Immune-Modulating Mucin Hydrogel Microdroplets for the Encapsulation of Cell and Microtissue

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Immune-modulating biomaterials used to encapsulate cells and microtissue transplants can be engineered to dampen the immune reaction and increase treatment efficacy. Mucin-derived materials have gained attention for their ability to modulate macrophage and dendritic cell activity, and to trigger mild foreign body response when implanted in vivo. In this study, the potential of mucin hydrogels (Muc-gels) as cell-encapsulating materials is investigated. When placed in contact with blood, Muc-gels trigger significantly lower complement activation, compared to clinical grade alginate hydrogels. Muc-gel is a size-selective barrier strongly hindering the diffusion of molecules with a hydrodynamic radius larger than 6 nm such as immunoglobulins. Muc-gels support the growth of MIN6m9 insulin-secreting cells into islet-like organoids and the survival of primary human pancreatic islets, which maintained glucose responsiveness. Muc-gels can be shaped into microdroplets in which MIN6m9 cells or cell aggregates can be encapsulated without loss of viability. Microdroplet encapsulation will allow transplants to be easily injected and improve their survival by favoring mass transport through the capsule. The combination of strong immune modulatory properties, appropriate selective barrier profile, biocompatibility for embedded cells Muc-gels of particular value for microencapsulating cells or microtissues for transplantation.

1. Introduction

Medical devices containing biomaterials that precisely orchestrate the host immune reaction post implantation could find valuable uses in several fields of medicine.[1] For instance, they would be able to decrease the foreign body reaction (FBR) of the immune system to implanted biomaterials by modulating immune cell recruitment and their activation, which otherwise typically leads to chronic inflammation, and fibrotic encapsulation detrimental to the implants’ function.[2,3] It would be particularly valuable to avoid the formation of a fibrous tissue barrier around microencapsulated cells or microtissues implanted to produce hormones, neurotransmitters, enzymes, growth factors, cytokines, and antibodies.[4] Immune-dampening materials that trap or lower the amount of inflammatory cytokines produced in a tissue can also participate in creating anti-inflammatory environments.
to promote tissue repair,[5,6] for cancer immunotherapy,[7,8] and to reduce chronic inflammations in ulcers or inflammatory bowel disease.[9,10]

As part of the innate immune system, the complement system has been increasingly investigated for its role in determining the biocompatibility of implantable biomaterials.[11] The complement system is activated by a proteolytic cascade, producing protein fragments which participate in the innate immune response by facilitating the ability of antibodies and phagocytic cells to clear foreign bodies such as microbes.[12] When the foreign body is an implantable material, the C3a and C5a protein fragments can participate in recruiting and activating acute immune cells, that is, polymorphonuclear leukocytes, neutrophils, and monocytes,[13] which drive the chronic inflammatory phase and foreign body response.[14,15] Thus, the biocompatibility of immune-modulating materials can be improved through their modulation of the complement activation system.[16]

The challenge remains to design the molecular features that will elicit the desired immune response. The great diversity of natural glycan structures offers opportunities to regulate important immune functions through multivalent binding to cell surface receptors. Mucin glycoproteins primarily compose the dense glyocalyx of mucosal epithelial cells or mucus layers that cover the wet epithelium.[17] In addition to protecting against shear stress and dehydration, mucins are also bioactive molecules. Transmembrane mucins are involved in various diseases, including cancers, in which their altered glycosylation is associated with poor prognosis.[18,19] This is at least in part due to their ability to provide damping signals to immune cells, allowing the tumors to evade immune recognition. For instance, secreted intestinal MUC2 mucins are heavily glycosylated, and their glycans imprint DCs with anti-inflammatory properties.[20] Mucins modulate immune cells through binding to cell surface receptors expressed on immune cells.[21] For example, mucin glycans bind scavenger receptors, and sialic acid-binding immunoglobulin-like lectins (Siglecs).[22] The rich sialic acid content of mucins or mucin materials also suggests they could inhibit complement activation.[23,24] However, the chemistry used in material assembly is key because sialic acid (Neu5Ac) immunological activity is primarily governed by structure.[25] The mucins of the glyocalyx coating the endothelial surface layer of the vascular system are likely important to prevent unwanted complement activation in healthy tissues, and alteration is associated with a number of impaired endothelial functions such as thrombosis.[26]

We have shown recently that bovine submaxillary mucins can be covalently cross-linked into hydrogels that show strong immune modulation in vitro, transiently activating but quickly damping macrophages in vitro likely through sialic acid-binding to cell surface receptors.[27] And when implanted in the peritoneal cavity of immunocompetent mice for 21 days, the mucin gels were not degraded within that timeframe and remained viable and functional when embedded in the Muc-gels over 28 or 10 days, respectively. We use human pancreatic islets as a model system to illustrate how other microtissues could also benefit from Muc-gel encapsulation prior to transplantation.[4] Long-term survival and function of the Muc-gel enclosed cells or tissues is dependent on the bio-compatibility of the component materials but also on the sufficient diffusion of nutrients and metabolic byproducts, but not the immunoglobulin antibodies, that is, immunoglobulins (IgG) and IgM, through the mucin matrix. Finally, we validate that a therapeutic protein produced by the islets, insulin, is able to diffuse through the gels and demonstrate our ability to form mucin microcapsules with a high specific surface area, which could serve to augment mass transport in and out of the material.

2. Experimental Section

2.1. Materials

Amine-derivatives of tetrazine (Tz) and norbornene (Nb) were purchased from Bioconjugate Technology Company and TCI Europe N.V., respectively. All other chemicals were purchased from Sigma-Aldrich. The Insulin High Range kit was purchased from CisBio. The resources of chemicals and instruments are indicated below. Human pancreatic islets were purchased from Lonza (USA).

2.2. Mucin Hydrogel Preparation

Bovine submaxillary mucin (Muc) Muc-Tz and Muc-Nb derivatives were synthesized following the authors’ previous publication.[28] Mucins were predissolved in a MES buffer (0.1 M MES, 0.3 M NaCl, and pH 6.5) at a concentration of 10 mg mL⁻¹. To this reaction mixture, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (4 mmol g⁻¹ of dry mucin) and N-hydroxysuccinimide (4 mmol g⁻¹ of dry mucin) were added and stirred for 15 min. Thereafter, 1 mmol of tetrazine-amine (Tz) and 2 mmol of norbornene-amine (Nb) were added individually to generate the BSM tetrazine (Muc-Tz) or BSM norbornene (Muc-Nb). The reaction mixtures were stirred overnight at 4 °C and then dialyzed in dialysis tubing (100 kD cutoff) against 150 mM NaCl for 2 days and then Milli-Q H₂O for 1 day. The samples were then freeze-dried and stored at −20 °C. Samples for cell culture were filtered through 0.45 µm syringe filters and then transferred into a tissue culture tube with screw caps with a 0.2 µm filter for lyophilization.
2.3. Rheological Characterization of Mucin Hydrogels

Rheological measurements were performed using a commercial shear rheometer (MCR302, Anton Paar) equipped with a plate-plate measuring geometry (measuring head: PP25, Anton Paar, Graz, Austria). The gap between the measuring head and the bottom plate (P-PTD200/Air, Anton Paar) was set to \( d = 150 \mu m \) for all measurements. Muc-Tz and Muc-Nb were predissolved in a PBS (pH 7.4) solution at the concentration of 15 and 25 mg mL\(^{-1}\), respectively, for 1.5% and 2.5% Muc-gels. The two components were thoroughly mixed and 100 \( \mu L \) of the sample were pipetted onto the rheometer plate. First, time-sweep was performed within a duration of \( t = 100 \) min. Both the elastic (\( G' \)) and loss modulus (\( G'' \)) were determined by performing a torque-controlled (\( M = 5 \mu \text{Nm} \)) oscillatory (\( f = 1 \text{ Hz} \)) measurement. Afterward, the frequency-dependent viscoelasticity was assessed by a strain-controlled frequency sweep (from \( f_{\text{start}} = 10 \text{ Hz} \) to \( f_{\text{end}} = 0.01 \text{ Hz} \)). A constant strain was used which was chosen as the average of the five last values determined from the prior torque-controlled measurement.

Both the average molecular weight (\( M_c \)), the molecular weight of chain segments between two adjacent cross-links or entanglement points) and mesh size (\( \xi \), the distance between two adjacent cross-links or entanglement points) were calculated following below equations:\[^{[31]}\]

\[
M_c = \frac{\rho cR T}{G'_{\ast}} \tag{1}
\]

\[
\xi = \left( \frac{G'_{\ast} N_A}{R T} \right)^{\frac{3}{2}} \tag{2}
\]

where \( c \) was the concentration of polymers (1.5% or 2.5% w/v), \( \rho \) was the density of water at 298 K (997 kg·m\(^{-3}\)), \( R \) was the molar gas constant (8.3144598 × 10\(^{-5}\) cm\(^3\) Pa K\(^{-1}\) mol\(^{-1}\)), \( T \) was the temperature (298 K), \( G'_{\ast} \) was the plateau value of elastic modulus, and \( N_A \) was the Avogadro constant.

2.4. Small Molecules Diffusion via a T-Shaped Small Molecule Diffusion-Microfluidic Device

Small molecule diffusion was conducted by using a polydiallylsiloxane microfluidic chip that was fabricated following a previous study:\[^{[32]}\] FITC-labeled dextran with a molecular weight of 4, 10, 40, and 70 kDa, IgG, and insulin (Sigma, catalog number: 13661) were used as model molecules for studying diffusion throughout 1.5% and 2.5% w/v Muc-gels. The chip was composed of eight finger-like channels and a reservoir channel at the cross direction. Finger-like channels were prefilled with premixed Muc-gel gelling components followed by incubation for 100 min to allow a complete cross-linking reaction. FITC-labeled molecules in a PBS buffer (pH 7.4, 0.1mg mL\(^{-1}\)) were then loaded into the reservoir channel. Fluorescent images at 10, 30, and 60 min were taken with 4× objective using an inverted fluorescence microscope (Nikon Eclipse Ti) with an LED lamp (CoolLED pE-300). The exposure time and gain value for acquiring images were set to get no saturation pixels in all images. The obtained images were then analyzed using ImageJ software (open domain, version 1.52e). The region of interest (ROI), a rectangle with a length of 1500 pixels and width of 40 pixels (1 pixel = 2 micrometers) of every single finger-like channel was chosen. The starting point was 40 pixels ahead of the Muc-gel interface, which was the area of dextran/IgG solutions and the fluorescence intensity of which was used as a reference to normalize the fluorescent intensity throughout the ROI. The approach for calculating the diffusion coefficient was adapted from the work of B. T. Henry et al.\[^{[33]}\]

The diffusion coefficients\[^{[33]}\] \( D \) were calculated based on the 50% fluorescence intensity points at 30 and 60 min by the following equation:

\[
D = \left( \frac{X}{2 \times 0.476936} \right) / T \tag{3}
\]

where \( X \) was the diffusion distance (\( \mu m \)) and \( T \) was the time point(s) used to calculate the diffusion coefficient.

2.5. Insulin-Secreting Cells Cultivation in Mucin Hydrogels

MIN6m9 cells\[^{[34]}\] were cultured in DMEM (Gibco) supplemented with 10% heat-inactivated FBS, and 1% penicillin and streptomycin (P/S), 50 mm \( \beta \)-mercaptoethanol, and 11 mm glucose and maintained in a humidified 37 °C incubator with 5% CO\(_2\). The medium was changed every second day. Cells were detached with an Accutase solution (Sigma-Aldrich) when reaching 80% confluence and then subcultured. For the encapsulation in macroscopic Muc-gels, cell pellets (cell density: 5 × 10\(^{4}\) mL\(^{-1}\)) were resuspended in presolubilized Muc-Tz and Muc-Nb with the MIN6m9 cell culture medium, at the concentration of 1.5% and 2.5 w/v. To make the Muc-gels, 25 \( \mu L \) of Muc-Tz and Muc-Nb with cells were mixed in a 1 mL syringe with the top cut. After 10 min incubation in the hood, gels were transferred into a 48 well plate. After 60 min incubation, 400 \( \mu L \) of MIN6m9 cell culture medium was added. The medium was exchanged every second day. To analyze the insulin secretion, MIN6m9 cells in Muc-gels were first washed followed by incubation with a HEPES buffer supplemented with 3 mm glucose for 2 h. Thereafter, samples were then incubated in a HEPES buffer with 16.5 mm glucose for 1 h. Supernatants were harvested and filtered with a 0.2 \( \mu m \) syringe filter and then stored at –80 °C.

2.6. Cell Metabolic Activity by Alamar Blue Assay

MIN6m9 cells in Muc-gels were incubated with 10% resazurin solution in a complete cell culture medium for 4 h in a humidified 37 °C incubator with 5% CO\(_2\), on days 1, 4, 6, 11, 22, and 28. The supernatants were then added into transparent 96 well plates. The 96 well plates were then covered with an optical film followed by centrifugation at 3500 rpm with a duration of 2 min. Fluorescence was measured with excitation at 544 nm and emission at 595 nm. The cell metabolic activity was presented as the percentage of fluorescent intensity relative to Alamar blue reagent without exposure to cells.
2.7. LIVE/DEAD Staining

LIVE/DEAD staining was used to stain islet-like organoids in Muc-gels following the instruction from the manufacturer (Sigma-Aldrich). In brief, a PBS solution containing 1 μL of solution A and 2 μL of solution B was prepared. 300 μL of the solution was added to a sample and incubated for 15 min at room temperature. Samples were washed twice with PBS and then transferred to a glass slide. Fluorescence images were obtained under a fluorescence microscope (inverted Nikon Eclipse Ti) which was equipped with an LED lamp (CoolLED pE-300). The green fluorophore indicated live cells and the red fluorophore indicated dead cells.

For cell aggregates in Muc-gel microdroplets, LIVE/DEAD staining assays were performed as follows: 5 μL of propidium iodide (1 mg mL⁻¹ in H₂O, Invitrogen), 5 μL of Hoechst 33342 (10 mg mL⁻¹ in H₂O, Invitrogen) and 1 μL calcein AM (1 mg mL⁻¹ in DMSO) were premixed in 1 mL of PBS. 100 μL of this staining solution was added to 200 μL of the sample and incubated for 15 min at room temperature in the dark before being transferred to a glass slide. Fluorescence images were obtained using a fluorescence microscope (inverted Nikon Eclipse Ti), equipped with a Sola Light Engine (Lumencor) and a Zyla 5.5 sCMOS (Andor).

2.8. Complement Activation Using Healthy Human Whole Blood

Human whole blood was drawn from a healthy donor who had no medication for at least 10 days directly into 6 mL vacutainer tubes (BD Vacutainer Z, Plymouth, UK) containing the specific thrombin inhibitor lepirudin (Refludan, Aventis Pharma, final concentration 50 μg mL⁻¹). Closed loops coated with heparin according to the manufacturer’s protocol (Corline System AB, Uppsala, Sweden) were then filled with the whole blood, followed by the Muc-gels or alginate gels (Alg-gels). The loops were then rotated at 22 rpm on a rotator at 37 °C and incubated for 1 h. The whole blood was then collected, the blood was transferred to Eppendorf tubes containing 4 mm EDTA, and then centrifuged at 3000 × g for 25 min, at 4 °C. The obtained plasmas were then stored at −70 °C until further analysis. C3a and sC5-b were then quantitatively analyzed by a conventional sandwich ELISAs, following the previous article.[35] To measure C3a or sC5b-9, samples were diluted 1:1000–1:3000 or 1:2–1:10, respectively, in a working buffer (PBS containing 0.05% Tween 20, 10 mg mL⁻¹ BSA, and 10 mM EDTA). C3a were first conjugated by anti-human C3a monoclonal antibodies (mAb) 4SD173 followed by detection with biotinylated polyclonal rabbit anti-C3a antibody and HRP-conjugated streptavidin. sC5-b were first conjugated to anti-human C5b-9 mAb AE11 (Diatex Monoclonals AS, Oslo, Norway) followed by the detection by anti-human C5 polyclonal rabbit antibody (Dako) and HRP-conjugated anti-rabbit IgG (Dako). Zymosan-activated serum calibrated against purified C3a, or sC5b-9 served as a standard. Values were expressed as ng mL⁻¹.

2.9. Primary Islets Cultivation in Mucin Hydrogels and Alginate Gels

Human pancreatic islets were obtained from Lonza (USA). Upon receipt, islets were pooled into a petri dish and hand-picked up under inverted microscopy using a 200 μL pipette, and then transferred into a new petri dish containing islet culture medium provided by the company. Islets were cultured in a humidified 37 °C incubator with 5% CO₂. Mediums were changed every second day under inverted microscopy. To encapsulate islets in Muc-gels, four islets were loaded into a 1 mL syringe with the top cut prefilled with 25 μL of Muc-Tz solution dissolved in islet culture medium (2.5% w/v). 25 μL of Muc-Nb solution (2.5% w/v) was then added and mixed on a vortex. To encapsulate islets in Alg-gels, four islets were loaded into a 1 mL syringe with the top cut prefilled with Alg solution dissolved in islet culture medium and then cross-linked with a BaCl₂ gelling solution following the authors’ previous publication.[29] Samples were incubated for 60 min in syringes before being transferred into a 48 well plate containing islets culture medium. The medium was changed every second day. Alamar blue assay was performed as described above on day 5 and day 10.

To analyze the glucose-stimulated insulin secretion (GSIS) for primary islets cultured in Muc-gels and Alg-gels, samples were incubated in a Kreb’s buffer (pH 7.4, supplemented with 2.5 mM CaCl₂) supplemented with 2.8 mM glucose for 1 h, as a prebaseline incubation, followed by the Kreb’s buffer supplemented with 28 mM for a GSIS for 1 h, and then incubation with the Kreb’s buffer with 2.8 mM glucose for recovery.[36] Between each incubation, samples were washed in the Kreb’s buffer supplemented with 2.8 mM for 1 h. Supernatants from three stages were then harvested and then stored at −80 °C.

2.10. Insulin Detection Assay

Insulin concentration was determined using an insulin high range kit that was based on homogeneous time-resolved fluorescence technology following the manufacturer’s instruction. In brief, the Insulin Eu Cryptate antibody and insulin XL665 antibody were reconstituted in 0.5 and 1 mL Milli-Q H₂O, respectively. Insulin standards were reconstituted with Milli-Q H₂O to obtain 500 ng mL⁻¹ followed by a serial dilution with the dilute buffer supplied by the kit. Those solutions were aliquoted and stored at −80 °C.

For the assay, 1 μL of samples and insulin standards were added into each well in a white 384 well plate followed by the addition of 15 μL premixed solution containing 10 μL insulin XL665 antibody working solution, and 5 μL of insulin Eu Cryptate antibody working solution. The plate was sealed with an optical film, and vortexed and centrifuged at 400 × g for 5 min. The reaction mixtures were incubated at room temperature overnight in the dark. The plate was then measured with a microplate reader (CLARIOstar, BMG LABTECH) at the emission of 665 and 620 nm.

The analysis was performed according to the instruction from the manufacturer. In brief, the ratio of signal at Emission 665 and Excitation 620 nm was obtained for both samples and standards. The standard curve was obtained based on the ratio of standards using Prism 9.0 using 4 Parameter Logistic (4PL) model: \( Y = \frac{Bottom + (X^{HillSlope} \times Top)}{1 + (X^{HillSlope} + EC50^{HillSlope})} \).
Where the value for “bottom” corresponded to the lowest value on the y-axis, “top” to the highest value on the y-axis. “Hillslope” was the slope between the top and bottom values. “EC50” corresponded to a concentration (ng mL⁻¹) that gave a response halfway between the top and bottom values.

2.11. Viscosity Measurements

The viscosity of Muz-Tz and Muc-Nb solution could influence the fluid resistance in the microfluidic circuit, thus was the critical parameter for the design of a microfluidic device. Herein, measurements for determining viscosities of Muc-Tz and Muc-Nb at the concentration of 2.5% w/v were conducted using a Lovis 2000 M/ME rolling-ball viscometer (Anton Paar GmbH, Austria), which measured a rolling time of a ball inside the capillary filled with samples following instructions from the manufacturer. The viscometer was first calibrated with water before and after each experiment. Capillary (capillary no. Φ = 1.59, no. 18957327) was prefilled by samples and a steel ball (diameter = 1.5 mm) without air bubbles. The smooth movement of the ball in the solution was essential for obtaining accurate results and checked by turning the capillary to a vertical position. The capillary was then loaded inside the Lovis block. The measurements were performed at 25 °C following the instruction of Anton Paar’s manual. Between each measurement, the capillary was washed with water twice, followed by 20%, and 70% ethanol. Capillary was then dried with compressed gas filtered by an adapted lab-made 0.45 μm syringe filter. The density of samples was measured by a density meter DMA 4500 M (Anton Paar GmbH, Austria) for calculating the dynamic viscosity. Triplicate measurements were performed independently for each sample.

2.12. The Design and Fabrication of a Gel Microdroplets Generating-Microfluidic Device

The design principle of the microfluidic device stemmed from the authors’ previous study.[37] Adjustments were made due to the lower viscosity of the Muc-Tz solution compared to the Muc-Nb solution (Table 1) which causes different hydrodynamic resistance in the microchannels. The amount of each gelling component at the meeting channel could be adjusted by increasing or decreasing the length of the microchannel leading to the meeting point. This affects the fluidic resistance in the microchannel and the mass transport, following a relationship described by Equation (4). To compensate for the viscosity difference and ensure equal injection rates of both Muc-gel components, the Muc-Tz microchannel was designed to be 1.6 times longer than the Muc-Nb microchannel to result in droplets with equal amounts of both gelling components.

\[
\frac{8 \mu_1 L_1}{\pi r^4} = \frac{8 \mu_2 L_2}{\pi r^4}
\]

(4)

The microfluidic device was composed of a 3D printed polylactic acid (PLA) chip bonded to a micromilled polymethyl methacrylate (PMMA) chip. For printing PLA-chips, the structure was first designed with SolidEdge software, then printed by a 3D printer (Ultimaker 2, Zaltbommel, Netherlands). For fabricating a PMMA-chip, a microfluidic circuit was designed in AutoCAD, then engraved on a 2 mm thick PMMA sheet by a computer numerically controlled (CNC) micromilling machine (MDX-40A 3D Milling Machine; Roland, Irvine, CA) with end mills of 200 μm. Microchannels’ dimensions were 200 μm width and 200 μm depth for the generation of the desired size of Muc-gel microdroplets. The inlets and the outlet were drilled with a 1 mm drill by the CNC micromilling machine. To remove plastic residues from all microchannels, the PMMA chip was first cleaned with a detergent using a cleaning brush, followed by rinsing thrice with Milli-Q water. After air-drying with a compressed airgun, the chip was then sealed with a pressure sensitive adhesive tape (ARCare 90445Q: Adhesives Research, Glen Rock, PA) at the side of microchannels. Three holes were precut on the double adhesive tape (Tesa, Hamburg, Germany) using a 2 mm Harris Uni-Core biopsy puncher. Then, the PLA-chip containing three reservoirs and outlet were aligned and adhered to the other side of the PMMA-chip by the tape as well as the holes in the tape. The microchannels were then treated with Aquapel to increase their hydrophobicity. The surface-modifying chemical was then removed by blowing compressed nitrogen through the channels. Hydrofluoroether (HFE) oil (Novec HFE-7500 Engineered Fluid; 3M, Two Harbors, MN) was passed through the microchannels to wash out any traces of Aquapel. The microchannels were then cleared and dried by compressed nitrogen.

2.13. Rapid Production and Recovery of MIN6m9 Cell Spheroids by a Droplet Microfluidics

A suspension of single MIN6m9 cells in complete cell culture medium (6 × 10⁶ cells/mL) was loaded into the inlet of a water-in-oil emulsions microdroplet microfluidic device.[37] The microdroplets were obtained using a negative-pressure driven box as described before.[37] After 7 h incubation at 37 °C, 5% CO₂, the emulsion was then transferred onto a UV-treated fluorinated oil-attracting (fluorophilic) and water-repelling (hydrophobic) PTFE membrane (PTFE filter, 0.45 μm pore size; Sartorius Biolgab Products, Göttingen, Germany) following the recovery method described in detail by Langer and Joensson.[37] De-emulsification occurred at the surface of the membrane which allowed to retrieve the cell clusters.

2.14. Mucin Hydrogel Microdroplet Generation in a Microfluidic Device

Muc-Tz and Muc-Nb were predissolved in a cell culture medium, supplemented with 10% FBS and 1% P/S at a

|                        | Dynamic Viscosity [mPa·s⁻¹] | Kinematic Viscosity [mPa·s⁻¹] |
|------------------------|-----------------------------|-------------------------------|
| Muc-Tz (2.5% w/v)      | 7.0189                      | 6.97340                       |
| Muc-Nb (2.5% w/v)      | 11.2530                     | 11.3260                       |

Table 1. Dynamic and kinematic viscosity of Muc-Tz and Muc-Nb.
concentration of 2.5% w/v, and then loaded to Muc-Tz and Muc-Nb reservoirs, respectively. 1 mL of fluorinated HFE oil (Novec HFE-7500 Engineered Fluid; 3M, Two Harbors, MN) supplemented with 2% w/v fluorosurfactant (Ran Biotechnologies, Boston, MA) was loaded in the oil reservoir. 1 mL pipette tip with a novel interface[37] was used to maintain a sealed connection at the outlet. As earlier described by Langer et al.,[38] to generate the Muc-gel microdroplets, the pipette was manually compressed before inserting into the outlet of the chip. The compression was gradually and generously released to aspirate liquids of HFE oil, Muc-Tz, and Muc-Nb solutions. Those liquids were passed through the corresponding circuit, equal amounts of Muc-Tz and Muc-Nb components met at the meeting point, and then were emulsified, as Muc-gel microdroplets into the HFE oil. The surfactant in HFE oil contributed to stabilizing the emulsion.

The coefficient of variation was calculated by the following equations:

$$\mu = \frac{\sum x}{n}, \sigma = \sqrt{\frac{\sum (x-\mu)^2}{n-1}}, \text{ and } C_v = \frac{\sigma}{\mu} \quad (5)$$

where $\mu$ was the average size of the droplets, $\sigma$ was the standard deviation, $C_v$ was the coefficient of variance, $x$ was the diameter of individual droplet measure, and $n$ was the total number of droplets.

To encapsulate MIN6m9 cells or MIN6m9 cell clusters in Muc-gels microdroplets, the microfluidic device was first sterilized with 70% ethanol. Ethanol was then removed and air-dried at sterile connotation in a laminar hood. Cells were resuspended in both Muc-Tz and Muc-Nb solutions from above at a density of $2 \times 10^6$ cells/mL and then loaded to the corresponding reservoir. For encapsulating cell clusters, the suspension of cell clusters in Muc-Tz dissolved in a complete cell culture medium was loaded into the device’s reservoir and stirred with a micromagnet.

The emulsion, that is, the MIN6m9 cells or MIN6m9 cell clusters entrapped in Muc-gels-in-oil droplets, was then incubated at 37 °C, in a humidified incubator with 5% CO2 for 60 min to allow complete gelation of the Muc-gel microdroplets. The emulsion was then transferred onto a fluorinated oil-attracting (fluorophilic) and water-repelling (hydrophobic) PTFE membrane (PTFE filter, 0.45 μm pore size; Sartorius Biolab Products, Göttingen, Germany). Cell or cell cluster laden-Muc-gel microdroplets were then collected by pipetting PBS onto the membrane, and then transferred onto a cell strainer (40 μm pore size), placed in 6 well plates, which enables easy medium exchange. PBS was then exchanged with a complete cell culture medium for MIN6m9 cells as mentioned above. The medium was exchanged every 3 days.

2.15. Statistical Analysis

Statistics analysis was determined by using GraphPad Prism 9.0, as indicated the statistical analysis and “*p”, “**p”, “***p”, and “****p” indicated $p$ value less than 0.05, 0.01, 0.0005, and 0.0001, respectively.

3. Results and Discussion

3.1. Mucin Hydrogels Dampened the Complement Activation

The Muc-gels are obtained by first introducing Tz or Nb functionalities onto bovine submaxillary mucin (Muc) protein backbones. The resulting components can form a stable gel network (Muc-gels) through “clickable” inverse electron demand Diels–Alder cycloaddition reactions.[29] We compare these Muc-gels to clinical-grade Alg-gels, which we consider a reference material with an extensive range of application areas and biocompatibility track record.[39] We first investigated whether the strong immune modulation observed previously for the Muc-gels in vitro[28] and in vivo,[29] were also accompanied by the modulation of complement activation. We introduced Muc-gels or Alg-gels, both at the 2.5% w/v concentration used in our previous studies,[29] in a closed-loop, prefilled with human whole blood, and then assessed complement activation by measuring the concentrations of complement effectors.[40] We showed that Muc-gels triggered significantly lower levels of fluid-phase complement activation products C3a and C5b-9 complement effectors than Alg-gels (Figure 1). Those results indicate that Muc-gels could have improved hemocompatibility compared to Alg-gels. To our knowledge, we are the first to show mucin-derived materials capable of dampening the complement activation. We hypothesize that the sialic acid (N-acetylmuraminic acid, Neu5Ac) presented on Muc-gels contributes to this dampening effect. Indeed, Neu5Ac is recognized as a “self” molecule by the factor H complement regulators which helps reduce complement activation triggered by implanted foreign objects.[24] We have also shown that sialic acid residues on Muc-gels are necessary for their immunomodulatory effect on macrophages through binding to Siglecs.[28] The C9 carbon of the polyhydroxylated tail and carboxyl groups on Neu5Ac was shown to be essential[26] for their immunological activity. This is in line with our previous data demonstrating that the addition of “clickable” Tz and Nb groups occurs on carboxyl groups

![Figure 1](image-url). Complement activation assessment. Muc-gels and Alg-gels (2.5% w/v) were loaded in a closed-loop prefilled with human whole blood collected from healthy donors. The loops were then loaded onto a rotator for 60 min. Final concentrations of complement effectors were then evaluated. The error bars denote the standard deviation as obtained from measurements of independent samples of $n = 3$ for Muc-gels and $n = 2$ for Alg-gels. Statistical analysis was determined by Prima 9.0 using the unpaired nonparametric Student’s t-test.
of the mucin protein backbone, leaving the sialic acid residues untouched.\cite{29} Inhibition of complement activation attenuated materials-triggered host inflammatory immune responses\cite{15,41,42} Thus, our data suggest that the ability of Muc-gels to evade the FBR\cite{29} can also be attributed to the dampening of complement activation, and thus that Muc-gels could be used in contact with blood or bodily fluids.

### 3.2. Pore Size and Mechanical Properties of Mucin Hydrogels Can be Modulated by Mucin Concentration

The immunomodulatory activities of Muc-gels we have demonstrated here and elsewhere could protect encapsulated cells and microorganisms from immune cells and immune-derived cytotoxic compounds and prevent fibrotic encapsulation post-implantation. Thus, Muc-gels have clear potential as encapsulation material to improve the effectiveness and duration of cell and organ transplants. However, in such a strategy, the long-term survival and function of cells or microtissues encapsulated into hydrogels rely on the diffusion of gases, nutrients, and metabolic byproducts, from the outside to the inner core of the gel, and vice versa in vitro, and in vivo prior engraftment.\cite{43} We thus aim to characterize if Muc-gels could meet the requirements to sustain the viability and function of embedded cells and microtissues for long periods.

The mucin concentration used to form those hydrogels can affect the properties of the materials, which in turn can affect the viability and function of cells embedded in the matrix. Comparing 1.5% and 2.5% w/v Muc-gels by time–sweep rheology revealed that both samples undergo a sol–gel transition at 5 min after mixing the two components, followed by a gradual increase for both the elastic modulus (G’) and storage modulus (G''), to reach plateau-like state at 40 min (Figure 2A,B, Left). This indicates that the gelation kinetics of Muc-gels are less prominently determined by the concentration of the mucin derivatives than by the kinetics of the cross-linking reaction and cross-linker density grafted onto the mucins. The frequency-sweep measurements confirmed proper cross-linking reaction occurred between the functionalities on mucin molecules, and showed that 1.5% Muc-gels have a lower elastic modulus than 2.5% Muc-gels (Figure 2A,B, Right). This is also reflected in the calculated average molecular weight between cross-links (Mc, Equation (1) and Figure 2C) and the average distance between two adjacent cross-links, mesh size,\cite{44,45} (ξ, Equation (2) and Figure 2D), which are significantly higher for 1.5% Muc-gels compared to 2.5% Muc-gels. Put together, this rheological characterization of the Muc-gels demonstrate that the mucin concentration can be lowered to 1.5% without compromising the gelation process. The 1.5% Muc-gels, although of weaker mechanical properties than 2.5% Muc-gels, can be shaped into various objects and handled without difficulty. The lower concentration also led to a larger mesh size, which could favor their ability to host cells by increasing the diffusivity of nutrients and other molecules through the gel.

![Figure 2. Rheological characterization of 1.5% and 2.5% Muc-gels. A,B) Time-dependent rheological assessment (left) and frequency-dependent viscoelastic behavior (right) of Muc-gels were assessed by a rheometer. The error bars denote the standard deviation as obtained from measurements of n = 3 independent samples. C,D) The estimated average molecular weight between the cross-links (Mc) and mesh size (ξ) of Muc-gels were calculated based on an average of elastic modulus (G’) values between 90 to 100 min in the time-dependent sweep assessment following the Equations (1) and (2). Statistical analysis was determined by Prima 9.0 using the unpaired parametric Student’s t-test.](image-url)
3.3. Mucin Hydrogels Hinder the Diffusion of Dextran 70 kDa and Limit the Diffusion of Immunoglobulins

We then assessed whether the differences in pore size could affect the diffusion of molecules through 1.5% and 2.5% Muc-gels using a T-shaped microfluidic device that allows the exposure of Muc-gel-filled channels to a central reservoir containing fluorescently labeled model molecules (Figure 3A insert). We exposed the gel to fluorescein-labeled dextran of various molar masses, IgG, and insulin, then measured the fluorescence intensity profile over time (Figure 3A and Figure S1, Supporting Information). We further fitted the time-dependent changes of fluorescence intensity in the Muc-gels to the 1D model of diffusion\[^{33,46}\] to estimate the diffusion coefficient of Muc-gels (Figure 3B). Uncharged dextrans are known to have limited interactions with mucins and have been used to assess mucosal permeability.[40] Thus, the speed at which dextrans diffuse through the Muc-gels should mostly be determined by the mesh size of the gels.[32] In both 1.5% and 2.5% Muc-gels, the diffusion coefficients of the dextrans decreased with increasing molar mass (Figure 3A,B). Dextran of 70 kDa was mostly excluded from the gel (Figure 3A), while dextran with molar masses below or ≈40 kDa and a hydrodynamic diameter \(R_h\) below or ≈4.50 nm diffused into the gel within the 60-min incubation (Figure 3B). The diffusion coefficients of 4 kDa dextran in both 1.5% and 2.5% Muc-gels were not significantly different (Figure 3B). Small 10 kDa dextrans diffused faster in Muc-gels than in 2.5% Alg-gels, ≈9 µm s\(^{-1}\) for 10 kDa dextran based on previous measurements.[48] This suggests a better diffusion profile for small molecules than Alg-gels, which have repeatedly been shown to sustain viable cells over the long term. Hydrogels with a mesh size ranging from 6 to 31 nm can allow unrestricted mass transfer of nutrients and metabolic byproducts.[49,50] Thus, we consider that Muc-gels meet the basic requirement for 3D cell culture based on the calculated mesh size and the small molecule diffusion assay.

IgG and IgM are the main components of the innate immune system and known to negatively affect the viability of transplanted encapsulated cells.[51,52] IgG denaturation induced by materials’ surface properties can also become a recognition protein of the complement system. We hypothesize the immuno-modulatory properties of Muc-gels can act as the first barrier against IgG activity, by lowering IgG antibody secretion by plasma cells. Indeed, sialic acids of mucins bind B cells’ surface receptor CD22 (Siglec-2),[53] which inhibit the antigen-induced activation of B cells.[23,54] However, physically preventing access of IgG to the encapsulated cells would be an essential second barrier. Both 1.5% and 2.5% Muc-gels hindered the diffusion of IgG with a greater effect for 2.5% Muc-gels. The IgG molecules with an \(R_h\) of 5.29 nm[55] diffused into Muc-gels with a diffusion coefficient between that of 40 kDa dextran \(R_h = 4.50 \text{ nm}\)[55] and 70 kDa dextran \(R_h = 6.00 \text{ nm}\). IgG proteins present 0–13 negative charges in physiological conditions (PBS, pH 7.4),[56] which could be repelled by the strong negative charge of Muc-gels and limit IgG partitioning into the Muc-gels. Our results also suggest that both our Muc-gels can effectively block the diffusion of IgM \(R_h = 12.65 \text{ nm}\).[54] Human insulins have a molar mass of 5.8 kDa,[57] and \(R_h\) ranging from 2.31 ± 0.07 to 2.75 ± 0.09 nm depending on the concentration.[58] We show human recombinant insulins (5.8 kDa) were able to diffuse through in both 1.5% and 2.5% Muc-gels with higher diffusion coefficient in 1.5% Muc-gels. Although half the size, insulin diffused slower than 10 kDa dextrans. The accumulation peak at the interface of the gel suggests that insulin interacts with the mucins, which could contribute to slowing its diffusion coefficient. Although both mucins and insulin are negatively

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Figure 3. Diffusion assessment by a microfluidic device. Horizontal finger-tip channels were filled by Muc-gels. Vertical channels were prefilled with FITC-labeled dextran having a molecular weight of 4, 10, 40, 70 kDa, IgG, or insulin in PBS after 60 min incubation. A) Normalized fluorescence intensity plotted as a function of diffusion distance throughout 1.5% or 2.5% Muc-gels. The error lines (dotted lines) indicate the standard deviations of each repeat (n = 3). B) The diffusion coefficient of Muc-gels. The approach of calculating the diffusion coefficient is adapted from the work of B. T. Henry et al. following Equation (3).[33] Two-time intervals (30 and 60 min) and points at \(C/C_0 = 0.5\) were used to calculate the diffusion coefficient. Statistical analysis was determined by Prima 9 using the unpaired parametric Student’s t-test.
charged at neutral pH, insulin has been shown to interact with negatively charged surfaces and aggregate at negatively charged lipid membranes.\[58,59\]

3.4. Mucin Hydrogel Supported the Formation of Insulin-Secreting Islet-Like Organoids

We next aimed to study if Muc-gels could provide a biocompatible microenvironment for cells and microtissues to be transplanted. We chose to use pancreatic islet transplantation for the treatment of type I diabetes as a model system to demonstrate the applicability of Muc-gels as cell encapsulation material for transplantation. The model is stringent, since the islets are fragile tissues, which require optimal culture conditions. In addition, the glucose-regulated insulin secretion is an ideal functional marker for encapsulated microtissues. In addition, current islet transplantation technologies suffer from fibrotic insulation that this Muc-gel system could overcome. First, we encapsulated and then cultured the insulin-secreting cell line MIN6m9 cells in 1.5% or 2.5% Muc-gels cylinder (diameter = 4.78 mm, height = 2.79 mm, 50 µL) for 28 days. 1 week after encapsulation, the single cells had grown into islet-like organoids in both 1.5% and 2.5% Muc-gels (Figure S2, Supporting Information). The proportion of islet-like organoids amongst the total objects detected was higher in 1.5% Muc-gels compared to 2.5% Muc-gels on days 11 and 14 (Figure 4A).

![Figure 4. Muc-gels promoted islet-like organoids formation. Bright-field images were obtained, and both the A) percentage of islet-like organoids to total objects on days 11 and 14 and B) the average diameter of MIN6m9 cells or islet-like organoids was measured by ImageJ. The quantification was done based on 18 images captured from three independent repeats (A,B). C) The metabolic activity of MIN6m9 cells or islet-like organoids was analyzed by Alamar blue assay and D) secreted insulin was analyzed by a FRET assay. The error bars denote the standard deviation as obtained from measurements of n > 12 independent samples from three-independent repeats. Statistical analysis was determined by Prima 9.0 using the unpaired parametric Student’s t-test.](image-url)
And after 3 weeks, no single cell could be observed (Figure S2, Supporting Information). The average diameter of organoids was not significantly different between 1.5% and 2.5% Muc-gels except for day 11 and day 28 (Figure 4B). Cells or islet-like organoids continuously increased in metabolic activity after day 4 as measured by Alamar blue assay (Figure 4C) and showed no sign of necrosis as indicated by LIVE/DEAD staining at day 28 (Figure 4E). The formation of islet-like organoids suggests MIN6m9 cells were able to migrate and proliferate within Muc-gels. In contrast to many other hydrogels supporting cell migration and proliferation, mucin gels are non-adhesive for various cell types,\[28,60\] including MIN6m9 cells. There is also no sign that the gel degrades over the course of the experiment. The gels do not swell after they are formed (Figure S3, Supporting Information), and we have shown that the gels remain intact after 21 days in vivo.\[29\] However, the migration of cells and formation of organoids in non-degradable gels have been shown before.\[61,62\] We thus hypothesize that the cells migrated through the Muc-gels by an amoeboid process which does not require adhesion and consists of cells squeezing through pores much smaller than the cells and deforming the Muc-gels network.\[62\]

We then measured insulin secretion to test the functionality of the MIN6m9 organoids and cells embedded in Muc-gels. Insulin levels rose to a detectable level from day 10, at which time the first islet-like organoids could be observed (Figure 4D). Although an increase in total cell numbers could lead to an increase in insulin secretion, there is prior evidence that clustering of insulin-secreting cells can enhance their insulin-secretion capacity compared to single cells, likely via restoring their phenotype.\[63\] Secretion levels were similar between the two Muc-gel concentrations on day 27, but higher for 1.5% Muc-gels on day 10 and day 13 (Figure 4D). The result also reflected the larger islet-like organoids within 1.5% Muc-gels compared to 2.5% Muc-gels on day 11 and day 14.

### 3.5. Primary Human Pancreatic Islets Embedded in Mucin Hydrogels Secrete Insulin in a Glucose-Regulated Manner

To demonstrate further the ability of Muc-gels to host sensitive transplantable microtissues, we next encapsulated human primary pancreatic islets obtained from healthy donors. We compared Muc-gels to Alg-gels as a gold-standard reference material (cylinder: diameter = 4.78 mm, height = 2.79 mm, 50 µL). Although alginate is prone to fibrotic encapsulation in vivo,\[29,64\] it has been validated as biocompatible for islets and allows insulin diffusion.\[65\] We assessed their viability and

![Figure 5](https://example.com/figure5.png)

**Figure 5.** A,B) Glucose-stimulated insulin secretion (GSIS) by human primary islets cultured 2.5% Muc-gels and Alginate gels (Alg-gel) was analyzed by a FRET assay. The ratio of the concentration of secreted insulin at the stimulation phase (stimulation with 28 mM glucose) to prebaseline incubation (incubation with 2.8 mM glucose) (A,B, black bar), and recovery phase (incubation with 2.8 mM glucose) to prebaseline incubation (A,B grey bar). C,D). The concentration of secreted insulin during prebaseline incubation, stimulation with 28 mM glucose, and recovery (incubation with 2.8 mM glucose), respectively. The standard deviation was obtained based on a total of five-independent samples for Muc-gels and four-independent samples for Alg-gels.
function in a GSIS assay on day 5 and day 10 of their culture in vitro. We selected 2.5% Muc-gels, considering that both 2.5% and 1.5% Muc-gels supported the growth and insulin secretion of MIN6m9 cells and that 2.5% Muc-gels\[28,29\] have been investigated substantially by our group, and exhibited excellent stability in vivo.\[29\] We showed that encapsulated human primary islets maintained their viability up to 10 days by Alamar blue assay (Figure S4, Supporting Information). Samples were subjected to 2.8 mM glucose as a prebaseline incubation, followed by 28 mM glucose, then back to 2.8 mM glucose to mimic the blood glucose followed by food intake. The insulin secretion of islets embedded both in Alg-gels and Muc-gels was stimulated by exposure to 28 mM glucose solutions, leading to an ≈4 increase in insulin concentration (Figure 5). These results indicated that the islets in both types of gels were functional and glucose-responsive after 10-day culture in Muc-gels. Importantly, the islets functioned comparably to those embedded in alginate, already validated in primate and human clinical trials as viable encapsulation material.\[65,66\]

3.6. Microencapsulating Insulin-Secreting Cells in Mucin Hydrogels Microdroplets by a Microfluidic Device

Another factor determining the success of gel-encapsulated cell transplantation is the shape of the encapsulating gel, which can strongly impact the immune response toward a positive outcome.\[39,67,68\]. In addition, microencapsulation increases the total specific surface area and increases the mass transfer of oxygen and nutrients into, and metabolic byproducts and therapeutic molecules produced by the cells out of the gel microsphere. Such microencapsulation strategies were successful in sustaining a large number of islets to correct the glycemia of T1D Mellitus patients (up to 500 000 IEQ).\[69–73\] Electrospray, 3D printing, and microfluidics-based methods can generate alginate microbeads.\[74,75\]. However, neither of these methods are suitable for generating covalently cross-linked gel microdroplets within a gelation time of ≈4 min.

We thus designed and fabricated a device that utilizes a microscale fluidic circuit to generate mucin droplets in immiscible oils at throughputs of hundreds to thousands of droplets per second.\[76\]. Droplets produced in these devices are monodisperse and compatible with encapsulated cell culture.\[77\]. The microfluidic chip is composed of a PMMA channel layer with microfluidic channels sealed with pressure sensitive adhesive and fluidically connected via through-holes to a 3D printed PLA interface layer containing three inlet reservoirs and an outlet port (Figure 6A–D). The PMMA layer and PLA layer are connected with double sided tape with through holes connecting channels in the PMMA layer to reservoirs in the PLA layer.

An equal amount of Muc-Tz and Muc-Nb components were aspirated into each microchannel by negative pressure applied to the outlet of the device following a reported micropipette-powered negative pressure-based method.\[38\]. At the cross channel intersections, Muc-gel microdroplets were formed in HFE oil containing 2% w/v emulsion stabilizing fluorosurfactant (RAN Biotechnologies) (Figure 6F). After incubation at room temperature for 60 min, the droplets were transferred to an aqueous buffer solution without losing their shape, suggesting they were well cross-linked (Figure 6G). The microdroplets were ≈262 ± 11 µm in diameter with a coefficient of variation lower than 4.2% (Figure 6H and Equation (5)). Thus,
Figure 7. Microencapsulation of MIN6m9 cells and cell clusters in Muc-gel microdroplets was performed using a negative pressure-driven droplet microfluidics device. MIN6m9 single cells were encapsulated in Muc-gel microdroplets. A,B) Muc-gel droplets supported the single cells (A, day 0) forming islet-like organoids (B, day 28). C) The metabolic activity of MIN6m9 cells or islet-like organoids was analyzed by Alamar blue assay and D) secreted insulin was analyzed by a FRET assay. The error bars denote the standard deviation as obtained from measurements of $n > 9$ independent samples. MIN6m9 cell clusters were generated in a high-throughput manner using a microfluidic device. E) MIN6m9 cells were encapsulated in microdroplets of cell culture medium in oil and incubated for 7 h, then retrieved into PBS. F) The process of generating MIN6m9 cells clusters was biocompatible, as indicated by LIVE/DEAD staining. MIN6m9 cell clusters were then microencapsulated in Muc-gel microdroplets using the negative pressure driven droplet microfluidics device and cultured over 28 days. G) The Muc-gel promoted the formation of islet-like organoids after 28 days. H) The metabolic activity of MIN6m9 cell clusters or islet-like organoids was analyzed by Alamar blue assay and I) secreted insulin was analyzed by a FRET assay. The error bars denote the standard deviation as obtained from measurements of $n > 6$ independent samples.
the size is suitable for encapsulating primary islets with an average diameter of 150 µm harvested from humans. However, the microdroplet generating microfluidic device design can be adapted to generate smaller or larger droplets, which will enable follow-up in vivo studies to find the optimal size for implant survival, immune response, and function. We could generate aqueous microdroplets with a total 100 µL at rates of up to 100 microdroplets per second. The devices also open the opportunity for the automation of droplet formation from any covalently cross-linked materials, which is scalable and reproducible.

3.7. Cell Encapsulation within Mucin Hydrogels Microdroplets by a Microfluidic Device

Thereafter, we encapsulated MIN6m9 single cells and cell clusters within Muc-gel microdroplets with the gel microdroplet generating microfluidic device. We added MIN6m9 single cells or cell clusters in solutions of Muc-Tz and Muc-Nb dissolved in a complete cell culture medium that we then used to load the device’s reservoirs. Cell-laden microdroplets were then transferred to the cell culture medium (Figure 7A) and cultured over 4 weeks. Encapsulated single cells grew into islet-like organoids (Figure 7B) and both their metabolic activity (Figure 7C) and insulin secretion (Figure 7D) increased over time, mirroring the results obtained for the macro-gels. To encapsulate MIN6m9 cell clusters, we first generated the clusters by culturing the cells in microdroplets of complete cell culture medium in oil (Figure 7E). The method allowed us to generate cell clusters without affecting the viability and in a high-throughput manner (Figure 7F). The MIN6m9 cell clusters were successfully encapsulated using the gel microdroplets generation-microfluidic device (Figure 7G) while maintaining high levels of cell viability as indicated by LIVE/DEAD staining (Figure 7G). Over 4 weeks of culture, the cell clusters matured into well-organized islet-like organoids (Figure 7G). Both their metabolic activity (Figure 7H) and insulin secretion (Figure 7I) increase over the cultivation period. If encapsulated into Muc-gel microdroplets, such large numbers of islets could be easily transplanted through simple needle injections. Furthermore, the gel microdroplets could be used to assemble encapsulated cells into various tissue mimics for tissue engineering approaches.

4. Conclusions

In this study, we demonstrate the successful micro-encapsulation of highly sensitive cells and cell clusters in Muc-gels using negative pressure-driven microdroplet generation-microfluidic devices. We demonstrate that Muc-gels trigger significantly lower amounts of fluid phase complement activation products and that Muc-gels can form a selective barrier, physically protecting embedded living objects from the immune destruction by the immune system components such as antibodies, while allowing nutrients and small therapeutic proteins like insulin to diffuse out. The Muc-gel meets the stringent physical property requirement to be used as encapsulation materials for cell and microtissue transplantation. However, the performance of the material as a transplant in specific tissue and animal model context should be studied further. In addition to the intrinsic immune-modulating properties of Muc-gel system, the free Tz or Nb click functionalities on the Muc-gels that remain after cross-linking could be used to incorporate growth factors that would further promote the cell growth, and differentiation, prior to transplantation to improve the engraftment of the transplants. For instance, EGF, or other molecules, to obtain peri-vascularized islets-like organoids, which has shown improved engraftment in transplantation. Many quality-control challenges remain before purified mucins are to be used in clinical trials as for such encapsulation, however, these are not insurmountable. In parallel to these efforts, this would continue to inform on important design principles for bottom-up approaches in which synthetic mucin and mucin mimic can be engineered for these specific purposes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords

complement system, insulin, microdroplets, microtissues, mucin hydrogels

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