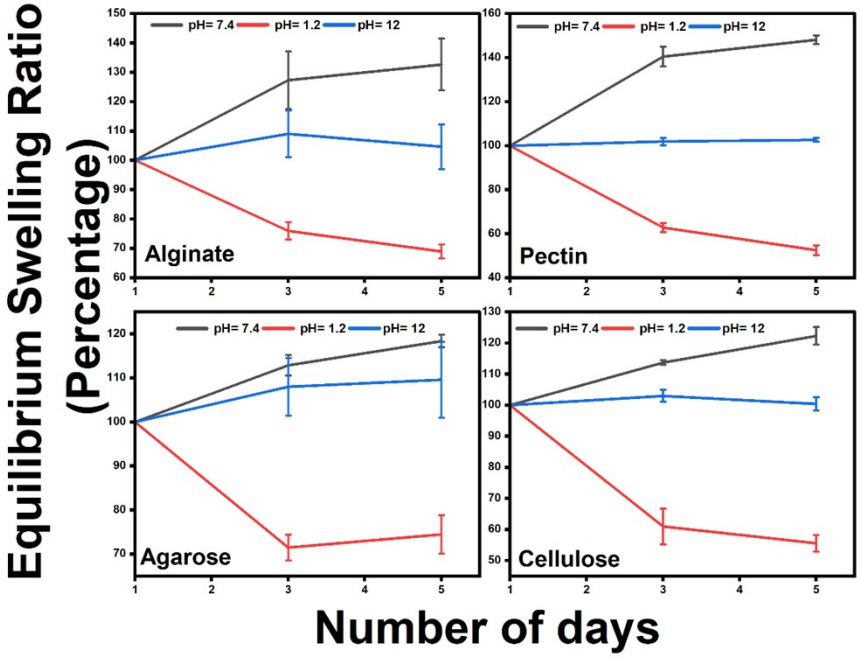
The refractive index (RI) of the selected hydrogel samples was determined using an Anton Paar refractometer (Abbemat-350). The instrument was calibrated daily using deionised water (RI-1.33), and the refractive index of the hydrogel was measured. All the measurements were taken at a controlled temperature of 37 °C.



Supporting Figure 1: Refractive index of respective hydrogel samples

Swelling study of hydrogel in different pH-

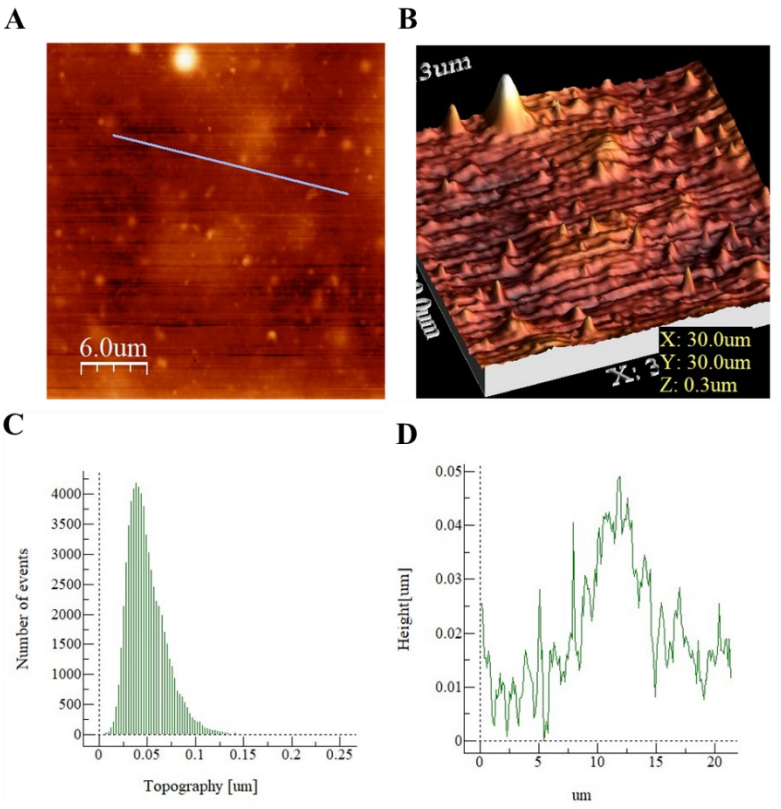
All the top four lead hydrogel samples were prepared according to the established protocol. The swelling behaviour of the four lead hydrogels (alginate, pectin, agarose, and cellulose) was checked in buffers of varying pH (1.2, 7.4, and 12) over 5 days to better approximate the range of environments that implants may experience. At physiological pH 7.4, all hydrogels exhibited a gradual increase in equilibrium swelling ratio, reaching approximately 130–150% for alginate and pectin, 115–120% for agarose, and approximately 120–125% for cellulose by day 5, indicating stable, moderately hydrated networks under near-physiological conditions. In contrast, at acidic pH 1.2, all four hydrogels exhibited a marked reduction in swelling (to ~60–75% by day 5), consistent with protonation of carboxyl groups and network contraction, whereas at alkaline pH 12, the swelling ratios were intermediate and relatively stable over time, reflecting high ionisation but partial structural relaxation of the networks.



Supporting Figure 2: Swelling study of the respective hydrogel sample at different pH levels.

Characterisation of lead pectin-

The AFM analysis of the pectin hydrogel reveals a nanoscale-smooth yet topographically heterogeneous surface, consistent with a homogeneous crosslinked network suitable for cell encapsulation. The height map over a 6 µm scan area shows only shallow peaks and valleys, with the line profile fluctuating between approximately 10–50 nm, indicating the absence of large defects, cracks, or sharp asperities that could mechanically stress encapsulated cells. The corresponding height histogram is narrow and unimodal, centred around ~50 nm, confirming that most surface points lie close to the mean height and that roughness originates from small-amplitude undulations rather than discrete irregularities. The calculated RMS roughness of 0.0222 µm (22.2 nm) quantitatively corroborates this observation and places the pectin surface in the low-roughness regime, which is generally favourable for stable protein adsorption and gentle cell–matrix interactions while maintaining sufficient



nano-scale texture for integrin engagement.

Supporting Figure 3- AFM imaging of pectin hydrogel cross-linked with $BaCl_2$. (A) Representative topographic AFM image of the pectin hydrogel surface (scale bar = 6 µm). (B) Three-dimensional surface topography rendering of the same pectin hydrogel region. (C) Height distribution profile corresponding to the blue cursor line indicated in panel A. (D) Surface roughness analysis of the pectin hydrogel derived from AFM measurements.