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Polymerized laminin-modified microcapsules improve pancreatic islet resilience towards cytokine induced inflammatory stress and lower chemoattractant cytokine secretion

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ABSTRACT

Encapsulation of human pancreatic islets in semipermeable membranes offers a promising, less invasive alternative to whole pancreas transplantation for Type 1 Diabetes (T1D), potentially reducing the need for immunosuppressive drugs and improving graft survival. However, the inflammatory environment during transplantation poses significant challenges, often leading to substantial graft loss. This study explores modifying the intracapsular environment with extracellular matrix (ECM) molecules – chondroitin sulfate (ChS), laminin (LN), and polymerized laminin (pLN) – to enhance islet cell resilience against cytokine-induced stress, associated with the post-transplantation environment. Encapsulated mouse insulinoma β-cells (MIN6) and human islets were exposed to pro-inflammatory cytokines (IL-1β, IFN-γ, TNF-α) over 1, 3, and 5 days. The results showed that LN and pLN in combination with ChS particularly improved outcomes, with LN reducing oxidative stress and pLN significantly lowering cell death and pro-inflammatory chemokine MCP-1 production. These findings highlight the potential of ECM-modified encapsulation to enhance the survival of transplanted islets, offering a more favorable approach for T1D treatment and allowing transplantation of pancreatic islets with fewer islets.

1. Introduction

Individuals with Type 1 diabetes (T1D) have to endure daily insulin injections and constant monitoring of blood glucose levels to manage their glucose metabolism. This is the consequence of the destruction of the insulin-producing β-cells in the pancreas by an autoimmune disease [1]. Despite the life-saving insulin therapy, frequent episodes of hyper- and hypoglycemia cannot be avoided. Over time, temporary but recurrent hyperglycemia can lead to serious diabetic complications, including blindness, kidney failure, cardiovascular diseases, and nerve damage, which can result in neuropathy, lower limb amputations, and even death [2]. Intensive insulin therapy may lower the frequency of hyperglycemic episodes but is associated with an enhanced risk of disabling hypoglycemic episodes [3]. Because of these side effects of insulin therapy, research efforts aim at the development of therapies that more closely mimic the natural, minute-to-minute regulation of glucose levels achieved by pancreatic islets [3].

Minute-to-minute regulation of blood glucose levels can be achieved by transplantation of insulin-producing tissue, such as the whole pancreas or pancreatic islets. While effective, whole pancreas transplantation requires major surgery and lifelong immunosuppression, posing significant risks and challenges [4,5]. Pancreatic islet transplantation offers a less invasive alternative. It requires no more than minor surgery and potentially allows for transplantation in the absence of immunosuppression, as technologies are being developed to protect the islets from the host immune system [6,7].

A promising strategy to prevent rejection of pancreatic islets is their encapsulation in semi-permeable membranes. Since the islets are located behind a physical barrier, the immune system cannot reach them, eliminating the need for immunosuppressant drugs. Another advantage is that the use of human cadaveric islets is not mandatory, as capsules have also been shown to protect islets from xenogeneic sources

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[8,9] as well as insulin-producing cells derived from progenitors or pluripotent stem cells [10]. Successes with microencapsulated islets have been demonstrated in both small [11] and large animal models [9] as well as in some safety studies in humans [12]. However, a significant issue has emerged: graft survival was typically limited to several months to a year and never permanent [12–14].

The survival of encapsulated islets post-transplantation is influenced by numerous factors, many of which contribute to their limited longevity and functionality. The majority of grafted islets are lost during the immediate peri-transplant period, largely due to hypoxic stress and the inflammatory environment at the transplant site [15–17]. This initial loss is often followed by a second phase of decline, characterized by β-cell exhaustion and functional impairment. This phase is driven by a combination of allo- and auto-immune destruction, as well as by non-immunological stressors such as ER stress, oxidative damage, and glucotoxicity [18,19]. Over time, these mechanisms contribute to a steady deterioration of β-cell function.

An important factor exacerbating this vulnerability is the disruption of interactions between islet cells and the extracellular matrix (ECM) [20,21]. During islet isolation, enzymatic digestion separates the endocrine tissue from the exocrine pancreas, leading to significant damage to the ECM, which is vital for maintaining islet structure, cell-cell communication, and β-cell survival [22–24]. To overcome this, we and others have modified the intracapsular microenvironment to restore ECM-islet cell interactions [25–34]. This is usually done by incorporating ECM components in microcapsules compositions [28,31, 33,34].

Many ECM molecules have been proposed to enhance the survival and function of islet cells, however, it has been shown that the effectiveness of this support depends greatly on the type and combination of ECM molecules [35–37]. Chondroitin sulfate has been proposed as a candidate molecule to be incorporated in alginate-based capsules [38–40]. Chondroitin sulfate is an important component of ECM and is abundant in various biological tissues [41]. It has been proposed for application in islet-transplantation due to its anti-inflammatory, anti-oxidant and immunomodulatory properties [42,43].

In addition, laminin (LN), which is abundant in pancreatic islet basement membranes, has been suggested as a beneficial ECM molecule for islets β-cells [44–46]. A derivate thereof, polylaminin (pLN) has the ability to mimic natural laminin matrices and might benefit islets in capsules [47]. At acidic pH and in the absence of cells, LN undergoes auto-polymerization to form a polymer resembling *in vivo* membranes, displaying neuritogenic properties in vitro and anti-inflammatory properties *in vivo* [48,49]. All these ECM components have been tested previously in alginate-based capsules [40,47,50]. However, their efficacy in maintaining cell viability and functionality in vitro over extended periods, as well as providing protection under pro-inflammatory conditions, has not been thoroughly investigated in both murine cells and human islets.

This study aimed to investigate the effects of the ECM molecules, chondroitin sulfate, LN, and pLN on the survival and function of mouse insulinoma β-cells (MIN6) and human islets under conditions that mimic the inflammatory environment post-transplantation. To achieve this, we exposed encapsulated MIN6 aggregates and human islets to pro-inflammatory cytokines interleukin 1-β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) for 1, 3, and 5 days. We assessed cell viability, functionality, and cellular stress using specific assays, and evaluated the expression of cellular stress and mitochondrial-related genes. Additionally, we quantified the secretion of pro-inflammatory cytokines and chemokines by human islets. This study contributes to a better understanding of how these different ECM components influence the performance of encapsulated pancreatic cells in an inflammatory setting, aiming to identify the most promising materials for future research and potential improvements in islet transplantation outcomes.

2. Materials and methods

2.1. Immune activation in reporter cells

THP1-reporter cells (THP1-XBlue™-MD2-CD14 Cells, InvivoGen), derived from a human monocytic THP-1 cell line that express an NF-kB- and AP-1-inducible SEAP reporter gene, were used to detect activation of the pro-inflammatory NF-kB pathway. THP1 cells, at a concentration of 1×10^5 cells per well in a 96-well plate, were co-incubated with 20 empty microcapsules of different compositions. PAM3CSK4, a TLR2/TLR1 ligand, served as a positive control, while Dulbecco's Phosphate-Buffered Saline (DPBS) was used as a negative control. The cells were incubated for 24 h at 37 °C and 5 % CO_2 to determine any potential activation of the NF-kB pathway by the empty capsules. The cells were co-incubated with the capsules for 24 h at 37 °C and 5 % CO_2. On the following day, 180 μl of QUANTI-Blue™ was added to a new 96-well plate containing 20 μl of supernatant from the stimulated THP1 cell cultures. The mixture was incubated for 1 h at 37 °C. SEAP activity, indicative of NF-κB/AP-1 activation, was then measured at a wavelength of 650 nm using a microplate spectrophotometer. This method allowed the detection of pro-inflammatory pathway activation in response to the tested conditions.

2.2. Atomic force microscopy

To measure the stiffness and roughness of the prepared capsules Atomic Force Microscopy (AFM) was applied. Capsules were prepared on PLL (Poly-L-Lysine) coated glass slides using a 0.01 % Merck solution and measured in a DPBS solution. The measurements were performed using a Bruker Catalyst AFM system in contact mode with a calibrated DNP-D cantilever ($K \approx 0.07$ N/m). Roughness was determined from AFM height images, by analyzing parameters of average roughness (Ra) and maximum roughness (Rmax). For stiffness measurement, ~30 force-distance curves were recorded on each capsule. These curves were fitted using the Sneddon model (conical indenter) to calculate the Young's modulus in kilopascals (kPa). All measurements were conducted three times.

2.3. MIN6 cell culture

MIN6 cells (ATCC, Manassas, VA, USA) were cultured in DMEM (Gibco, Thermo Fisher) supplemented with 10 % fetal bovine serum, 50 μmol/L β-mercaptoethanol, 2 mmol/L L-glutamine, 50 U/mL penicillin, and 50 mg/L streptomycin (all from Sigma-Aldrich, St. Louis, MO, USA) and maintained in 5 % CO_2 at 37 °C in a humidified atmosphere.

2.4. Human pancreatic islets

Pancreatic islets obtained from deceased donors were received from the Islet Isolation Laboratory at the Leiden University Medical Center. All related procedures involving human islets received approval and were performed according to the Dutch code for proper secondary use of human tissue, as established by the Dutch Federation of Medical Scientific Societies. Following their transport to the University Medical Center Groningen, these islets were maintained in CMRL-1066 medium (Pan Biotech, Germany), 10 % fetal bovine serum, glucose up to 8.3 mM, 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mM L-glutamine (Gibco), and 50 μg/ml penicillin and streptomycin (Gibco). for 24 or 48h prior to use, according to methodologies previously reported [51]. The pertinent patient information is shown in Table 1.

2.5. Microcapsule production and cell encapsulation

Intermediate-α-L-guluronic acid (G) alginate [42 % (G)-chains, 58 % β-D-mannuronic acid (M)-chains, 23 % GG chains, 19 % GM-chains, 38

Table 1
Human islet donor information.

Donor Age	Gender	BMI	Blood type	Cause of death	Estimated purity (%)
46	M	30	A -	Euthanasia	90
66	F	31	O +	SAB	80
59	M	23	B	Non-cardiac	60
79	M	26	O +	Non-cardiac	75
67	M	26	AB -	Cardiac	93–98
51	F	20	A +	CVA	45
58	M	26	O	Non-cardiac	65

SAB: Subarachnoid bleeding; CVA: Cerebral Vascular Accident.

% MM-chains, Mw = 428 kDa] was obtained from ISP Alginates (Girvan, UK). Chondroitin sulfate sodium salt and Laminin 111 were purchased from Sigma Aldrich.

The compositions tested were Alginate 3.4 % (Alg), or Alginate 3.4 %+Chondroitin sulfate 0.85 % (Alg+ChS), or Alginate 3.4 %+Chondroitin sulfate 0.85 %+Laminin 10 μg/ml (Alg+ChS+LN), or Alginate 3.4 %+Chondroitin sulfate 0.85 %+polylaminin 10 μg/ml (Alg+ChS+pLN). Each composition was prepared by mixing alginate with the mentioned components, in proportions based on previous studies [40,50]. Capsules containing laminin were prepared by the addition of 10 μg of LN 111 (Sigma Aldrich) to the polymer mixture. For the preparation of pLN, an aliquot of 10 μg of LN 111 was polymerized in 200 μl of polymerization buffer, consisting of 20 mM sodium acetate, 1 mM $CaCl_2$, and a pH of 4.0. After washing with Krebs-Ringer-Hepes (KRH) 25 mM Ca^{2+}free buffer, the supernatant was completely removed, and the pellet was homogenized in 1 ml of the respective composition.

The polymers preparations were converted into droplets using an air-driven droplet generator and the alginate droplets were transformed into beads by gelling in a 40 mM $CaCl_2$ solution (10 mM HEPES, 2 mM KCl) for 5 min. All capsules were washed 3 times with KRH 25 mM buffer containing $CaCl_2$. Capsules were handpicked and used in further experiments. For capsules containing islets, each composition was homogenized with 2.000 islets per mL of the polymer (mixture). MIN6 cells were aggregated for encapsulation using a Corning® Elplasia® Plate, with 800 cells seeded per microwell of the 6-well plate (Supplementary Fig. S1A). The cells were maintained on the 6-well plate for 48 h to allow for aggregate formation. Following this period, the aggregates were removed and homogenized with the polymer preparations at a concentration of 2.000 aggregates per mL of the polymer (mixture). The encapsulated islets and MIN6 aggregates (Supplementary Fig. S1A and B) were then cultured in the presence or absence of a cytokine cocktail. For human islets, the pro-inflammatory cytokines used were recombinant human IL-1β at 50 U/mL, IFN-γ at 1000 U/mL, and TNF-α at 1000 U/mL. For MIN6 aggregates, mouse recombinant IL-1β at 25 U/mL, IFN-γ at 500 U/mL, and TNF-α at 500 U/mL were utilized (all from ImmunoTools, Friesoythe, Germany). The encapsulated islets or MIN6 aggregates were maintained in cell type-specific culture medium, as detailed in Sections 2.1 and 2.2, either under standard conditions or under inflammatory conditions, for durations of 1, 3, or 5 days.

2.6. Cell viability assays

The cell viability of encapsulated MIN6 aggregates and human islets was quantitatively evaluated using the WST-1 assay (5015944001, Roche), a method designed to assess mitochondrial functionality. For this procedure, encapsulated aggregates and islets from each capsule composition were handpicked, with 15 capsules added to each well of a 96-well plate. These samples were then subjected to incubation periods of 1, 3, and 5 days, in the presence and absence of the cytokine cocktail. Following each incubation period, the culture medium was removed and replaced with fresh medium containing the WST-1 reagent at a 1:10 dilution ratio and incubated for 4h in 5 % CO_2 at 37 °C under humidified air conditions. To establish a baseline for absorbance readings, wells without capsules served as the blank control. The absorbance was measured at 450 nm using a Bio-Rad Benchmark Plus microplate spectrophotometer reader (Bio-Rad Laboratories B.V, Veenendaal, The Netherlands).

Cell survival was assessed using Calcein AM staining to identify live cells and Ethidium Bromide staining for dead cells, employing the LIVE/DEAD Cell Viability/Cytotoxicity Assay Kit (Invitrogen, Thermo Fisher). Following each incubation period, 15 handpicked capsules containing islets or MIN6 aggregates were stained with 1 mM Calcein AM and 2 mM Ethidium Bromide and subsequently diluted in the appropriate cell type-specific culture medium as detailed in Sections 2.1 and 2.2. These samples were then incubated for 30 min in a 5 % CO_2 atmosphere at 37 °C under humidified air conditions. Subsequently, the encapsulated cells were washed four times with KRH buffer and resuspended in the same buffer. The images of the stained cells were captured using a confocal microscope (TCS SP8, Leica) equipped with a HC PL FLUOTAR 20x/0.5 dry objective. OPSL lasers 488 nm and 552 nm were chosen for excitation. Emission was detected in green (PMT1: 494–547 nm) and red (PMT2: 595–647 nm), respectively. The images obtained were further examined using ImageJ software (National Institutes of Health, USA). Images obtained from the confocal microscope were analyzed using two macros. Raw images of the green (live) and red (dead) fluorescence channels were exported from the Leica software. The first macro (available at NickCondon/fiji_IMB_ChannelMerge.git) was used to merge single-channel images into a stacked image. Next, thresholds for each channel (red and green) were determined based on several randomly selected images to remove background signal. The second macro (custom made) processed the stack images and then measured the area of positive signal for each channel. These measurements were used to determine the percentage of red fluorescence, representing the proportion of dead cells in the analysis. The percentage of cell death was calculated using the following formula:

$$\%Cell\ death = \frac{Total\ red\ area}{Sum\ of\ total\ areas\ (red + green)} \times 100$$

2.7. In vitro glucose-stimulated insulin secretion (GSIS)

MIN6 aggregates and human islets were submitted to different concentrations of glucose to assess their capacity to respond by secreting insulin. First, 25 encapsulated MIN6 aggregates, or human islets, were handpicked and distributed in a 24-well plate. They were first incubated in KRH buffer with a low glucose concentration of 2.75 mM, followed by incubation with a high glucose concentration of 16.5 mM in KRH buffer. Afterward, the cells were incubated once more with a low glucose concentration of 2.75 mM. The incubation time of each step was 30 min for MIN6 aggregates and 1 h for human islets. At the end of each incubation period, the supernatant was collected, and the insulin content was measured using an ELISA assay (Mercodia AB, Sweden), the assay was done following the supplier's recommendation. The insulin concentration was calculated by interpolating sample absorbance values from the standard curve. The DNA from each sample submitted to the GSIS was quantified using a fluorescent Quant-iT PicoGreen double-strand DNA assay kit (Invitrogen, Thermo Fisher). The insulin values were normalized to the total DNA content for each of the samples.

2.8. Oxidative stress assays

Intracellular ROS was measured using the DCFDA Cellular ROS Detection Assay Kit (#ab113851, Abcam), and nitric oxide (NO) was measured using the Total Nitric Oxide Assay Kit (#EMSNOTOT, Invitrogen, Thermo Fisher), following the manufacturers' instructions. Briefly, 15 encapsulated islets or MIN6 aggregates of each composition were handpicked into a 96-well plate and cultured in the presence or absence of the cytokine cocktail for 5 days. On the 5th day, the medium

was collected and stored at −20 °C for NO measurement, and the cells were washed with 1x Buffer provided in the DCFDA Cellular ROS kit. After washing, the buffer was removed, and the cells were incubated with 20 μM DCFDA solution for 45 min at 37 °C in the dark. The DCFDA solution was then removed, and 1x Buffer was added to each well. Fluorescence was immediately measured using a CLARIOstar Plus fluorescence plate reader (BMG Labtech) with an excitation/emission wavelength of 485/535 nm. Wells containing 1x Buffer alone were used as blanks.

NO concentration was measured in the collected supernatants using the Total Nitric Oxide Assay Kit. This assay employs nitrate reductase to convert nitrate to nitrite, which is then detected as a colored azo dye product of the Griess reaction. Standards and samples, diluted 1:2 in the provided 1X Reagent Diluent, were added to a 96-well plate. Culture medium was used as a blank. Diluted NADH and Nitrate Reductase were added to the appropriate wells, and the plate was gently mixed and incubated at 37 °C for 30 min. Griess Reagents I and II were then added sequentially to the wells, and the plate was mixed gently and incubated at room temperature for 10 min. Optical density was measured at 550 nm using a Bio-Rad Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands).

2.9. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

To determine the gene expression of pro-inflammatory cytokines, mitochondrial dynamics and function as well as stress response-related genes in MIN6 aggregates and human islets, qRT-PCR was performed. The alginate encapsulation was removed using an EDTA buffer (50 mM EDTA and 10 mM HEPES) prepared in RNase-free water. Capsules were first washed with cold DPBS without Ca^{2+} and Mg^{2+}, followed by the addition of the EDTA solution. The samples were vortexed to facilitate capsule dissolution. After the capsules were dissolved, the samples were centrifuged. The cell pellet from MIN6 cells or human islets was then homogenized in TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) for RNA extraction. Total RNA was isolated following the manufacturer's instructions, followed by obtaining reverse transcribed cDNA using SuperScrit II Reverse Transcriptase (Invitrogen, Thermo Fisher). qPCR assays were performed with a FastStart Universal SYBR-Green Master Kit (Roche) for the genes listed in Table 2. Reactions were carried out in 384-well PCR plate using the ViiA7 Real-Time PCR System (Applied Biosystems). Delta Ct values were calculated and normalized relative to the housekeeping gene Gapdh. The $2^{-\Delta\Delta Ct}$ method was used for the comparative quantification of gene expression. Cells encapsulated in alginate with no exposure to cytokines were used as the control group for normalization.

2.10. Quantification of proinflammatory cytokines and chemokines

To assess the impact of each capsule's composition on the secretion of pro-inflammatory cytokines and chemokines in human islets after 5 days of exposure to cytokines or in the absence of it, we quantified the levels of CCL20, MCP1, IL-13, IL-33, and TSLP in the supernatant. This quantification was performed using magnetic Luminex® Assays (#LXSAHM-07, R&D Systems). The reagents were prepared following the manufacturer's instructions. For the assay, 50 μl of a 1:2 dilution of each supernatant sample was added per well, followed by the addition of the microparticle cocktail. The plate was incubated overnight at 4 °C on a horizontal orbital microplate shaker at 800 rpm. The next day, the plate was washed according to the manufacturer's protocol, and the biotin-antibody cocktail was added to each well and incubated for 1 h. After another washing step, streptavidin-PE was added and incubated for 30 min. Following this incubation, an additional wash step was performed, and the microparticles were resuspended in washing buffer. The samples were then analyzed using a 200 System, and the data were processed using the Luminex xPONENT software.

2.11. Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). The Shapiro-Wilk test was employed to assess the normality of data distribution. For parametric distributed data, statistical analyses were performed using either one-way or two-way ANOVA. For non-parametric data, the Kruskal-Wallis test was used with Dunnett's post-hoc test for multiple comparisons. These analyses utilized the GraphPad Prism software (version 10.1.2). A p-value of less than 0.05 was considered to indicate statistical significance. For both the mitochondrial activity and oxidative stress assays, data were normalized within each experimental replicate. Alginate capsules served as the control group for each condition in presence or absence of cytokines and were set to 1, expressed as fold change relative to the control.

Table 2
Primer sequences for qRT-PCR.

Gene	Species	Forward sequence 5′-3′	Reverse sequence 5′-3′
Gapdh	Mouse	TCTCCTGCGACTTCAACA	TGTAGCCGTATTCATTGTCA
Mfn1	Mouse	TGGTCTTCCCTTGTACATCG	GAGCAGTAGGAGTTGAAGCT
Mfn2	Mouse	GTGATCAATGCCATGCTCTG	TGGTTCACAGTCTTGACACT
Drp1	Mouse	CTGCCTCAAATCGTCGTAGTG	GAGGTCTCCGGGTGACAATTC
Opa1	Mouse	TGTGAGGTCTGCCAGTCTTTA	TGTCCTTAATTGGGGTCGTTG
Fis1	Mouse	GTCCAAGAGCACGCAGTTTG	ATGCCTTTACGGATGTCATCATT
Foxo1	Mouse	ACTTCAAGGATAAGGGCGAC	TTGAGCATCCACCAAGAACT
Hspa5	Mouse	CCACCAAGATGCTGACATTG	AGGGCCTGCACTTCCATAGA
Xpb1	Mouse	GGAGTTAAGACAGCGCTTGGGG	TGTTCTGGAGGGGTGACAACTGGG
GAPDH	Human	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
MFN1	Human	TGGCTAAGAAGGCGATTACTGC	TCTCCGAGATAGCACCTCACC
MFN2	Human	CTCTCGATGCAACTCTATCGTC	TCCTGTACGTGTCTTCAAGGAA
DRP1	Human	CTGCCTCAAATCGTCGTAGTG	GAGGTCTCCGGGTGACAATTC
OPA1	Human	TGTGAGGTCTGCCAGTCTTTA	TGTCCTTAATTGGGGTCGTTG
FIS1	Human	GTCCAAGAGCACGCAGTTTG	ATGCCTTTACGGATGTCATCATT
FOXO1	Human	CTACGAGTGGATGGTCAAGAGC	CCAGTTCCTTCATTCTGCACACG
HSPA5	Human	CCACCAAGATGCTGACATTG	AGGGCCTGCACTTCCATAGA
XPB1	Human	GGAGTTAAGACAGCGCTTGGGG	TGTTCTGGAGGGGTGACAACTGGG
MCP1	Human	TCTCGCCTCCAGCATGAAAG	GGCATTGATTGCATCTGGCT
IL-6	Human	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
IL-8	Human	ACTCCAAACCTTTCCACCCC	TTCTCAGCCCTCTTCAAAAACTTC
IL-33	Human	GCCTTGTGTTTCAAGCTGGG	ATCATAAGGCCAGAGCGGAG

3. Results

3.1. Different capsules compositions show absence of NF-κB-dependent immune activation in vitro

To assess whether the different capsules compositions, Alg, Alg+ChS, Alg+ChS+LN, and Alg+ChS+pLN, can activate immune responses, we co-incubated empty capsules with the THP-1 reporter cell line and measured NF-κB activation. The results indicated no induction of NF-κB activation regardless of the capsule composition. The levels of NF-κB activation were similar to those of the control group (Supplementary Fig. S2).

3.2. Different capsules compositions influence surface stiffness and roughness

To assess the impact of adding ChS, LN, and pLN to alginate-based capsules on their physicochemical properties, surface stiffness and roughness were analyzed using atomic force microscopy (AFM). The results indicated that the addition of chondroitin sulfate increased surface stiffness, while the inclusion of laminin to the chondroitin-based composition decreased surface stiffness, compared to Alg capsules (Supplementary Fig. S3A). Additionally, we assessed the capsule's surface roughness (Supplementary Fig. S3B). We found average values of 135.2 nm up to a maximum average of 1205.7 nm for Alg, 105.1 nm up to a maximum of 785.7 nm for Alg+ChS, 100.7 nm up to a maximum of 537 nm for Alg+ChS+LN, and 102.7 nm up to a maximum of 684.6 nm for Alg+ChS+pLN. These results suggest that the inclusion of laminin,

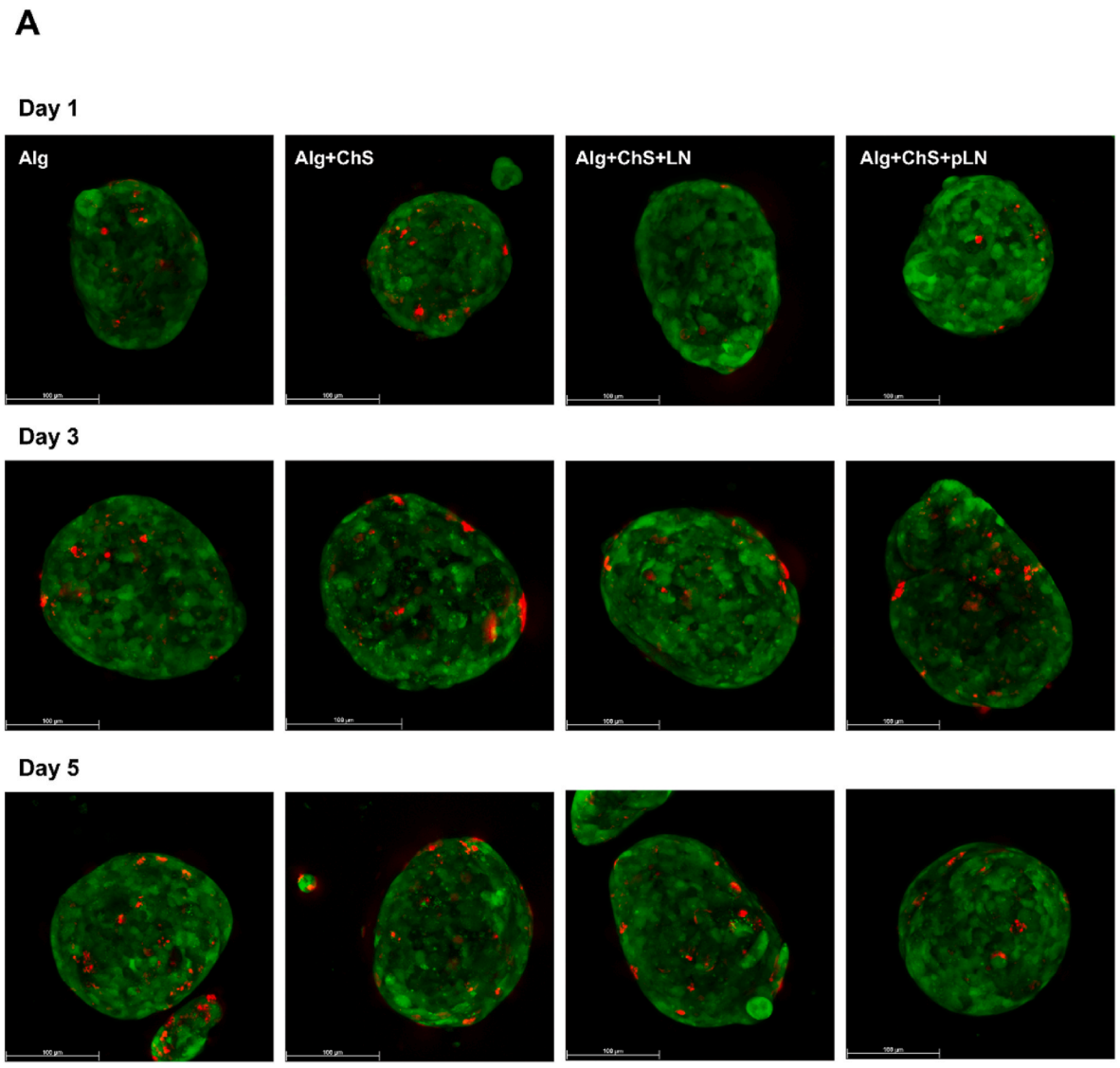

Fig. 1. Impact of various capsules compositions on encapsulated MIN6 aggregates over 5 days in the absence of pro-inflammatory cytokine exposure. Four capsules compositions were tested: alginate (Alg), alginate with chondroitin sulfate (Alg+ChS), alginate with chondroitin sulfate and laminin (Alg+ChS+LN), and alginate with chondroitin sulfate and poly-laminin (Alg+ChS+pLN). (A) Live and dead cell staining of MIN6 aggregates. Green indicates live cells stained with Calcein AM and red indicates dead cells stained with Ethidium Bromide (scale bar = 100 μm). (B) Live and dead staining was quantified using ImageJ software. (C) Mitochondrial activity was measured using the WST1 assay in absorbance at 450 nm. (D) Glucose-stimulated insulin secretion (GSIS) was assessed under low glucose (LG) and high glucose (HG) conditions. (E) Intracellular reactive oxygen species (ROS) were measured using the 2′,7′-dichlorofluorescin diacetate (DCFDA) ROS kit. (F) Nitric oxide levels were measured using the Total Nitric Oxide Kit. Statistical differences were quantified using one-way ANOVA or two-way ANOVA analysis with Dunnett's *post hoc* test compared with the control group (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$), N = 5. Data are expressed as mean ± standard error of the mean (SEM). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.).

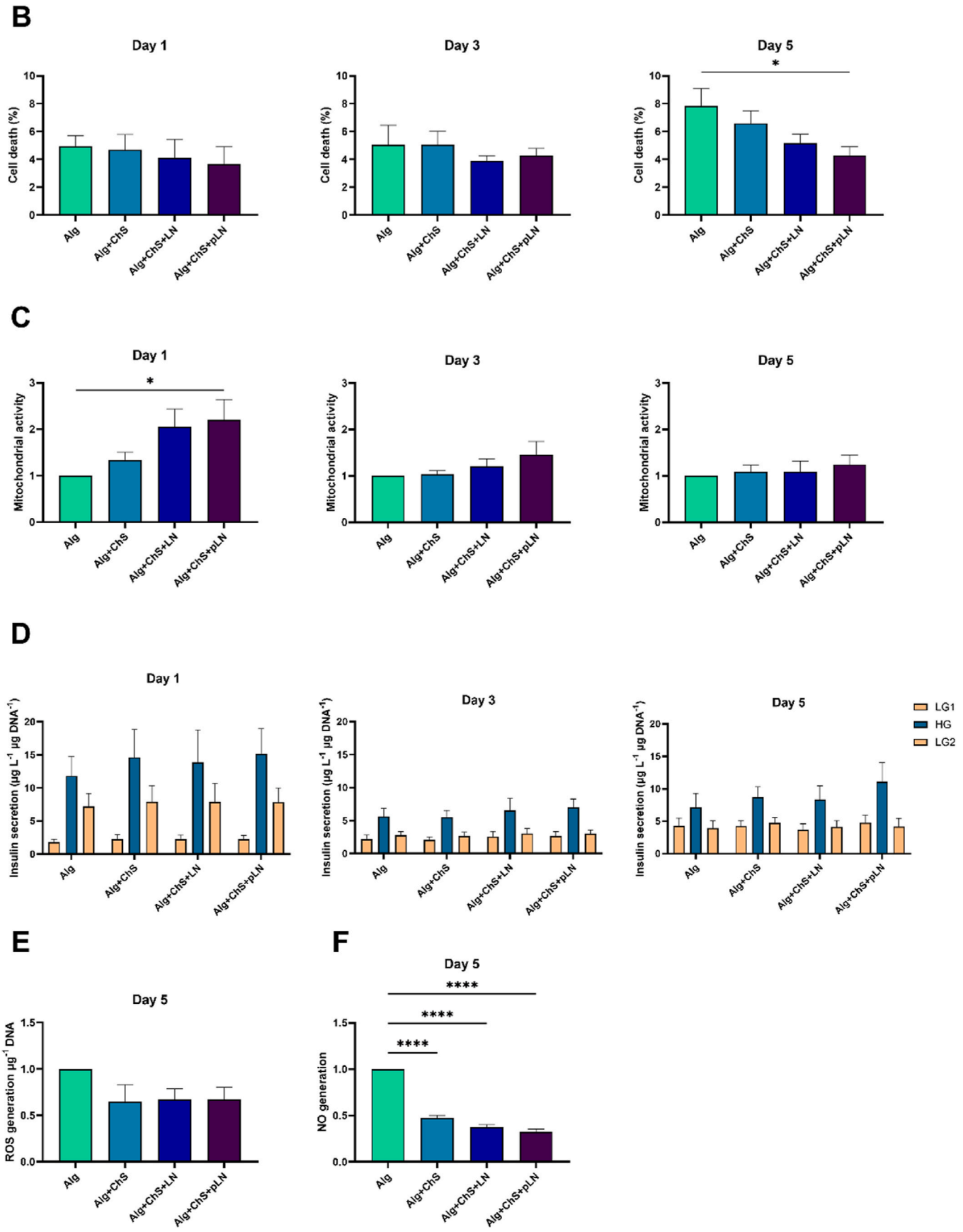

Fig. 1. (*continued*).

despite reducing surface stiffness, resulted in a smoother surface when compared to Alg-only capsules.

3.3. Laminin and poly-laminin capsules maintain MIN6 aggregates viability over five days of culturing in the absence of cytokines

As a first set of experiments, we assessed the impact of inclusion in the capsules of ChS, LN, and pLN on the viability, functionality and cellular stress of MIN6 aggregates under standard culture conditions, in the absence of cytokines (Fig. 1). Fig. 1A are representative images of MIN6 cell aggregates stained for live and dead cells, shown for each composition at time points of days 1, 3, and 5. The results indicate that MIN6 aggregates encapsulated in Alg+ChS+LN and Alg+ChS+pLN exhibited a lower cell death rate over the experimental period (Fig. 1B). Initially, on days 1 and 3, the variations in cell death percentages among the different capsules compositions were minimal. By day 5, we observed a trend showing that adding ChS, LN, and pLN led to progressively lower cell death rates. Specifically, MIN6 aggregates in Alg+ChS+pLN capsules demonstrated a statistically significantly lower cell death than those in Alg, with a 45.2 ± 9.65 % decrease ($p = 0.02$).

The mitochondrial activity assay (Fig. 1C) revealed a peak in metabolic activity on day 1 for cells encapsulated in Alg+ChS+LN and Alg+ChS+pLN, showing a significant increase in mitochondrial activity (2.2-fold, $p = 0.03$) of MIN6 aggregates in Alg+ChS+pLN capsules compared to those in Alg capsules. Mitochondrial activity increased over time (Supplementary Fig. S4A); however, no significant differences were detected between groups on days 3 and 5. The enhanced mitochondrial activity on day 1 suggests that cells encapsulated with Alg+ChS+LN and Alg+ChS+pLN might adapt more efficiently to the microcapsule environment. This might explain the reduced cell death over time, especially

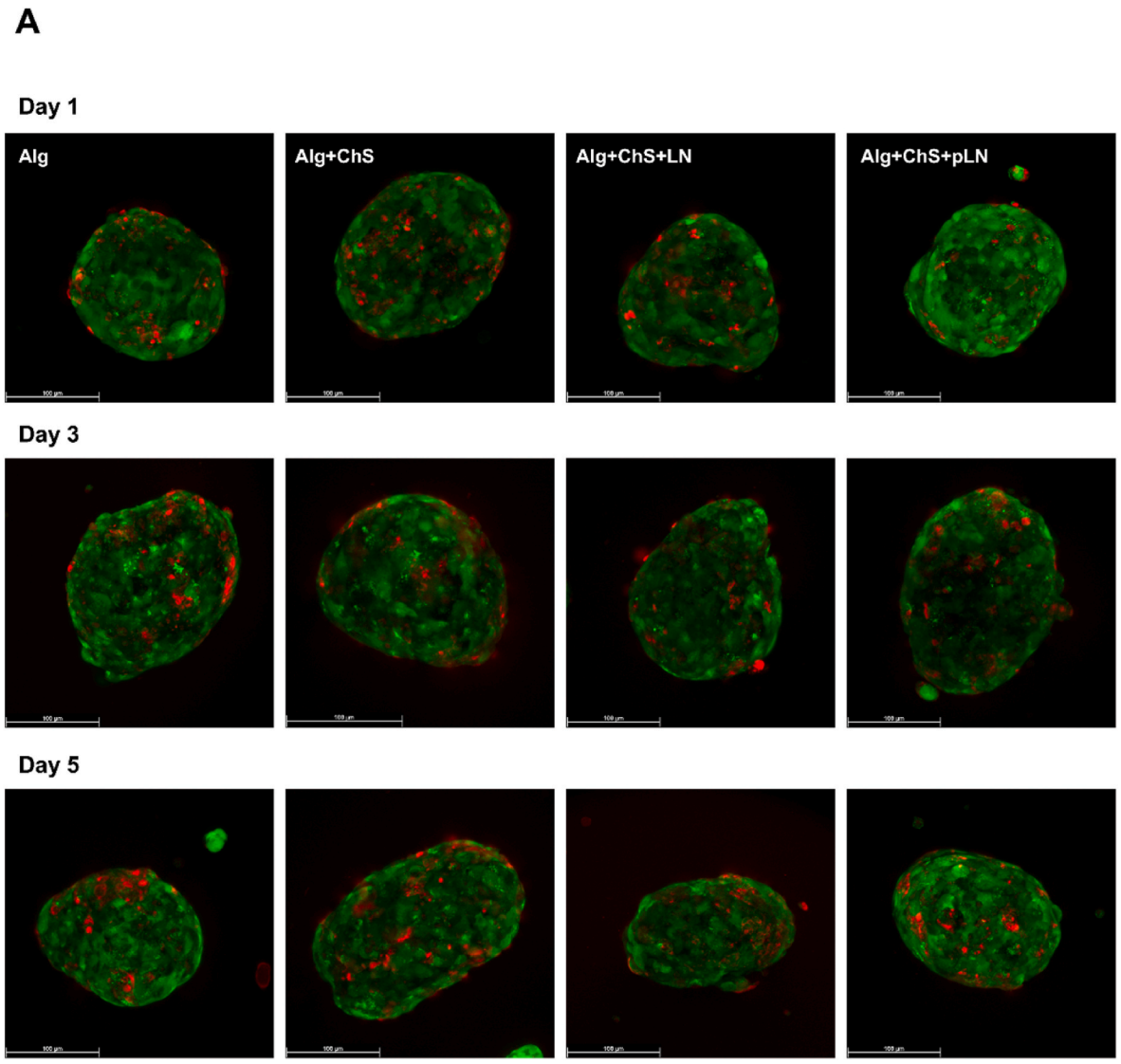

Fig. 2. Impact of various capsules compositions on encapsulated MIN6 aggregates over 5 days in the presence of pro-inflammatory cytokines. Four capsules compositions were tested: alginate (Alg), alginate with chondroitin sulfate (Alg+ChS), alginate with chondroitin sulfate and laminin (Alg+ChS+LN), and alginate with chondroitin sulfate and poly-laminin (Alg+ChS+pLN). (A) Live and dead cell staining of MIN6 aggregates. Green indicates live cells stained with Calcein AM and red indicates dead cells stained with Ethidium Bromide (scale bar = 100 μm). (B) Live and dead staining was quantified using ImageJ software. (C) Mitochondrial activity was measured using the WST1 assay in absorbance at 450 nm. (D) Glucose-stimulated insulin secretion (GSIS) was assessed under low glucose (LG) and high glucose (HG) conditions. (E) Intracellular reactive oxygen species (ROS) were measured using the 2′,7′-dichlorofluorescin diacetate (DCFDA) ROS kit. (F) Nitric oxide levels were measured using the Total Nitric Oxide Kit. Statistical differences were quantified using one-way ANOVA two-way ANOVA analysis with Dunnett's *post hoc* test compared with the control group (*p < 0.05; **p < 0.01), N = 5. Data are expressed as mean ± standard error of the mean (SEM). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.).

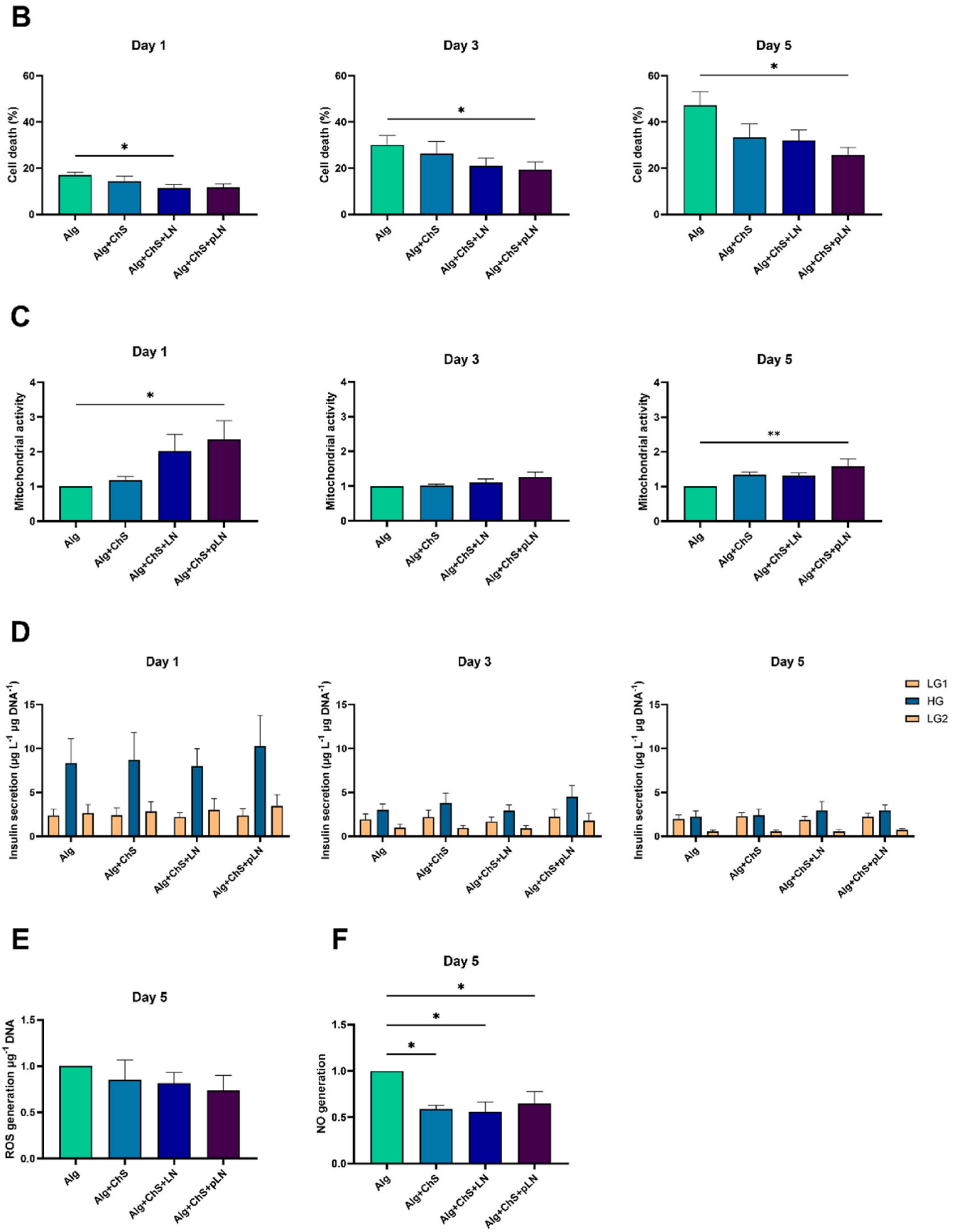

Fig. 2. (*continued*).

on day 5, for cells in Alg+ChS+pLN capsules.

The insulin response to glucose, evaluated by the glucose-stimulated insulin secretion (GSIS) assay (Fig. 1D), decreased from day 1 to day 3 in MIN6 aggregates, regardless of the capsule composition. However, from day 3 to day 5, the insulin response either stabilized or increased, suggesting cellular adaptation to the intracapsular environment. By day 5, cells in Alg+ChS+pLN capsules were the only ones that maintained significant differences in insulin secretion between high glucose and the first low glucose (LG1) incubation stimulation ($p = 0.01$) and between high glucose and the second low glucose (LG2) incubation stimulation ($p = 0.006$) (Supplementary Fig. S5A). On the last day, MIN6 aggregates in pLN-containing capsules also showed a 55.3 ± 22.7 % ($p = 0.20$) of increase on insulin secretion upon high glucose stimulation compared to alginate-only capsules.

To evaluate whether the intracapsular environment could prevent cellular stress, we measured the generation of intracellular reactive oxygen species (ROS) (Fig. 1E and Supplementary Fig. S4C) and secreted nitric oxide (NO) (Fig. 1F and Supplementary Fig. S4E). We observed that adding ChS, LN, or pLN to alginate-based capsules reduced ROS generation in encapsulated MIN6 aggregates, when compared to those in alginate-only capsules. Furthermore, the nitric oxide (NO) assay revealed that cells in all types of modified capsules significantly reduced NO production compared to cells in alginate-only capsules ($p < 0.001$).

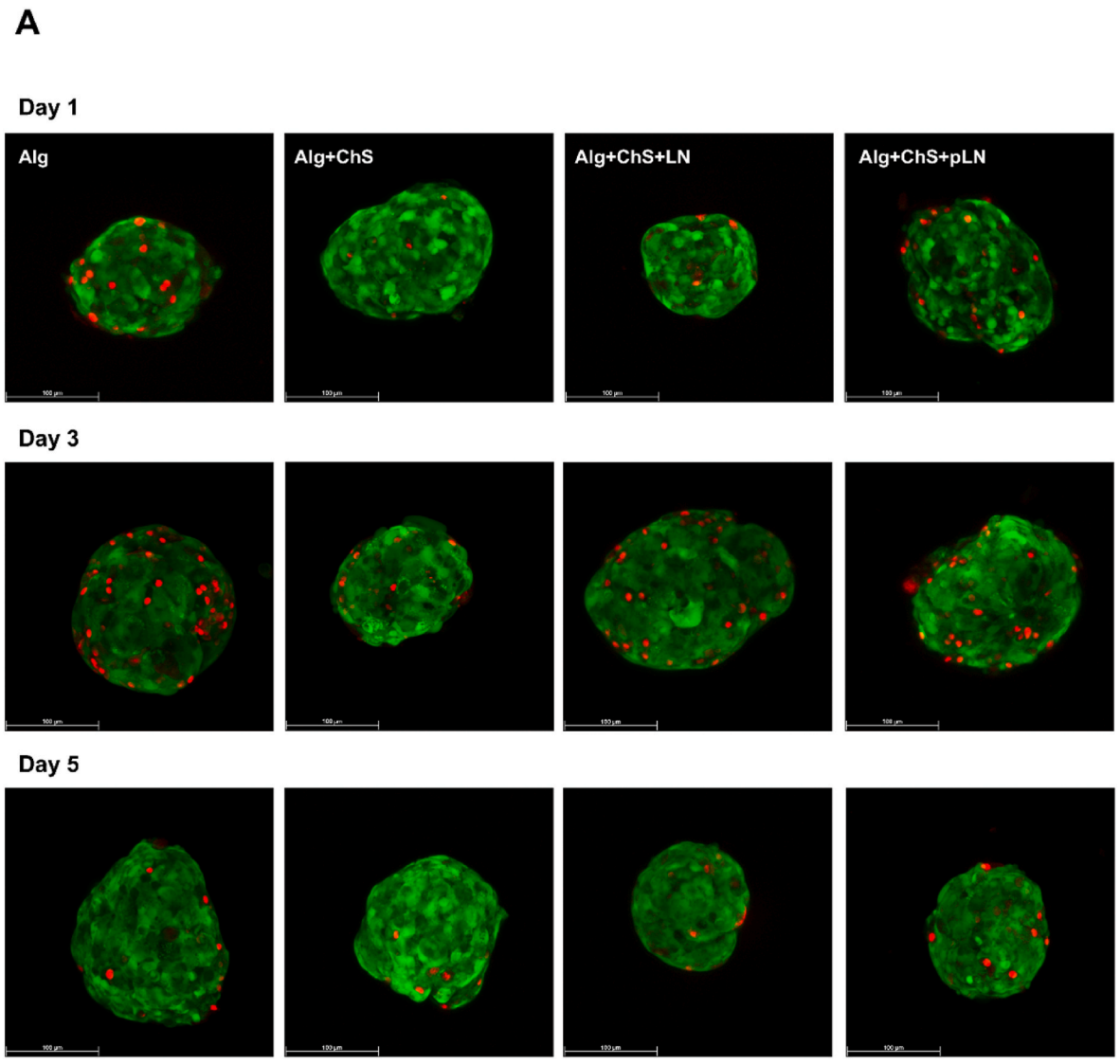

Fig. 3. Impact of various capsules compositions on encapsulated human islets over 5 days in the absence of pro-inflammatory cytokine exposure. Four capsules compositions were tested: alginate (Alg), alginate with chondroitin sulfate (Alg+ChS), alginate with chondroitin sulfate and laminin (Alg+ChS+LN), and alginate with chondroitin sulfate and poly-laminin (Alg+ChS+pLN). (A) Live and dead staining of human islets is shown. Green indicates live cells stained with Calcein AM and red indicates dead cells stained with Ethidium Bromide (scale bar = 100 μm). (B) Live and dead staining was quantified using ImageJ software (N = 6). (C) Mitochondrial activity was measured using the WST1 assay in absorbance at 450 nm (N = 6). (D) Glucose-stimulated insulin secretion (GSIS) was assessed under low glucose (LG) and high glucose (HG) conditions (day 1 N = 5, day 3 and 5 N = 6). (E) Intracellular reactive oxygen species (ROS) were measured using the 2′,7′-dichlorofluorescin diacetate (DCFDA) ROS kit (N = 5). (F) Nitric oxide levels were measured using the Total Nitric Oxide Kit (N = 4). Statistical differences were quantified using one-way ANOVA or two-way ANOVA (D) analysis with Dunnett's *post hoc* test compared with the control group (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001) and Kruskal-Wallis test for Nitric oxide levels. Data are expressed as mean ± standard error of the mean (SEM). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.).

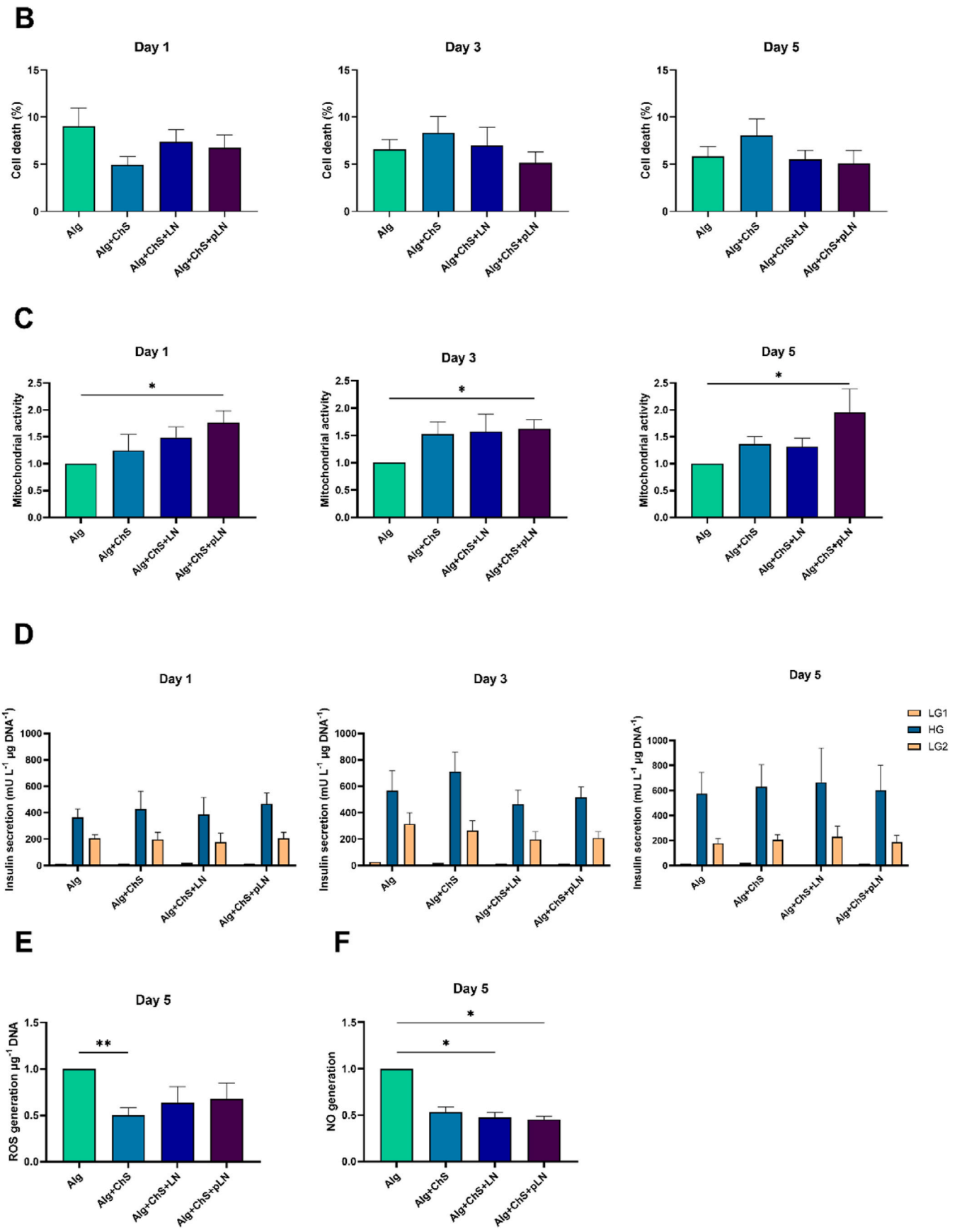

Fig. 3. (*continued*).

3.4. The addition of ECM molecules to capsule composition enhances MIN6 cell viability and reduces oxidative stress under inflammatory conditions

Exposure to the pro-inflammatory cytokines IL-1β, IFN-γ, and TNF-α resulted in increased cell death across all capsules compositions (Fig. 2A and B). On the first day, MIN6 aggregates in capsules containing Alg+ChS+LN and Alg+ChS+pLN exhibited lower percentages of cell death, with Alg+ChS+LN capsules showing the significantly lowest percentage ($p = 0.02$). At day 3, cells in Alg+ChS+pLN capsules showed a significantly lower cell death rate (35.6 ± 7.53 % less), compared to alginate-only capsules ($p = 0.03$). By day 5, this reduced cell death was even more pronounced, with a 45.2 ± 7.94 % lower cell death for MIN6 aggregates in Alg+ChS+pLN capsules ($p = 0.01$).

Mitochondrial activity was also influenced by the capsule composition (Fig. 2C). On the first day of exposure, cells in Alg+ChS+pLN capsules demonstrated a 2.78-fold increase in mitochondrial activity compared to alginate-only capsules ($p = 0.01$). Over time, mitochondrial activity decreased for cells in both LN and pLN-containing capsules (Supplementary Fig. S4B), but after 5 days, it remained significantly higher for cells in pLN-containing capsules compared to those in alginate-only capsules ($p = 0.008$).

In the GSIS assay (Fig. 2D), cytokine exposure resulted in a marked decrease in the functionality of MIN6 aggregates over time across all compositions. No statistical significance was observed between groups upon low and high glucose level exposure. After 5 days of cytokine exposure, cells encapsulated in the different compositions exhibited a reduced LG1 to HG response (Supplementary Fig. S5B), with the highest difference in response (1.57-fold) being observed in cells encapsulated in Alg+ChS+LN capsules.

In the oxidative stress assays, compared to alginate-only capsules, MIN6 aggregates encapsulated in either ECM-based compositions exhibited lower ROS generation (Fig. 2E and Supplementary Fig. S4D). Cells in Alg+ChS+pLN demonstrated the lowest ROS generation (1.37-fold). Additionally, NO generation was significantly reduced 1.70-fold ($p = 0.01$) in the same group (Fig. 2F and Supplementary Fig. S4F). Cells encapsulated in capsules of Alg+ChS+LN also showed a significant 1.78-fold ($p = 0.01$) lower NO generation. MIN6 aggregates in Alg+ChS+pLN capsules had a 1.55-fold lower NO ($p = 0.03$).

3.5. Laminin and poly-Laminin containing microcapsules enhances human islet cell survival and improves function over time

We also determined whether the capsules compositions could influence the viability and functionality of human islets in the absence of inflammatory stress. In the live and dead assay (Fig. 3A and B), compared to Alg-only, islets in Alg+ChS showed the lowest cell death of 45.5 ± 12.8 % on the first day. On day 3, human islets encapsulated in Alg+ChS+pLN capsules exhibited the lowest cell death percentage, with 22 ± 6 % lower cell death, compared to Alg-only. By day 5, islets in Alg+ChS+pLN maintained the lowest cell death, with 13.31 ± 4.3 % lower cell death compared to islets in Alg-only.

Mitochondrial activity analysis revealed that, while all other compositions exhibited reduced activity by day 5, human islets encapsulated in Alg+ChS+pLN maintained their activity consistently over days 3 and 5 (Supplementary Fig. S6A). Notably, islets in pLN-containing microcapsules exhibited significantly higher mitochondrial activity compared to those in alginate-only capsules, with a 1.76-fold increase ($p = 0.04$) on day 1, a 1.62-fold increase ($p = 0.03$) on day 3, and a 1.95-fold increase ($p = 0.01$) on day 5 (Fig. 3C).

In the GSIS assay (Fig. 3D and Supplementary Fig. S7A), in the absence of cytokine stress, insulin secretion increased upon high glucose stimulation across all compositions over the days. On day 3 human islets in Alg+ChS capsules showed the highest insulin secretion upon high glucose levels with 25.5 % ± 8.62 % higher responses when compared to those in alginate-only capsules. No major differences were observed between the groups on the other days.

The generation of intracellular ROS in human islets after 5 days (Fig. 3E and Supplementary Fig. S6C), in the absence of cytokines, was reduced in all capsules compositions, compared to alginate-only capsules. Human islets in Alg+ChS capsules showed a 2-fold ($p = 0.007$) decrease in ROS generation. Moreover, islets in Alg+ChS+LN and Alg+ChS+pLN showed a significantly lower NO generation compared to those in Alg, of 2.12-fold ($p = 0.01$) and 2.22-fold ($p = 0.02$) (Fig. 3F and Supplementary Fig. S6E).

3.6. Poly-laminin capsules minimize cytokine-induced human islet cell death and laminin capsules reduce oxidative stress

Upon exposure to the cytokines IL-1β, IFN-γ, and TNF-α, human islets encapsulated in Alg+ChS, Alg+ChS+LN, and Alg+ChS+pLN showed progressively lower cell death rates on the first and third days (Fig. 4A and B). The strongest effects were found for islets in Alg+ChS+pLN capsules, with 43 ± 12.6 % lower cell death than in Alg-only capsules on day 1 ($p = 0.01$) and 55 ± 18.6 % on day 3 but did not reach statistical significancy. On the final day of the experiment, although the differences between compositions were reduced, islets in pLN-containing capsules continued to induce the lowest cell death (15.1 ± 3.5 %).

Importantly, the compositions that demonstrated higher survival rates in the live/dead assay also showed increased mitochondrial activity (Fig. 4C and Supplementary Fig. S6B), indicating a progressive increase in mitochondrial activity across islets in Alg+ChS, Alg+ChS+LN, and Alg+ChS+pLN. Islets in LN and pLN-containing capsules had a similar increase in mitochondrial activity on day 1 (1.4-fold). Human islets in Alg+ChS+pLN showed a 1.63-fold increase in mitochondrial activity on day 3 compared to islets in alginate-only capsules. Similarly to the live/dead assay, the differences between compositions were reduced on day 5, with the highest mitochondrial activity observed in islets encapsulated in Alg+ChS capsules, showing a 1.34-fold increase compared to islets in alginate-only capsules.

The GSIS assay indicates that the detrimental effect of pro-inflammatory cytokines on islet functionality became evident only by day 5 (Fig. 4D). On day 5, islets in Alg+ChS+LN capsules exhibited the highest response to high glucose stimulation (35.8 ± 17.5 %) compared to those in Alg-only capsules. This condition also showed the highest statistical significance between both low and high glucose treatments on the same day ($p = 0.0002$ for LG1 and $p = 0.004$ for LG2) (Supplementary Fig. S7B). Although all compositions exhibited a decrease in insulin response to high glucose stimulation on day 3, islets in Alg+ChS capsules showed an increase in the response when compared to day 1 (29.8 ± 13 %), which subsequently dropped by day 5.

The ROS generation in human islets upon five days of exposure to cytokines showed that islets encapsulated within Alg+ChS+LN showed a significantly lower ROS generation (1.85- fold, $p = 0.01$), when compared to islets in Alg-only (Fig. 4E and Supplementary Fig. S6D). Islets in Alg+ChS also reduced ROS generation, but to a lower extent (1.17-fold) compared to alginate-only. Regarding NO generation, human islets showed a similar pattern as that observed in the absence of cytokines with a significantly lower NO generation for islets encapsulated in Alg+ChS+LN (1.85-fold, $p = 0.02$) and Alg+ChS+Pln (1.72-fold, $p = 0.04$) (Fig. 4F and Supplementary Fig. S6F).

3.7. MIN6 modulation of mitochondrial dynamics and stress response genes in ECM-modified microcapsules exposed to cytokines

To assess the impact of each capsule composition on gene expression under cytokine-induced stress, we analyzed genes associated with mitochondrial dynamics and cellular stress (Table 3). Exposure to cytokines for 5 days led to an increased expression of genes associated with both mitochondrial fusion (*Mfn1, Mfn2, Opa1*) and fission (*Drp1, Fis1*) (Fig. 5A), which is an important process for the maintenance of mitochondrial integrity and homeostasis especially under metabolic and

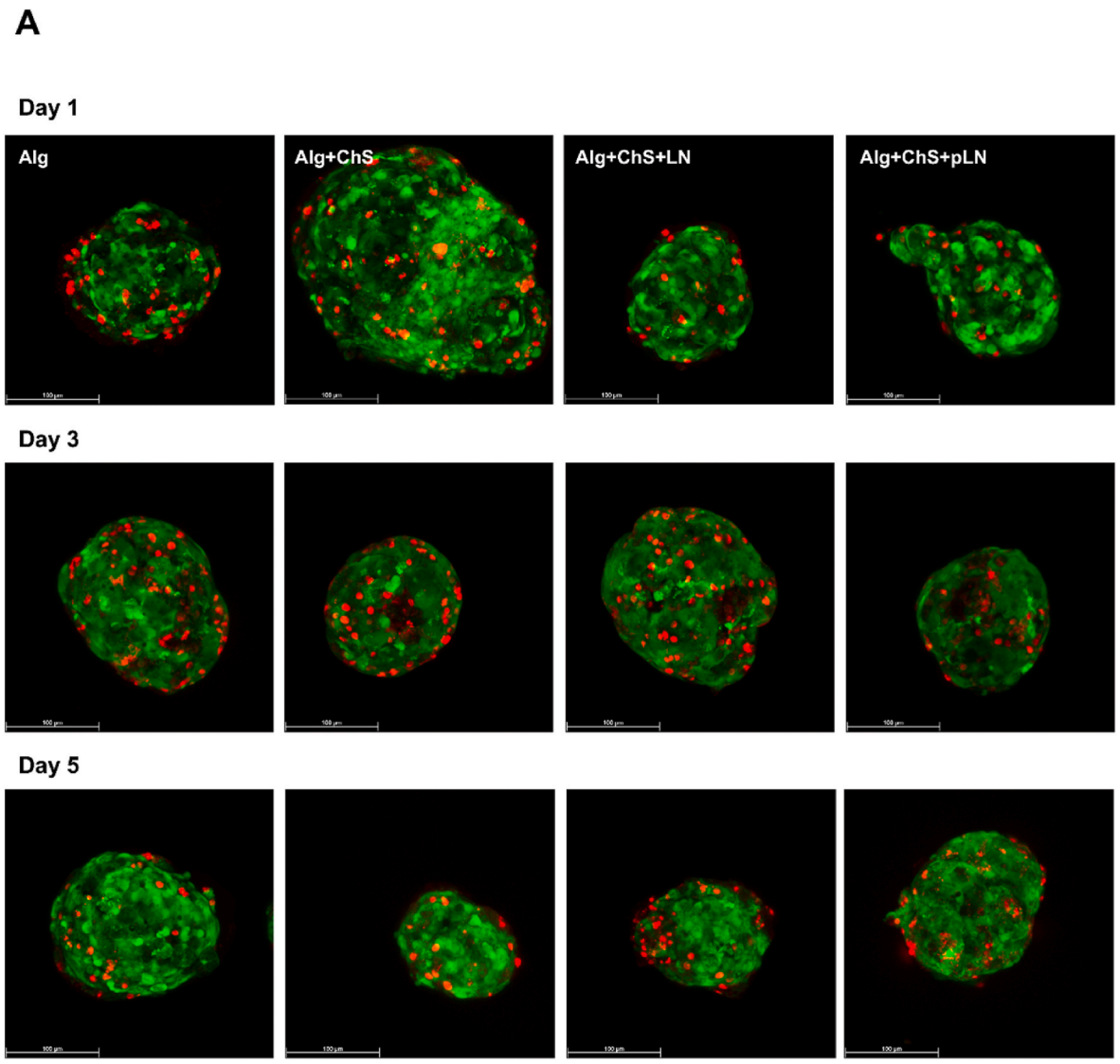

Fig. 4. Impact of various capsules compositions on encapsulated human islets over 5 days in the presence of pro-inflammatory cytokines. Four capsules compositions were tested: alginate (Alg), alginate with chondroitin sulfate (Alg+ChS), alginate with chondroitin sulfate and laminin (Alg+ChS+LN), and alginate with chondroitin sulfate and poly-laminin (Alg+ChS+pLN). (A) Live and dead staining of human islets is shown. Green indicates live cells stained with Calcein AM and red indicates dead cells stained with Ethidium Bromide (scale bar = 100 μm). (B) Live and dead staining was quantified using ImageJ software (N = 6). (C) Mitochondrial activity was measured using the WST1 assay in absorbance at 450 nm (N = 6). (D) Glucose-stimulated insulin secretion (GSIS) was assessed under low glucose (LG) and high glucose (HG) conditions (day 1 N = 5, day 3 and 5 N = 6). (E) Intracellular reactive oxygen species (ROS) were measured using the 2′,7′-dichlorofluorescin diacetate (DCFDA) ROS kit (N = 5). (F) Nitric oxide levels were measured using the Total Nitric Oxide Kit (N = 4). Statistical differences were quantified using one-way ANOVA or two-way ANOVA (D) analysis with Dunnett's *post hoc* test or Kruskal-Wallis with Dunn's *post hoc* test (F) compared with the control group (*$p < 0.05$) for Nitric oxide levels. Data are expressed as mean ± standard error of the mean (SEM). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.).

environmental stress [52]. Among these genes, the fusion-related genes *Mfn1* and *Mfn2* showed the most pronounced upregulation across all capsules compositions. MIN6 aggregates encapsulated in Alg+ChS+pLN exhibited an overall lower expression of mitochondrial fusion and fission genes compared to the other compositions. Specifically, the expression of *Mfn1* and *Mfn2* of cells in pLN-containing capsules was markedly reduced compared to alginate-only capsules, with a 1.82-fold decrease for *Mfn1* ($p = 0.008$) and a 1.81-fold decrease for *Mfn2* ($p = 0.002$).

To understand how the added ECM molecules influence stress-related genes in MIN6 aggregates exposed to cytokines, we evaluated the expression of three critical genes, *Foxo1, Xbp1*, and *Hspa5*, which indicate stress-related disruptions in β-cell function. Our results revealed that exposure to cytokines led to an increased expression of the *Foxo1* gene, which regulates insulin secretion and is associated with β-cell apoptosis upon endoplasmic reticulum (ER) stress (Fig. 5B). This occurred in cells encapsulated in all compositions, when compared to MIN6 aggregates in alginate capsules in the absence of cytokine exposure. In contrast, no remarkable differences were observed in the expression of *Xpb1,* a gene which is involved in the unfolded protein stress response (UPR). Concerning *Hspa5* expression, an indicator of ER stress, cytokine exposure induced a reduced expression across all capsules compositions compared to cells in alginate-only capsules in the absence of cytokine exposure. However, differences were observed among the compositions were observed, with progressively higher *Hspa5* expression in capsules containing ChS, LN, and pLN. Among

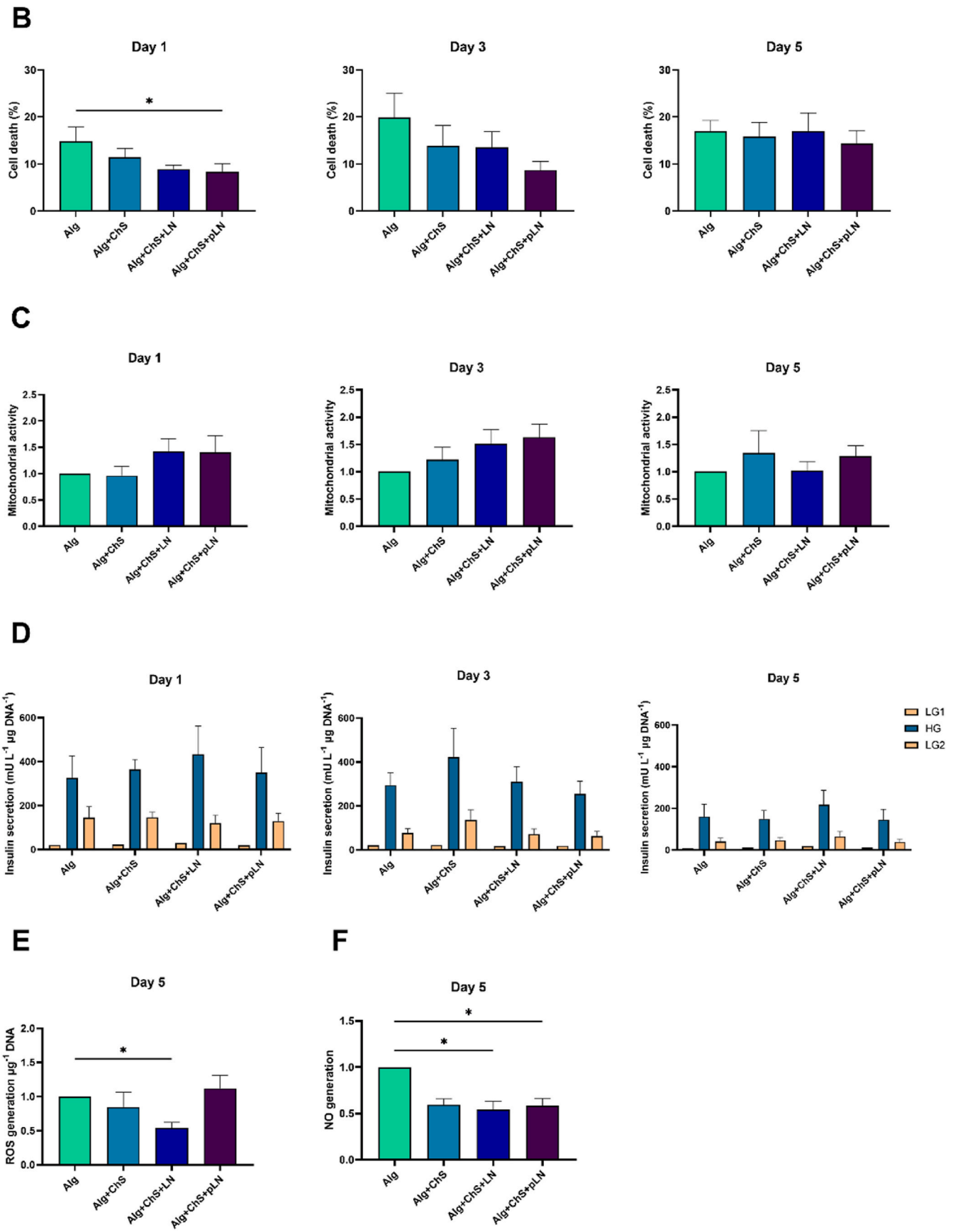

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Table 3
Summary of gene categories and functions analyzed in MIN6 aggregates and human islets.

Gene	Category	Role in cellular process	References
MFN1	Mitochondrial fusion	Mediates outer mitochondrial membrane fusion	[53]
MFN2	Mitochondrial fusion	Mediates outer mitochondrial membrane fusion	[53]
DRP1	Mitochondrial fission	Mediates mitochondrial and peroxisomal division	[54]
OPA1	Mitochondrial fusion	Regulates inner mitochondrial membrane fusion	[55]
FIS1	Mitochondrial fission	Regulates part of the mammalian fission machinery	[56]
FOXO1	Transcription Factor	The main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress	[57]
HSPA5	Molecular Chaperone	Involved in the folding and assembly of proteins in the ER and is a master regulator of ER homeostasis	[58]
XPB1	Transcription Factor	Modulates the cellular response to ER stress by regulating the UPR	[59]
MCP1	Chemokine	Monocyte chemoattractant protein-1; recruits monocytes to sites of inflammation.	[60]
IL-6	Cytokine	Involved in inflammation and immune response, can act as both pro-inflammatory and anti-inflammatory mediator.	[61]
IL-8	Chemokine	Mediates inflammatory response by attracting neutrophils to the inflammation site.	[62]
IL-33	Cytokine	Functions as an alarmin released upon cell damage; involved in Th2 immune responses.	[63]

ER: Endoplasmic reticulum; UPR: Unfolded protein response.

these, MIN6 aggregates encapsulated in the Alg+ChS+pLN capsules exhibited the highest expression, showing a 1.27-fold increase when compared to alginate-only capsules.

3.8. Modulation of inflammatory responses in human islets encapsulated in poly-laminin based capsules

The same genes analyzed in MIN6 cells were also evaluated in human islets, with the addition of inflammation-related genes (Table 3). Encapsulated human islets exposed to pro-inflammatory cytokines for 5 days exhibited lower expression of genes related to mitochondrial dynamics compared to islets in alginate-only capsules in the absence of cytokine exposure. Notably, islets in Alg+ChS+pLN capsules demonstrated the lowest expression levels for mitochondrial fusion and fission genes, similar to that observed for MIN6 aggregates. Human islets encapsulated in Alg+ChS capsules showed a significant upregulation of the *FIS1* gene, associated with mitochondrial fission ($p = 0.041$), compared to those in alginate-only capsules upon cytokine exposure (Fig. 6A).

For stress-related genes, cytokine exposure induced a significant downregulation of the *FOXO1* gene in human islets encapsulated in Alg-only capsules ($p = 0.001$) when compared to islets in Alg capsules in the absence of cytokine exposure (Fig. 6B). The expression of *XPB1* gene in human islets was reduced in islets encapsulated in both Alg+ChS+LN (1.23-fold) and Alg+ChS+pLN (1.25-fold) capsules. Moreover, islets in Alg+ChS+pLN showed the lowest *HSPA5* gene expression, with 1.34-fold compared to islets in alginate exposed to cytokines.

In encapsulated human islet samples, we also evaluated the expression of the proinflammatory cytokines *IL-33, IL-6, IL-8*, and *MCP1* to gain insight into how different capsules compositions influence the islet's inflammatory response under cytokine-induced stress and their potential to mitigate the immune-mediated damage. Cytokine treatment led to an upregulation of the *IL-33*, *IL-6*, *IL-8*, and *MCP-1* genes across all capsules compositions compared to alginate capsules in the absence of cytokine exposure (Fig. 6C). Notably, *IL-6* expression significantly increased by 1.5-fold in islets encapsulated in Alg+ChS+pLN capsules ($p = 0.02$). In contrast, the expression of the other pro-inflammatory genes, *IL-33*, *IL-8*, and *MCP-1*, was downregulated in islets encapsulated in both Alg+ChS+LN and Alg+ChS+pLN compared to those in Alg-only capsules exposed to cytokines.

After 5 days of cytokine exposure, the secretion of various cytokines and chemokines into the supernatant of encapsulated human islets was assessed to determine the impact of each capsule composition on immune-related responses (Fig. 7). Notably, human islets in alginate-only capsules exhibited a significantly higher secretion of IL-33 in the supernatant compared to islets encapsulated in alginate capsules in the absence of cytokine exposure ($p = 0.004$) (Fig. 7A). Additionally, IL-33 levels were higher in alginate-only capsules compared to islets encapsuled in other capsules compositions exposed to cytokines. The lowest secretion was observed in islets encapsulated in Alg+ChS, with 32.6 ± 8.3 % lower responses compared to islets encapsulated in Alg-only capsules. This pattern was consistent for IL-13 and CCL20 secretion, where islets in Alg+ChS capsules showed levels 17.85 ± 2.54 % and 57 ± 28 % lower responses, when compared to Alg capsules exposed to cytokines (Fig. 7B and D).

The secretion of MCP-1 was significantly increased in human islets encapsulated in Alg capsules exposed to cytokines compared to those in the absence of cytokines exposure (Fig. 7, C). However, the addition of ChS, LN, and pLN to the capsule composition resulted in progressively lower MCP-1 secretion, with the lowest levels observed in islets encapsulated in Alg+ChS+pLN capsules. This decrease was statistically significant, with levels being 41.7 ± 7.2 % lower than those in Alg-only capsules exposed to cytokines ($p = 0.001$). Similarly, the secretion of the alarmin TSLP by human islets was lower in Alg+ChS+pLN capsules compared to those encapsulated in other compositions exposed to cytokines, showing a 27.5 ± 13.3 % decrease compared to Alg-only capsules (Fig. 7E).

4. Discussion

It is well recognized that the inflammatory milieu pancreatic islets encounter immediately after transplantation significantly impacts graft survival and overall transplantation outcomes [18,64–66]. Reduction of the graft mass by up to 60 % has been reported in the first weeks after implantation for both naked and encapsulated islets [67]. A way to reduce this loss is by enhancing the resilience or lowering the susceptibility of islet cells to the cytokine stress to which the β-cells are exposed in the immediate period after transplantation [68]. This study underscores the importance and success of changing the intracapsular environment to mitigate the detrimental effects of the inflammatory environment on transplanted β-cells, thereby enhancing the efficacy of transplanted pancreatic islets. However, our study does also show that the efficacy of the modifications depends on the type of ECM molecule applied.

Incorporating ChS, LN, and pLN into alginate-based microcapsules altered their physicochemical properties. The inclusion of ECM molecules can modulate the mechanical characteristics of the capsules, which, in turn, influence cellular behavior by activating mechanosensitive signaling pathways [69–71]. ChS increased surface stiffness, enhancing resistance to deformation under shear stress, potentially providing better mechanical protection for encapsulated cells. However, higher stiffness in alginate matrices can negatively impact cell survival and protein secretion [72]. In contrast, adding LN or pLN reduced stiffness, likely improving metabolite and nutrient diffusion to the capsule core. This softer surface may benefit encapsulated cells by maintaining metabolic activity and reducing mechanotransduction effects [73,74], which could explain the lower cell death rates and higher mitochondrial activity observed in MIN6 aggregates and human islets encapsulated with these polymers. Furthermore, the reduced roughness

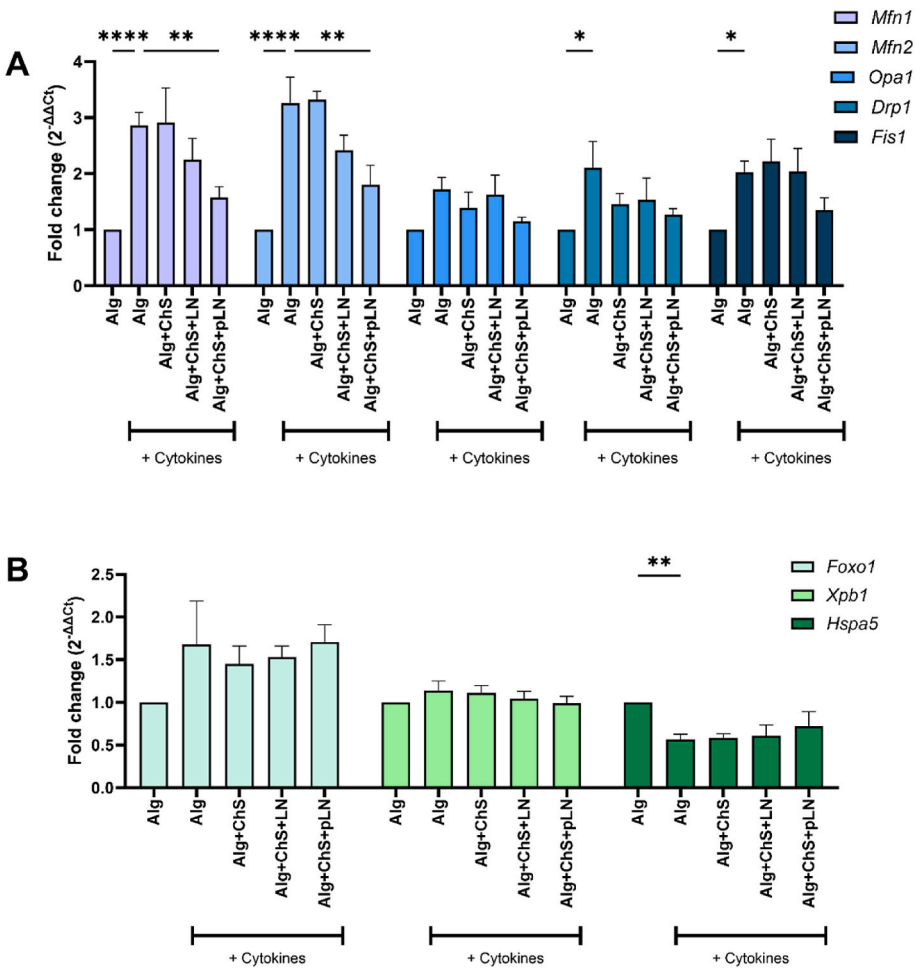

Fig. 5. Effects of various capsules compositions on encapsulated MIN6 aggregates on mitochondrial dynamics genes (A) and cell stress response (B) after 5 days of exposure to cytokine. The gene expression was quantified using qRT-PCR analysis. Statistical differences were quantified using two-way ANOVA analysis with Dunnett's *post hoc* test compared to the control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$), N = 5. Data are expressed as mean ± standard error of the mean (SEM).

observed on Alg+ChS+LN and Alg+ChS+pLN capsules could contribute to enhanced biocompatibility *in vivo* [75] and is therefore considered to be beneficial.

In the absence of cytokines, the inclusion of ChS, LN and pLN ECM molecules to the capsule composition significantly enhanced the viability of both MIN6 aggregates and human islets throughout the 5-day experiment. Also, it lowered the oxidative stress levels measured on the final day of the experiment. The results indicate that the potential cellular stress caused by the encapsulation process and/or cell immobilization within the capsules can be mitigated especially by the presence of LN and pLN. The ECM components appear to create a supportive environment that helps to mitigate the detrimental effects of prolonged culture. This is particularly significant for human islets, which are known to experience reduced functionality during extended culture periods. In fact, it has been shown that capsules containing ECM molecules can increase islet function over 14 days culture [34] and the presence of laminin in silk-based capsules was also reported to induce higher stimulation indexes after 7 days [33].

The ECM-dependent effects on the capsules were also noticeable when MIN6 and human islets were exposed to the cytokines IL-1β, IFN-γ, and TNF-α. The results indicate that the inclusion of ChS, LN, and pLN exerts a progressively protective effect, as evidenced by a consistent reduction in cell death and increased mitochondrial activity. One possible mechanism underlying this protective effect is the buffering action of ECM components on cytokine concentrations near the encapsulated cells. Additionally, ECM molecules, especially glycosaminoglycans like ChS, have a natural affinity for binding cytokines [76–78]. By sequestering cytokines within the ECM, their interaction with encapsulated cells is reduced, mitigating harmful effects [62,63]. The effects could work in synergy with integrin-ECM binding which activates intracellular signaling pathways. These interactions can modulate the cellular response to cytokines, altering how the cells perceive and react to inflammatory signals [79]. This modulation of intracellular signaling can diminish the cytotoxic effects of cytokines, making the cells more resistant to inflammatory damage [26,27,34].

Our study highlights ChS as a promising ECM component for alginate-based β-cell encapsulation. ChS reduced ROS generation in human islets after five days of culture in the absence of cytokines and decreased the secretion of IL-33 and CCL20 upon cytokine exposure, key mediators of immune cell recruitment and inflammation. ChS has been shown to exhibit notable anti-inflammatory properties by modulating inflammatory cytokines activity and reducing the expression of pro-inflammatory mediators [80,81]. Additionally, ChS-containing capsules showed increased *Fis1* gene expression, particularly in human islets upon cytokine exposure. *Fis1* is involved in mitochondrial fission, a process that may play a crucial role in the removal of damaged mitochondria and the maintenance of mitochondrial function [52]. This increased *Fis1* expression suggests that the cells were actively engaged in repairing mitochondrial damage.

The addition of LN or pLN to the Alg+ChS composition supported cell viability and reduced oxidative stress response in a pro-inflammatory environment. The incorporation of non-polymerized LN

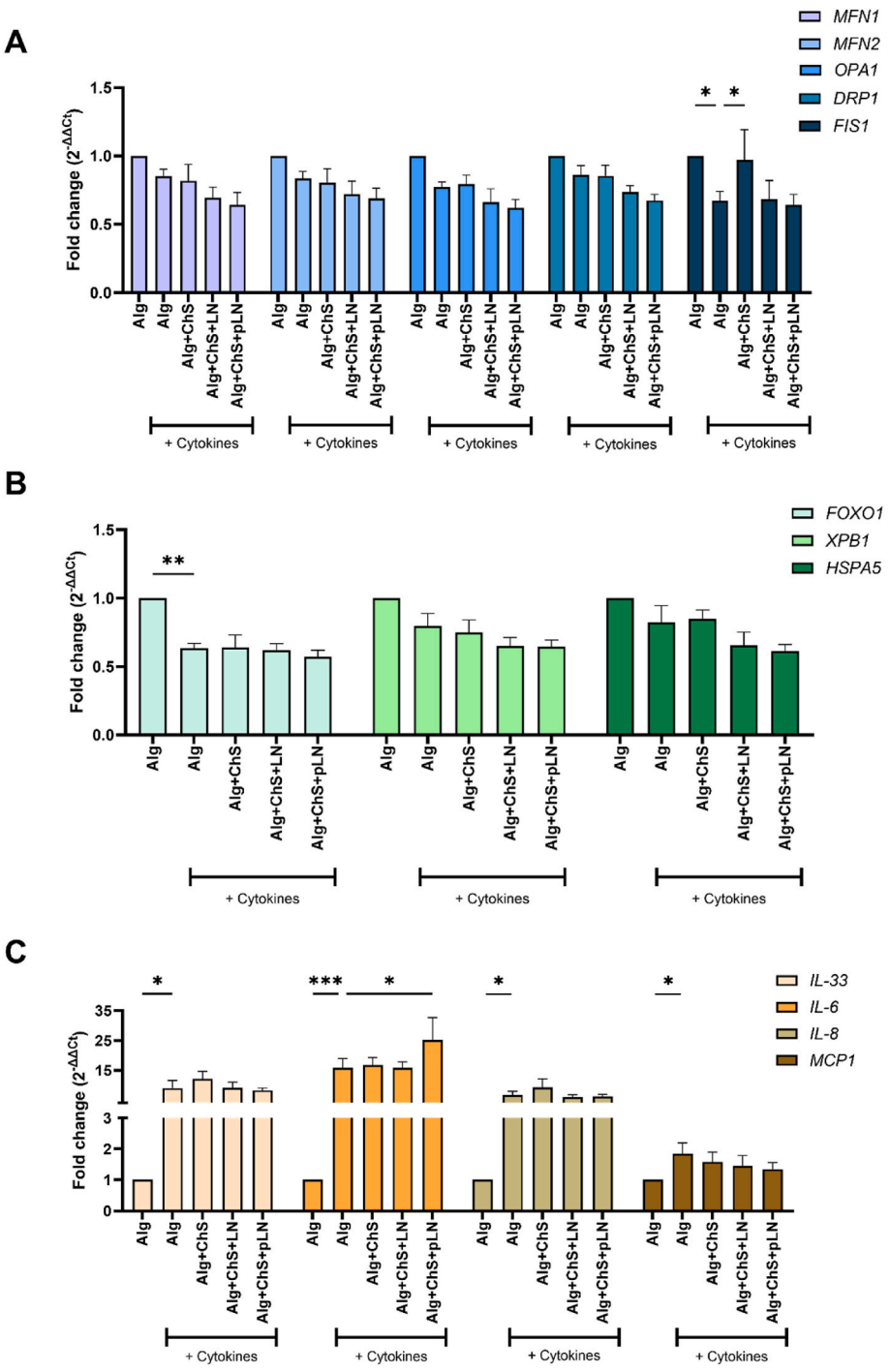

Fig. 6. Effects of various capsules compositions on encapsulated human islets on mitochondrial dynamics genes (A), cell stress response (B), and immune response (C) after 5 days of cytokine exposure. Gene expressions were quantified using qRT-PCR analysis. Statistical differences were quantified using two-way ANOVA analysis with Dunnett's *post hoc* test compared with the control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$), N = 5. Data are expressed as mean ± standard error of the mean (SEM).

in the capsule composition had a significant effect on reducing ROS generation in human islets after 5 days of cytokine exposure. This reduction in oxidative stress may have helped the cells to maintain their functionality more effectively, as the Alg+ChS+LN composition showed the highest insulin secretion response to high glucose stimulation on both the first and fifth days of cytokine exposure. Both human islets and MIN6 aggregates in pLN-containing capsules exhibited the lowest cell death rates over time, accompanied by increased mitochondrial activity. The expression of mitochondrial dynamics-related genes in MIN6 cells and human islets suggests that pLN may exert an anti-inflammatory effect, reducing the necessity for increased mitochondrial fusion or fission. This indicates a balanced state between these processes. By alleviating inflammatory signaling, pLN is likely to decrease cellular stress, diminishing the need for compensatory mitochondrial dynamics.

Stress-related genes play crucial roles in maintaining cellular homeostasis under adverse conditions. HSPA5 (BiP) is a molecular chaperone essential for the unfolded protein response (UPR), promoting protein folding and preventing misfolded protein accumulation [82].

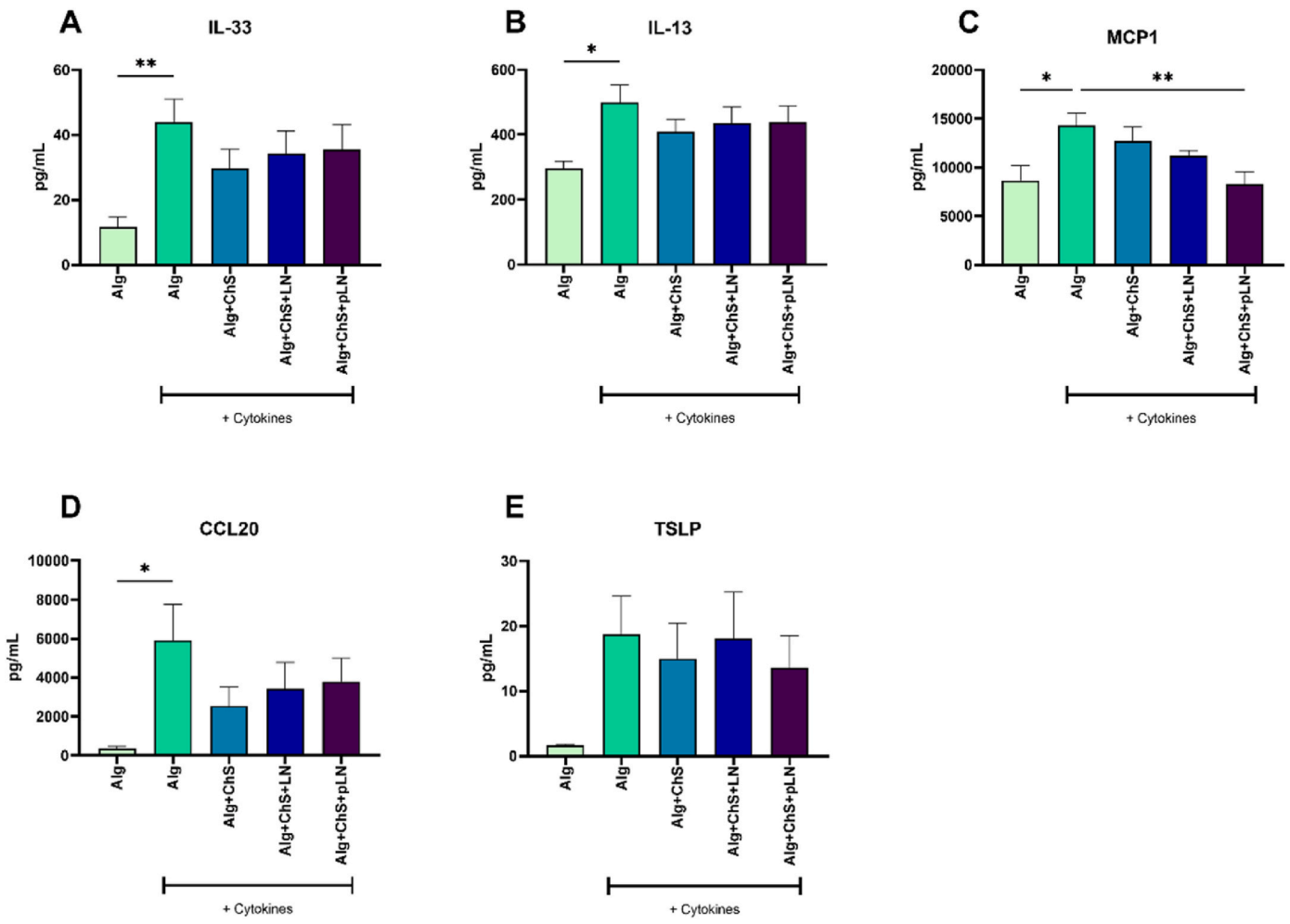

Fig. 7. Effects of various capsules compositions on cytokine and chemokine secretion by human islets after 5 days of cytokine exposure. Secreted (A) interleukin 33 (IL-33), (B) interleukin 13, (C) monocyte chemoattractant (MCP-1 or CCL2), (D) CC motif chemokine ligand 20 (CCL20), (E) thymic stromal lymphopoietin (TSLP). The concentrations of these molecules in the supernatant were measured using the Luminex assay. Statistical differences were quantified using one-way ANOVA analysis with Dunnett's *post hoc* test compared with the control group (*$p < 0.05$; **$p < 0.01$), $N = 6$. Data are expressed as mean ± standard error of the mean (SEM).

XBP1 enhances the UPR by regulating genes involved in protein folding and degradation [83], while FOXO1 governs cellular stress responses, including oxidative stress and apoptosis [84]. Islets encapsulated in all compositions showed a downregulation of these genes upon cytokine exposure, with no major differences between compositions. This likely reflects the impact of prolonged cytokine exposure (5 days), overwhelming adaptive stress responses [85]. In contrast, encapsulated MIN6 aggregates showed increased expression of mitochondrial-related genes, as well as stress-related genes such as *Foxo1* and *Xbp1*, under cytokine exposure compared to unexposed cells. This suggests an active attempt to adapt to stress. However, this response does not appear to translate into effective adaptive mechanisms, as indicated by higher cell death and reduced functionality in these cells.

A key effect of pLN incorporation in capsules was the significant reduction in MCP-1 release and lower MCP-1 gene expression in human islets after 5 days of cytokine exposure. MCP-1, a pro-inflammatory chemokine, recruits immune cells like monocytes and macrophages to sites of inflammation, contributing to islet inflammation and graft failure [60,86]. Additionally, human islets in pLN-containing capsules exhibited significantly increased IL-6 gene expression under cytokine exposure compared to other capsule compositions. Acute IL-6 signaling in β-cells has been shown to link the antioxidant response to autophagy, helping preserve β-cell mass under oxidative stress [87]. This increased IL-6 expression may represent a protective mechanism to maintain redox balance and reduce β-cell loss [88].

Importantly, the cytokines IL-33, IL-13, MCP1, CCL20, and TSLP were reduced in ECM-containing capsules compared to those released by cells in capsules in alginate-only. Among these, CCL20 exhibited the most considerable decrease. CCL20's is a major immune cell attracting cytokine contributing to β-cell destruction and diabetes development [89]. Additionally, NO production, a major mediator of oxidative stress and inflammation, was also reduced in all ECM-containing compositions. NO is a key factor in cytokine-induced β-cell dysfunction, contributing to oxidative damage and impaired β-cell function [90,91] The lower NO levels observed suggest a protective effect of ECM components, which likely supports the enhanced cell viability seen in these capsules. This highlights the potential of ECM incorporation to mitigate inflammatory and oxidative stress of encapsulated cells.

As a major component of the basement membrane, LN forms a supportive scaffold essential for cellular interactions and tissue organization [92]. The biological effects attributed to laminin depend on the formation of an appropriate supramolecular structure. Poly-laminin, an artificial polymer formed through in vitro LN polymerization, closely mimics the stability and matrix structure of natural membranes found *in vivo* [93,94]. Alg+ChS+pLN, also known as polyLN-Biodritin, has been shown to have a significant potential in attenuating the immune response against implanted microcapsules [47]. Importantly, laminin isoform 111 (L-111) contains the β1 chain, a critical component for

interacting with β1 integrin-containing receptors. These interactions play a pivotal role in mediating cell adhesion, survival, and function, as β1 integrins serve as essential mediators of ECM signaling and structural support in pancreatic β-cells [21,95,96]. However, it is important to note that the use of L-111 may not fully replicate the native ECM conditions of human islets. Future research should explore L-511 or L-521, isoforms naturally expressed in adult human islets, to assess their potential for improved protection and compatibility. This includes investigating their polymerization capacity and impact on islet survival and function.

Previous studies have also demonstrated that ECM incorporation improved β-cell viability and function in alginate microcapsules, which influenced the overall resilience of encapsulated cells in inflammatory environments [25–27]. Previous studies from our group demonstrated through transcriptomic analysis that different LN sequences can influence gene expression and activate various pathways in islets, potentially contributing to improved longevity [28]. This same study revealed that LN treatments resulted in lower expression of inflammatory signals, such as IL-33, an alarm signal released by cells under stress or damage [97]. This finding aligns with our current study, where both LN-based compositions and ChS were shown to reduce IL-33 secretion.

Our data suggests that there may be species-specific differences in the efficacy of ECM components in protecting cells when incorporated into microcapsules. Human islets seem to exhibit greater resistance to pro-inflammatory cytokines compared to MIN6 aggregates as evidenced in our study by lower cell death rates, a lesser decline in cell function over time and differences in gene expression. Species differences in susceptibility of pancreatic cells to injury are well recognized [98,99]. MIN6 cells, due to their transformed nature, may exhibit a distinct integrin profile compared to primary β-cells. While both MIN6 cells and human islets express β1-integrin mRNA and other integrin subunits, with β1-integrin localized on their plasma membranes [45], MIN6 cells, being a transformed cell line, may exhibit a distinct integrin profile compared to primary β-cells. This difference could influence their adhesion properties and responsiveness to ECM signals, potentially contributing to the observed variations in their responses.

5. Conclusion

The ability of encapsulated cells to withstand and adapt to inflammatory stress is essential for the success of cell-based therapies. Incorporating ChS, LN, and pLN into alginate microcapsules offers notable benefits by modulating stress responses and maintaining mitochondrial function, thereby improving the resilience and survival of encapsulated β-cells. These results also underscore the specificity and complexity of ECM proteins in supporting β-cells within a microcapsule environment. Together, these findings demonstrate the potential of ECM-modified microcapsules to enhance the outcomes of pancreatic islet transplantation.

CRediT authorship contribution statement

Isaura Beatriz Borges Silva: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Theo Borghuis:** Writing – review & editing, Validation, Software, Investigation. **Tian Qin:** Writing – review & editing, Validation, Methodology. **Mari Cleide Sogayar:** Writing – review & editing, Supervision, Funding acquisition. **Paul de Vos:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and grammar checking. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2025.101812.

Data availability

Data will be made available on request.

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