

Full length article

Basement membrane proteins improve human islet survival in hypoxia: Implications for islet inflammation

Daniel Brandhorst^{a,b,1,*}, Heide Brandhorst^{a,b,1}, Shannon Lee Layland^c, Samuel Acreman^{a,b}, Katja Schenke-Layland^{c,d,e,f,g}, Paul R.V. Johnson^{a,b}

^a Research Group for Islet Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford OX3 9DU, United Kingdom

^b Oxford Consortium for Islet Transplantation, Oxford Centre for Diabetes, Endocrinology, and Metabolism (OCDEM), Churchill Hospital, University of Oxford, Oxford, United Kingdom

^c Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University, Tübingen, Germany

^d Department of Bioengineering, Eberhard Karls University, Tübingen, Germany

^e Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University, Tübingen, Germany

^f NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

^g Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA



ARTICLE INFO

Article history:

Received 6 May 2021

Revised 6 October 2021

Accepted 7 October 2021

Available online 12 October 2021

Keywords:

Extracellular matrix proteins

Human islet isolation

Hypoxia

Inflammation

Marginal pancreases

ABSTRACT

Enzymatic digestion of the pancreas during islet isolation is associated with disintegration of the islet basement membrane (IBM) that can cause reduction of functional and morphological islet integrity. Attempts to re-establish IBM by coating the surface of culture vessels with various IBM proteins (IBMP) have resulted in loss of islet phenotype and function. This study investigated the capability of Collagen-IV, Laminin-521 and Nidogen-1, utilised as single or combined media supplements, to protect human islets cultured in hypoxia.

When individually supplemented to media, all IBMP significantly improved islet survival and *in-vitro* function, finally resulting in as much as a two-fold increase of islet overall survival. In contrast, combining IBMP enhanced the production of chemokines and reactive oxygen species diminishing all positive effects of individually added IBMP. This impact was concentration-dependent and concerned nearly all parameters of islet integrity.

Predictive extrapolation of these findings to data from 116 processed human pancreases suggests that more than 90% of suboptimal pancreases could be rescued for clinical islet transplantation increasing the number of transplantable preparations from actual 25 to 40 when adding Nidogen-1 to pretransplant culture.

This study suggests that media supplementation with essential IBMP protects human islets from hypoxia. Amongst those, certain IBMP may be incompatible when combined or applied at higher concentrations.

Statement of significance

Pancreatic islet transplantation is a minimally-invasive treatment that can reverse type 1 diabetes in certain patients. It involves infusing of insulin-producing cell-clusters (islets) from donor pancreases. Unfortunately, islet extraction is associated with damage of the islet basement membrane (IBM) causing reduced islet function and cell death. Attempts to re-establish the IBM by coating the surface of culture vessels with IBM proteins (IBMP) have been unsuccessful. Instead, we dissolved the most relevant IBM components Collagen-IV, Laminin-521 and Nidogen-1 in media routinely used for clinical islet culture and transplantation. We found human islet survival and function was substantially improved by IBMP,

* Corresponding author at: Research Group for Islet Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford OX3 9DU, United Kingdom.

E-mail address: daniel.brandhorst@nds.ox.ac.uk (D. Brandhorst).

¹ These authors contributed equally to this work.

particularly Nidogen-1, when exposed to a hypoxic environment as found *in vivo*. We also investigated IBMP combinations. Our present findings have important clinical implications.

© 2021 The Authors. Published by Elsevier Ltd on behalf of Acta Materialia Inc.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Over the past 30 years, human islet allotransplantation has been established as a successful and safe treatment for reversing life-threatening hypoglycaemia unawareness and restoring euglycemia in prone patients with type 1 diabetes mellitus [1]. The outcome of islet allotransplantation alone for selective non-uremic patients has now reached a success rate similar to whole pancreas alone transplantation [2]. Nevertheless, 72% of the islet recipients still require two or more successfully processed islet preparations to receive an islet yield that is sufficient for post-transplant success [3].

The high demand for several pancreases to successfully treat one single patient with diabetes can be partly explained by the harmful techniques that are utilised for the extraction of islets from human donor pancreases. This particularly involves the dissociation of the islet basement membrane (IBM) during pancreas digestion by means of collagenolytic enzyme blends [4,5]. The cleavage of the IBM destroys the integration of islets within the extracellular matrix and interrupts the communication between IBM proteins (IBMP) and islet-expressed integrins [6–9]. The loss of interaction between the external microenvironment and the cellular interior also interrupts the transmission of pro-survival signals that are essential for the morphological as well as functional integrity of isolated islets [10–13]. In combination with the omnipresence of hypoxia, islet isolation finally results in enhanced expression of numerous stress kinases, that subsequently activate pro-inflammatory and pro-apoptotic pathways [14–18].

Nearly all studies that have been undertaken so far, have clearly suggested that IBMP are not re-established during islet culture [5,19–21]. As a result, a significant increase in apoptosis is observed during human islet culture particularly when serum-free media are used [22,23]. Attempts to increase the survival of isolated human islets during culture by coating the surface of culture vessels with various IBMP, have mostly failed, since islets, when attached to the IBMP-treated surface, lose their spherical structure as well as their ability to respond to glucose challenge [24–27].

In this study, we present an innovative approach using the most prominent components of the human IBM as supplements dissolved in culture media to minimise islet attachment to plastic surfaces and to protect cultured human islets from dysfunction and cell death [28,29]. In order to mimic the post-transplant environment of grafted islets, all experiments were performed in hypoxic atmosphere.

2. Materials and methods

2.1. Human islet isolation

All donor pancreases were voluntarily donated with written consent according to the Declaration of Istanbul. Ethical approval for using isolated human islets for research was given by the NHS National Research Ethics Service (09/H0605/2). Pancreases were retrieved from 30 human multi-organ donors with brain death with a mean age of 48.1 ± 1.4 years (\pm standard error) and a mean body mass index of 28.2 ± 0.8 kg/m². All pancreases were preserved with University of Wisconsin solution (Bridge to Life, London, U.K.) for a mean cold ischaemia time of 6.2 ± 0.3 h. The male-to-female ratio of donors was 13 to 17. Human islets were isolated and purified

using standard isolation techniques as previously described in detail [5].

2.2. Human islet culture

After isolation and purification, every islet suspension was divided into aliquots of approximately 300 or 600 islet equivalents (IEQ) according to the number of experimental groups assessed which were placed in 12- or 24-well plates (Greiner Bio-One, Stonehouse, U.K.) and suspended in 500 μ L or 1000 μ L of the culture medium CMRL 1066, respectively. Culture medium was supplemented with 20 mmol/L HEPES, 2 mmol/L L-glutamine, 200 units/mL penicillin, 200 μ g/mL streptomycin (all reagents from Life Technologies, Paisley, United Kingdom), 2.5% fetal calf serum (PAA Laboratories, Pasching Austria) and various IBMP. Islet culture was performed for three to four days in hypoxic atmosphere (1.5% oxygen) prior to islet characterisation.

2.3. Experimental design

In the first part of the study, different Laminin isoforms, namely Laminin-521 (L-521), Laminin-511 (L-511) and Laminin-411 (L-411) were compared for their efficiency to protect human islets from hypoxia-induced damage. Islet aliquots were suspended in CMRL 1066 supplemented as described above, and treated with 10 μ g/mL of either L-521, L-511 or L-411 according to the recommendation of the manufacturer (Biolamina, Stockholm, Sweden). Islets cultured in hypoxia without Laminins or other IBMP served as hypoxic vehicle-treated controls.

The second part of the study was split into two series of experiments. In series 2A, islet aliquots were treated with either Collagen-IV (COL-4, Sigma-Aldrich, Dorset, U.K.), L-521 (Biolamina) or Nidogen-1 (NID-1). Recombinant NID-1 was kindly provided by Professor Schenke-Layland of the Eberhard Karls University Hospital Tübingen (Tübingen, Germany) [30]. IBMP were used either alone or in combination administering a concentration of 80, 10, 25 and 115 μ g/mL for COL-4, L-521, NID-1 and the IBMP combination, respectively. The most suitable dose for COL-4 and NID-1 had been determined in pilot experiments (data not shown). The experiments were repeated in series 2B, using a reduced concentration of 40 μ g/mL COL-4 and 12.5 μ g/mL NID-1 whilst keeping the L-521 concentration constantly at 10 μ g/mL. The experimental design of the study is shown in Fig. 1.

2.4. Islet characterisation

Islet characterisation was performed according to the release criteria for clinical islet transplantation as defined for the U.S. National Institutes of Health (NIH)-sponsored Clinical Islet Transplantation Consortium (CITC) trial [31]. Before and after culture, islet number was quantified as islet particle number (IN) and number of IEQ as previously described in detail [32]. Islet morphological integrity was determined calculating the islet fragmentation index (IN/IEQ) [33]. Islet viability was assessed utilising 0.67 μ mol/L of fluorescein diacetate (FDA, Sigma-Aldrich) and 4.0 μ mol/L of propidium iodide (PI, Sigma-Aldrich) for staining of viable and dead cells, respectively [34]. The fluorescence intensity (FI) of FDA-PI was quantified utilising a fluorometric plate reader as previously described [28]. Islet overall survival was calculated considering the

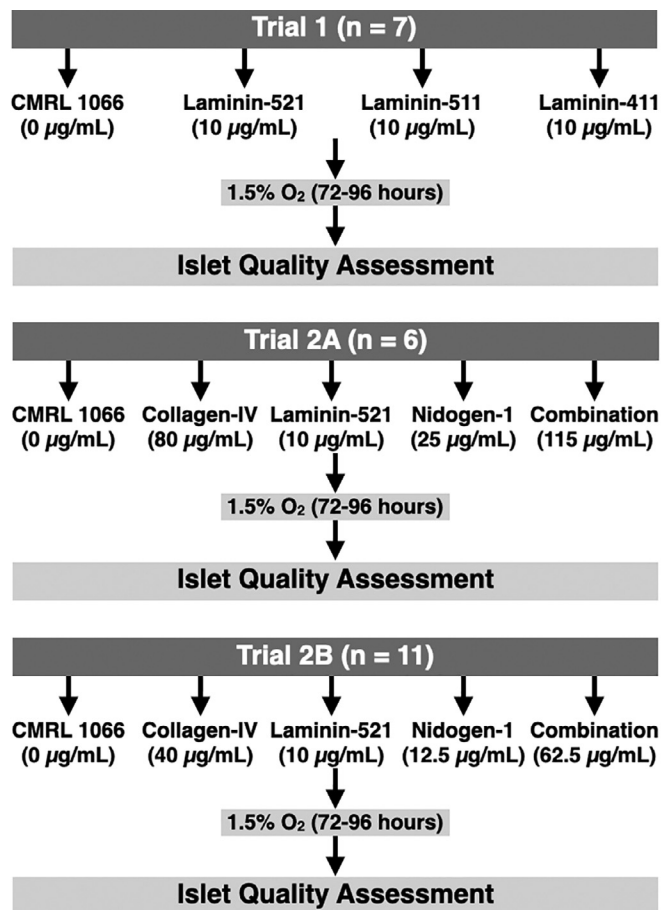


Fig. 1. Experimental study design of various IBMP dissolved in CMRL 1066 medium prior to islet characterisation.

recovery of viable cells only, stained exclusively by FDA and not being penetrated by PI.

In vitro function of 20 hand-picked islets of similar size was assessed in duplicate during static glucose incubation. Islets were seeded on 3 µm-pore size filter inserts, transferred into 24-well plates and sequentially incubated for 45 min in 1 mL of Krebs-Ringer buffer supplemented with 2.0 mmol/L glucose followed by 45 min at 20 mmol/L glucose. After stimulation, islets were recovered and sonified in distilled water prior to insulin extraction in acid ethanol and for subsequent determination of DNA content using the Pico Green assay (Life Technologies, Paisley, U.K.). [35] Secreted insulin was determined utilizing an enzyme immunoassay specific for human insulin (Mercodia, Uppsala, Sweden). The glucose stimulation index (GSI) was calculated dividing the insulin release at 20 mmol/L glucose by insulin secreted during the basal period.

Production of reactive oxygen species (ROS) was determined in duplicate by measuring the intra-islet conversion of dichlorofluorescein diacetate into fluorescent dichlorodihydrofluorescein as previously described in detail [36]. After culture in hypoxic atmosphere, islet-preconditioned supernatants were collected and assessed for secretion of hypoxia- and inflammation-related chemokines. Release of Interleukin-1 beta (IL-1β), IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNFα) and vascular endothelial growth factor A (VEGF-A) was detected utilising enzyme immunoassays specific for human chemokines (Invitrogen/Thermo Fisher, Rochford, United Kingdom). Early apoptosis in hypoxic islets was determined by simultaneous staining with Annexin-V FITC (Becton-Dickinson Biosciences, Oxford, United Kingdom) and PI used at a concentration of 450 ng/mL and 4.0 µmol/L, respectively.

ROS production as well as chemokine release, glucose-stimulated insulin secretion, and expression of early apoptosis markers were measured in duplicate samples and related to islet DNA content measured as described above. In order to avoid sample contamination with DNA released by dead and fragmenting cells every sample was washed twice to carefully remove the supernatant with cell debris and DNA.

2.5. Extrapolation of clinical transplantability

In order to estimate the potential impact of culture media supplementation with IBMP to improve the use of islet preparations for transplantation that fail to fulfil the product release criteria for islet transplant alone, we extrapolated decisive isolation variables measured post-culture during the observation period 2016–2018. The product release criteria were defined as $\geq 300,000$ IEQ of islet yield, $\geq 50\%$ of islet purity and $\geq 70\%$ of islet viability.

Because the variables subjected to extrapolation were normalised to non-treated controls ([post-culture islet survival of simulated treatment groups ÷ post-culture islet survival of non-treated controls] $\times 100$), extrapolation was performed using the geometric mean which is acknowledged as variable to average ratios. The geometric mean served as the multiplier to calculate hypothetically assumed values obtained after simulated i.e. extrapolated protection through NID-1 treatment and was calculated from data collected in series 2B.

2.6. Statistical analysis

Statistical analysis and graphical presentations were performed using Prism 9.0.0 (GraphPad, La Jolla, USA). Analysis of data was carried out using the nonparametric Friedman test followed by Dunn's test for multiple comparisons or by the Wilcoxon test for subsequent insulin release at 2 and 20 mmol/L of glucose. Comparisons between corresponding parameters of experimental series 2A and series 2B were done using the Mann-Whitney test. Correlation analysis was performed calculating the nonparametric Spearman's correlation coefficient (r). Where appropriate, data were normalised to data determined pre-culture or to hypoxic vehicle-treated controls. Differences were considered significant at P less than 0.05. P -values larger than 0.05 were termed nonsignificant (NS). Results are generally expressed as mean \pm standard error (SEM).

3. Results

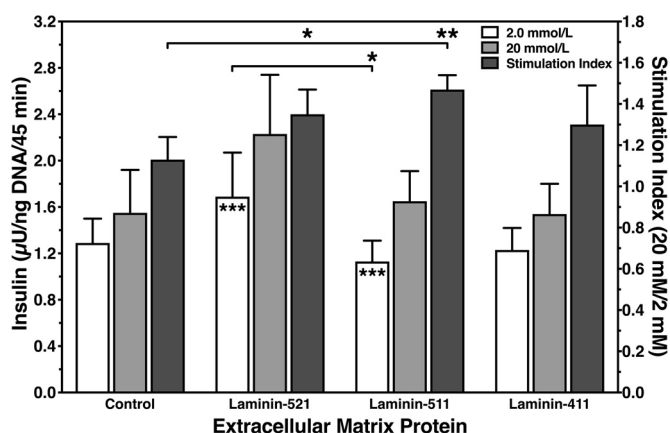
3.1. Comparison of different Laminin isoforms

After 3 – 4 days of culture in hypoxic atmosphere, no cell attachment or cellular spreading on the surface of the culture vessels was observed. Harvesting of free-floating islets could be performed without applying mechanical stress. As shown in Table 1, recovery of human islets was significantly increased by a similar extent when Laminin isoforms had been present during culture ($p < 0.01$ vs hypoxic controls). The islet fragmentation index was doubled by severe hypoxia from 1.0 ± 0.2 as measured pre-culture to 2.4 ± 0.5 in controls ($p < 0.001$). The post-culture fragmentation index could be significantly improved using L-521 ($p < 0.01$ vs controls) or L-511 ($p < 0.05$). In contrast, the effect of L-411 on this variable was statistically not significant and was significantly lower compared with L-521 ($p < 0.05$). The lack of oxygen also substantially reduced pre-culture islet viability from $75 \pm 2\%$ to $49 \pm 4\%$ in controls ($p < 0.001$) which was significantly ameliorated by means of L-521 and L-411 ($p < 0.05$). L-511 supplementation resulted in a higher and statistically significant protective effect ($p < 0.001$ vs controls) (Table 1).

Table 1Effect of different Laminin isoforms (10 µg/mL) on human islet characterisation after 3 – 4 days of culture in hypoxia ($n = 7$).

Laminins	IEQ Yield (%)	Fragmentation (IN/IEQ)	Viability (%)	ROS (FI/ng DNA)	Overall Survival (%)
Control (0 µg/mL)	40 ± 3 (100%)	2.4 ± 0.5 (100%)	49 ± 4 (100%)	36 ± 6 (100%)	20 ± 2 (100%)
L-521 (10 µg/mL)	68 ± 9 ^b (170 ± 22)	1.6 ± 0.4 ^b (66 ± 7)	61 ± 6 ^a (125 ± 4)	14 ± 2 ^a (41 ± 6)	40 ± 5 ^a (213 ± 29)
L-511 (10 µg/mL)	66 ± 5 ^b (166 ± 11)	1.6 ± 0.4 ^a (66 ± 7)	64 ± 7 ^c (130 ± 4)	11 ± 3 ^c (40 ± 7)	41 ± 4 ^b (216 ± 16)
L-411 (10 µg/mL)	68 ± 10 ^b (173 ± 28)	1.8 ± 0.4 ^d (80 ± 11)	61 ± 7 ^a (124 ± 8)	15 ± 3 ^a (46 ± 9)	38 ± 4 ^a (215 ± 38)

Islet yield is normalised to preculture yield; figures normalised to hypoxic vehicle-treated controls are shown in parentheses.

^a $p < 0.05$ ^b $p < 0.01$ ^c $p < 0.001$ vs control^d $p < 0.05$ vs L-521.**Fig. 2.** Glucose-stimulated insulin release after 3 – 4 days of culture in hypoxic atmosphere utilising 10 µg/mL of different Laminin isoforms ($n = 7$). Basal (white bars) and stimulated (grey bars) insulin release of 20 human islets is normalised to ng islet DNA and expressed as glucose stimulation index (black bars). Symbols inside bars indicate *** $p < 0.001$ for 2.0 vs 20 mmol/L of glucose. * $p < 0.05$ for basal insulin release of L-521 vs L-511; ^a $p < 0.05$, ** $p < 0.01$ for GSI of hypoxic vehicle-treated controls vs L-521 and L-511 as indicated.

As anticipated, the responsiveness of hypoxia-exposed islets towards glucose challenge was minimised and resulted in a low GSI in controls (Fig. 2). The presence of L-521 ($p < 0.05$) and L-511 ($p < 0.01$) significantly increased the insulin response after glucose stimulation whilst the protective effect of L-411 was marginal compared with controls (NS).

Another specific characteristic of hypoxia is the increase of mitochondrial ROS generation (Table 1). The presence of laminin isoforms substantially reduced the intra-islet ROS production by approximately 50% when compared with controls ($p < 0.05$). Again, this effect had a higher statistical significance utilising L-511 ($p < 0.001$) than after use of L-521 or L-411.

In order to compare the protective potency of the different Laminin isoforms, islet overall survival was determined by calculating the survival of viable islet cells only (Table 1). Compared with controls, the overall survival of Laminin-treated islets was significantly doubled showing only small differences between the different isoforms. Contrasting the different isoforms with respect to the protective effects on in vitro function, overall survival and to financial costs, it was decided to use L-521 for the subsequent experimental series.

3.2. Assessment of various islet basement membrane proteins

3.2.1. High islet basement membrane protein concentration (series 2A)

In the second part of the study, we compared members of different extracellular matrix protein families. We selected COL-4, L-521 and NID-1 and used these individually or in combination. As observed in the Laminin isoform study, no cell attachment was observed in any of the experimental groups. As noted in the first part of the study, L-521 significantly increased post-culture islet yield compared with controls ($p < 0.01$) as shown in Table 2A. The other IBMP, such as COL-4 ($p < 0.05$) or NID-1 ($p < 0.01$) enhanced recovery of islets in a hypoxic environment to a nearly identical extent. When the IBMP were combined, harvesting of islets resulted in a significantly lower outcome ($p < 0.05$ vs COL-4; $p < 0.01$ vs L-521, NID-1) compared with individually used IBMP. A similar observation was made analysing the fragmentation index after culture. When COL-4, L-521 and NID-1 had been combined, the protective effect of the individual IBMP was completely diminished, resulting in a fragmentation index equal to or larger than that of controls (Table 2A).

The finding that a combination of different IBMP has detrimental effects on islet morphology, applied also to intra-islet ROS production. As demonstrated in Table 2A, a substantial reduction of ROS generation was measured comparing individually used IBMP with controls ($p < 0.01$ vs COL-4; $p < 0.05$ vs NID-1). Among the IBMP applied, L-521 was characterised by the lowest ROS production measured ($p < 0.001$). In contrast, the ROS generation was significantly stimulated, when COL-4 ($p < 0.05$ vs combination), L-521 ($p = 0.001$) and NID-1 ($p < 0.05$) had been combined.

Accordingly, the low viability of controls was significantly increased when COL-4 ($p < 0.05$), L-521 ($p < 0.05$) or NID-1 ($p < 0.001$) had been dissolved in the culture medium. Again, combining these IBMP lowered islet viability to the level of controls. Likewise, the binding of the early apoptosis marker Annexin-V in controls was significantly reduced in the presence of COL-4 ($p < 0.05$), L-521 ($p < 0.01$) or NID-1 ($p < 0.01$) when used alone, but was maintained on a relatively high level after administration of combined IBMP ($p < 0.05$ vs COL-4; $p < 0.01$ vs L-521, NID-1). A nearly identical pattern was observed for overall survival, which was more than doubled when hypoxic islets were treated with individual COL-4 ($p < 0.05$), L-521 ($p < 0.05$) or NID-1 ($p < 0.01$) and compared with controls or with the combination group.

As was to be expected, an insulin response towards glucose challenge was not present in controls resulting in a GSI of less than 0.8 (Fig. 3A). Islet functional capacity could be significantly re-established when COL-4 ($p < 0.01$), L-521 ($p < 0.001$) or NID-1

Table 2A

Effect of high IBMP concentrations on human islet characterisation after 3 – 4 days of culture in hypoxia (n = 6).

IBM Proteins	IEQ Yield (%)	Fragmentation (IN/IEQ)	ROS (FI/ng DNA)	Viability (%)	Early Apoptosis (FI/ng DNA)	Overall Survival (%)
Control (0 µg/mL)	25 ± 6 (100%)	0.8 ± 0.1 (100%)	65 ± 20 (100%)	47 ± 5 (100%)	56 ± 32 (100%)	16 ± 4 (100%)
COL-4 (80 µg/mL)	48 ± 11 ^{a,d} (198 ± 24)	0.7 ± 0.1 ^{a,d} (85 ± 4)	23 ± 7 ^{b,d} (35 ± 5)	59 ± 2 ^a (135 ± 15)	31 ± 20 ^{a,d} (53 ± 8)	38 ± 7 ^{a,d} (262 ± 32)
L-521 (10 µg/mL)	50 ± 10 ^{b,e} (242 ± 66)	0.6 ± 0.1 ^{c,f} (72 ± 5)	15 ± 6 ^{c,f} (28 ± 7)	57 ± 4 ^a (127 ± 9)	24 ± 13 ^{b,e} (54 ± 12)	38 ± 6 ^{a,d} (302 ± 83)
NID-1 (25 µg/mL)	50 ± 10 ^{b,e} (228 ± 53)	0.7 ± 0.1 ^d (89 ± 5)	30 ± 10 ^{a,d} (39 ± 7)	64 ± 4 ^{c,f} (146 ± 14)	31 ± 19 ^{b,e} (56 ± 7)	43 ± 6 ^{b,e} (332 ± 86)
Combination (115 µg/mL)	30 ± 10 (116 ± 15)	0.8 ± 0.1 (104 ± 4)	65 ± 24 (95 ± 16)	48 ± 6 (104 ± 4)	46 ± 24 (95 ± 7)	18 ± 6 (120 ± 16)

Islet yield is normalised to preculture yield; figures normalised to hypoxic vehicle-treated controls are shown in parentheses.

^a $p < 0.05$

^b $p < 0.01$

^c $p < 0.001$ vs control

^d $p < 0.05$

^e $p < 0.01$

^f $p = 0.001$ vs combination.

($p < 0.001$) were present in the hypoxic environment. Calculation of the GSI of the combination group revealed that these islets had a significantly lower GSI than those treated with L-521 and NID-1. As demonstrated in Fig. 3A, the magnitude of the insulin response was similar in all treatment groups with the exception of the combination group.

As shown in Table 3A, a significant accumulation of various chemokines was detected in the culture medium of human islets cultured over a period of 3 – 4 days at 1.5% oxygen. In agreement with previous human islet studies [18], IL-8 was the cytokine that substantially exceeded the production of the other chemokines. Despite the huge variability between the secretion of these pro-inflammatory mediators, it was found that the presence of single IBMP substantially reduced the production of TNF α , IL-1 β , IL-6, and MCP-1 by almost one third or even more. Out of all IBMP assessed, NID-1 consistently reduced cytokine release to the largest extent. In congruence with the other variables presented, no significant reduction of chemokine release could be observed when IBMP were used in combination (Table 3A). Remarkably, the reduction of islet chemokine production did not only concern pro-inflammatory mediators, it also applied to pro-angiogenic factors such as IL-8 and VEGF-A (Table 3A).

The close linkages between the different chemokines that were assessed is confirmed by matrix correlation analysis which revealed a correlation coefficient of $r = 0.83, 0.95, 0.95$, and 0.89 between TNF- α and IL-1 β , IL-6, IL-8, and VEGF-A ($p < 0.001$), respectively. Although the correlation coefficient of MCP-1 was lower compared with the other chemokines, it was still highly significant ($r = 0.66, p < 0.001$). When the chemokines were correlated with ROS, the coefficient ranged from $r = 0.76$ for MCP-1 ($p < 0.001$) to $r = 0.93$ for IL-6 ($p < 0.001$).

The impact of chemokine production on human islet survival is displayed in Fig. 4A utilising exemplarily the release of TNF- α as a central component of the chemokine network. As expected, a strong inverse correlation was found for islet yield ($r = -0.75, p < 0.001$) whilst early apoptosis was positively correlated with TNF- α ($r = 0.83, p < 0.001$). In contrast, no correlation was noted when TNF- α was opposed to islet viability as measured with the FDA-PI assay suggesting a pro-apoptotic rather than a pro-inflammatory effect of secreted chemokines.

3.2.2. Reduced islet basement membrane protein concentration (series 2B)

In this part of the study, series 2A was repeated by decreasing the concentration of COL-4 and NID-1 from 80 to 40 µg/mL and

from 25 to 12.5 µg/mL, respectively. L-521 concentration was kept constant in both series. The total concentration of the IBMP combination was thereby reduced from 115 in series 2A to 62.5 µg/mL in series 2B. As noted in series 2A, no cell attachment was observed in any of the experimental groups and islet yield was almost doubled by IBMP when used individually (Table 2B). In contrast to series 2A, where we applied nearly the twofold IBMP concentration, combined IBMP significantly increased islet yield ($p < 0.001$ vs controls; $p < 0.01$ vs series 2A). A similar finding was made with respect to islet fragmentation ($p < 0.001$ vs controls) and islet overall survival ($p < 0.001$ vs controls; $p < 0.01$ vs series 2A). As demonstrated in Fig. 3B, islet in vitro function was significantly improved as well, when islets were treated with the lower amount of combined IBMP ($p < 0.05$ vs series 2A). The binding of Annexin-V followed the same pattern as observed in series 2A, showing a significantly reduced early apoptosis for islets treated with COL-4 ($p < 0.05$ vs controls), L-521 ($p < 0.05$) or NID-1 ($p < 0.001$), but no improvement when IBMP were combined at a reduced dosage (Table 2B). When compared with the other individually used IBMP, NID-1 reduced early apoptosis to the largest extent ($p < 0.05$). The amelioration of islet morphological and functional integrity clearly correlated with a massively reduced ROS production in the combination group (Table 2B, $p < 0.01$ vs series 2A).

As shown in Table 3B, the concentration of accumulated chemokines produced by controls was in a similar range when compared with series 2A. Nevertheless, a trend towards a decreased chemokine production was observed for the halved concentration of NID-1 (NS vs series 2A) resulting in the largest inhibitory effect on islet chemokine release amongst all individually administered IBMP (Table 3B). Likewise, using the combination at a reduced concentration, lowered the production of every chemokine assessed to a similar level as measured for individually used IBMP.

Matrix correlation analysis revealed a tight correlation between TNF- α and the other chemokines analysed. With the exception of IL-1 β ($r = 0.37, p = 0.072$) and VEGF-A ($r = 0.92, p < 0.001$), the correlation coefficient for IL-6, MCP-1 and IL-8 varied in a narrow range of $r = 0.81, r = 0.83$ and $r = 0.87$ ($p < 0.001$), respectively. The substantially reduced production of ROS in the combination group and in NID-1 treated islets of series 2B was associated with a slightly weaker correlation between ROS and TNF- α ($r = 0.69, p < 0.001$), IL-1 β ($r = 0.43, p < 0.05$), IL-6 and IL-8 ($r = 0.52, p < 0.01$), MCP-1 ($r = 0.53, p < 0.01$) or VEGF-A ($r = 0.70, p < 0.001$).

Table 2B

Effect of reduced IBMP concentrations on human islet characterisation after 3 – 4 days of culture in hypoxia (n = 11).

IBM Proteins	IEQ Yield (%)	Fragmentation (IN/IEQ)	ROS (FI/ng DNA)	Viability (%)	Early Apoptosis (FI/ng DNA)	Overall Survival (%)
Control (0 µg/mL)	41 ± 7 (100%)	1.0 ± 0.2 (100%)	55 ± 7 (100%)	43 ± 4 (100%)	33 ± 6 (100%)	19 ± 4 (100%)
COL-4 (40 µg/mL)	64 ± 7 ^c (198 ± 40)	0.7 ± 0.1 ^c (75.4 ± 3.5)	17 ± 4 ^{d,e} (32 ± 7)	58 ± 5 ^b (136 ± 7.8)	20 ± 6 ^{a,e} (56 ± 11)	39 ± 6 ^c (274 ± 58)
L-521 (10 µg/mL)	57 ± 6 ^b (162 ± 20)	0.7 ± 0.1 ^b (77 ± 6)	15 ± 4 ^{d,e} (29 ± 8)	55 ± 5 ^b (127 ± 9)	19 ± 3 ^{a,e} (65 ± 8)	33 ± 6 ^a (201 ± 21)
NID-1 (12.5 µg/mL)	65 ± 6 ^c (208 ± 44)	0.8 ± 0.2 ^b (80 ± 4)	5 ± 1 ^c (9 ± 1)	58 ± 4 ^c (137 ± 8)	13 ± 4 ^c (36 ± 6)	38 ± 5 ^c (302 ± 80)
Combination (62.5 µg/mL)	61 ± 7 ^c (174 ± 22)	0.7 ± 0.1 ^c (78 ± 4)	6 ± 2 ^c (14 ± 5)	57 ± 4 ^c (135 ± 5)	27 ± 7 ^f (84 ± 13)	36 ± 5 ^c (245 ± 39)

Islet yield is normalised to preculture yield; figures normalised to hypoxic vehicle-treated controls are shown in parentheses.

^a *p* < 0.05^b *p* < 0.01^c *p* < 0.001 vs control^d *p* < 0.05 vs combination^e *p* < 0.05^f *p* < 0.01 vs NID-1.**Table 3A**

Effect of high IBMP concentrations on islet chemokine production (ng/ng DNA) during 3 – 4 days of culture in hypoxia (n = 6).

IBM Proteins	TNF-α	IL-1β	IL-6	IL-8	MCP-1	VEGF-A
Control (0 µg/mL)	0.6 ± 0.2 (100%)	1.4 ± 0.5 (100%)	21.0 ± 6.4 (100%)	145.5 ± 45.9 (100%)	16.4 ± 7.7 (100%)	47.6 ± 14.5 (100%)
COL-4 (80 µg/mL)	0.3 ± 0.1 ^a (68 ± 16)	0.9 ± 0.3 ^b (62 ± 7)	10.5 ± 3.3 ^{b,c} (55 ± 7)	65.0 ± 21.0 ^{b,c} (50 ± 6)	9.7 ± 4.6 ^a (64 ± 10)	21.5 ± 6.8 ^{b,c} (50 ± 8)
L-521 (10 µg/mL)	0.3 ± 0.2 (63 ± 12)	0.8 ± 0.3 ^a (63 ± 9)	10.6 ± 3.5 ^{a,c} (59 ± 10)	71.8 ± 25.3 (61 ± 12)	7.7 ± 3.5 ^a (59 ± 9)	19.0 ± 5.6 ^{b,c} (49 ± 10)
NID-1 (25 µg/mL)	0.3 ± 0.1 ^a (51 ± 13)	0.8 ± 0.3 ^b (59 ± 8)	11.6 ± 4.0 ^{b,d} (54 ± 7)	77.2 ± 28.8 ^{b,c} (51 ± 8)	9.2 ± 5.1 ^b (56 ± 8)	20.2 ± 6.0 ^{b,c} (45 ± 5)
Combination (115 µg/mL)	0.4 ± 0.1 (84 ± 17)	1.1 ± 0.4 (88 ± 9)	17.1 ± 5.1 (85 ± 11)	111.6 ± 33.5 (83 ± 13)	15.6 ± 10.1 (78 ± 15)	32.0 ± 9.5 (75 ± 14)

Figures normalised to hypoxic vehicle-treated controls are shown in parentheses.

^a *p* < 0.05^b *p* < 0.01 vs control^c *p* < 0.05^d *p* < 0.01 vs combination.**Table 3B**

Effect of reduced IBMP concentrations on islet chemokine production (ng/ng DNA) during 3 – 4 days of culture in hypoxia (n = 5).

IBM Proteins	TNF-α	IL-1β	IL-6	IL-8	MCP-1	VEGF-A
Control (0 µg/mL)	0.7 ± 0.2 (100%)	1.0 ± 0.5 (100%)	27.0 ± 14.1 (100%)	160.3 ± 67.7 (100%)	24.1 ± 14.2 (100%)	47.9 ± 15.3 (100%)
COL-4 (40 µg/mL)	0.3 ± 0.1 ^{a,c} (47 ± 13)	0.7 ± 0.4 ^{a,c} (47 ± 13)	18.2 ± 8.9 ^{a,c} (54 ± 14)	99.4 ± 44.9 ^{a,c} (58 ± 12)	18.4 ± 13.2 ^{a,c} (52 ± 15)	24.9 ± 9.9 ^a (49 ± 11)
L-521 (10 µg/mL)	0.3 ± 0.1 ^{a,c} (47 ± 11)	0.7 ± 0.4 ^{a,c} (48 ± 12)	14.1 ± 5.8 ^{a,c} (51 ± 12)	80.9 ± 29.3 ^{a,c} (51 ± 14)	10.6 ± 5.4 ^{a,c} (46 ± 6)	22.6 ± 7.4 ^{a,c} (50 ± 12)
NID-1 (12.5 µg/mL)	0.2 ± 0.1 ^b (36 ± 11)	0.6 ± 0.4 ^b (39 ± 11)	9.3 ± 3.6 ^b (36 ± 11)	59.5 ± 23.0 ^b (40 ± 14)	7.0 ± 3.4 ^b (30 ± 8)	15.1 ± 6.3 ^b (35 ± 12)
Combination (62.5 µg/mL)	0.3 ± 0.1 ^{a,c} (53 ± 12)	0.6 ± 0.4 ^{a,c} (50 ± 10)	16.6 ± 7.4 ^{a,c} (53 ± 15)	87.0 ± 36.1 ^a (51 ± 1)	11.9 ± 6.2 ^{a,c} (49 ± 10)	24.6 ± 9.3 ^c (51 ± 12)

Figures normalised to hypoxic vehicle-treated controls are shown in parentheses.

^a *p* < 0.05^b *p* < 0.001 vs control^c *p* < 0.05 vs NID-1.

As demonstrated in Fig. 4B, the accumulated release of TNF-α in series 2B was reduced by approximately 30% whilst the dimension of early apoptosis reached approximately 25% when compared to series 2A (Fig. 4A). As a consequence, no significant correlation was detected when TNF-α and islet yield were analysed (*r* = -0.31, NS). In agreement with series 2A, TNF-α and viability were not significantly correlated (*r* = 0.13, NS), whilst a strong correlation between TNF-α and early apoptosis was calculated (*r* = 0.88, *p* < 0.001) (Fig. 4B). These findings suggest again that chemokine

production in hypoxic human islets is associated with apoptosis rather than with inflammation.

3.3. Extrapolation of clinical transplantability

In order to predict the potential translation of culture media supplementation with IBMP into a clinical setting, we extrapolated decisive isolation variables collected during the observation period 2016–2018 particularly focussing on NID-1. This period cov-

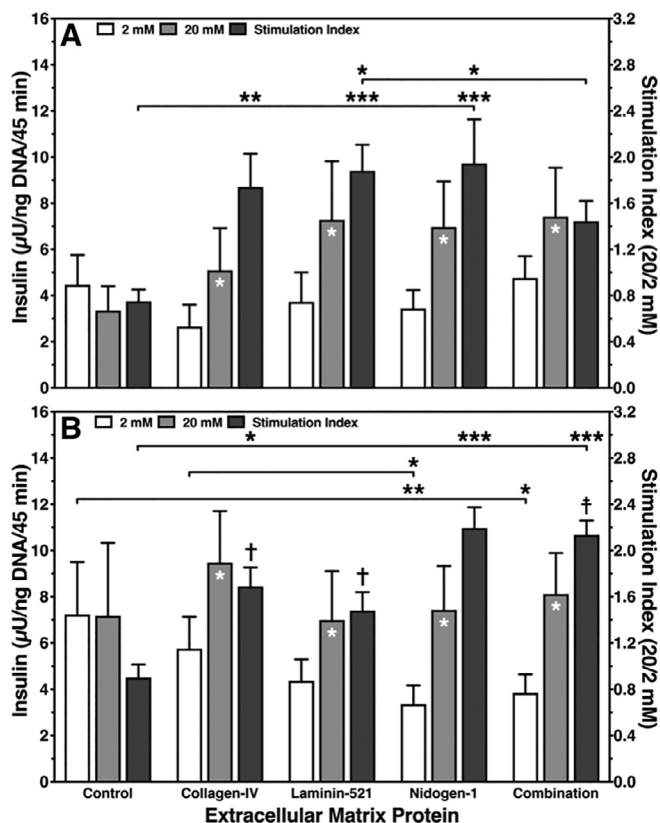


Fig. 3. Glucose-stimulated insulin release after 3–4 days of culture in hypoxic atmosphere utilising a high (A, series 2A, $n = 6$) or reduced (B, series 2B, $n = 11$) concentration of COL-4 and NID-1. Basal (white bars) and stimulated (grey bars) insulin release of 20 human islets is normalised to ng islet DNA and expressed as glucose stimulation index (GSI, black bars). Symbols inside bars indicate $p < 0.05$ for 2.0 vs 20 mmol/L of glucose. (A) $*p < 0.05$ for stimulation index of combination vs L-521 and NID-1; $**p < 0.01$, $***p < 0.001$ for GSI of hypoxic vehicle-treated controls vs COL-4, L-521 and NID-1. (B) $*p < 0.05$, $**p < 0.01$ for basal insulin release as indicated; $*p < 0.05$, $***p < 0.001$ for GSI of hypoxic vehicle-treated controls vs COL-4, NID-1 and combination. $\dagger p < 0.05$ for GSI of COL-4 and L-521 vs NID-1; $\ddagger p < 0.05$ for GSI of combination vs L-521.

ered the processing of 116 human pancreases in total. Islet preparations that had been retrieved from donors after cardiac death (DCD) or that had been transplanted were excluded from further analysis. The product release criteria for clinical islet transplantation were $\geq 300,000$ IEQ of islet yield, $\geq 50\%$ of islet purity and $\geq 70\%$ of islet viability. Seventeen of the remaining islet preparations were cultured for subsequent clinical islet transplantation but failed to completely fulfil the product release criteria as assessed post-culture due to a reduction of islet yield and/or decrease of islet viability.

Simulated culture media supplementation with NID-1 increased islet yield from $237,140 \pm 19,570$ IEQ ($n = 17$), as actually measured post-culture, to $417,370 \pm 34,440$ IEQ after simulated NID-1 treatment ($p < 0.001$). At the same time, post-culture islet viability increased from actually $70 \pm 3\%$ to extrapolated $89 \pm 2\%$ ($p < 0.001$). As a result, the number of suboptimal islet preparations, that were eligible for clinical islet transplantation, could be increased from actually 0 to 16 out of 17 which is equivalent to a proportional increase of 94.1% ($p < 0.001$ by Fisher's Exact test). One out of 17 islet preparations (5.9%) still failed to fulfil product release criteria after extrapolation. Considering the total number of 80 marginal and suboptimal islet preparations processed during the observation period, the number of islet preparations suitable for islet allotransplantation could be increased by simulated NID-

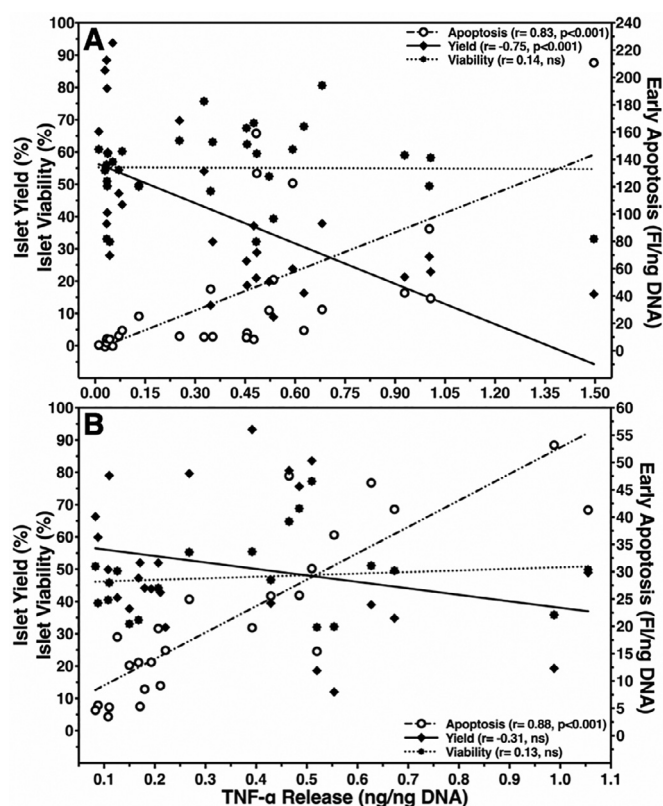


Fig. 4. Effect of islet TNF- α production in hypoxic atmosphere on post-culture islet yield (\blacklozenge), viability (\bullet) (left y-axis) and early apoptosis (\circ) (right y-axis) applying a high (A, series 2A, $n = 30$) or reduced (B, series 2B, $n = 25$) concentration of COL-4 and NID-1. The correlation coefficient (r) was calculated using Spearman's rank correlation.

1 treatment from a proportion of actual 0 to 20% ($p < 0.001$ by Fisher's Exact test).

4. Discussion

Pancreatic islet isolation essentially requires the separation of the hormone-producing cell clusters from the non-endocrine exocrine matrix of the pancreas. This process intrinsically involves the cleavage of the IBM, the most proximal extracellular structure for islets, by means of specific enzymes [4,5]. In addition to the mechanical and structural peri-islet support that is lost when the IBM is enzymatically dissociated, numerous junctions transferring pro-survival signals from the out- to the inside of islet cells are disconnected [6–13,20]. These pro-survival signals are transmitted via binding of IBM components to specific integrins located at the surface of islet cells [37]. For that reason, human islet isolation, even when performed according to currently established clinical standards, is associated with the induction of pro-inflammatory and pro-apoptotic pathways in isolated islets [14–17]. Growth factors, such as VEGF, are certainly amongst the most relevant mediators to trigger protective pathways, particularly when islets are exposed to the non-physiological hypoxic conditions present during and after the isolation procedure [38]. It has been shown that several extracellular matrix proteins such as Fibronectin, Perlecan, Laminin or Nidogen have binding domains for VEGF and other growth factors or chemokines [37,39–41].

Nearly all of previously published attempts to increase survival of isolated human islet cells by re-establishing the viable interaction between beta cells and the extracellular matrix, have used surface treatment of the islet microenvironment with different IBMP.

Experiments with isolated mouse islets have revealed that the utilisation of soluble Laminin or Fibronectin is superior to IBMP coating to preserve glucose-stimulated insulin release after 48 hours of cytokine incubation [42]. However, IBMP-coating of culture vessels results in the attachment of islets to the plastic surfaces which essentially requires enzyme treatment or mechanical scraping for subsequent tissue harvesting. In addition to these harmful manipulations, which can increase islet cell death [43], the strategy of surface-coating also incorporates the significant risk that attached islet cells de-differentiate and lose their specific phenotype [25–27,44]. In order to find a feasible solution to this problem, our initial study investigated the protective capability of the most prominent components of the human IBM when used as supplements dissolved in culture media. Indeed, neither attachment nor spreading was observed in our study, when human islets were cultured for several days in the presence of dissolved IBMP.

The protective potency of different IBMP was assessed by exposing isolated islets to hypoxia for a prolonged period of time to mimic the situation during and after islet processing for clinical transplantation. In support of the findings of our study, it has previously been shown that hypoxic islets generate large amounts of cytokines that significantly contribute to mitochondria dysfunction resulting in a massive production of ROS [45–48], which, in turn, mediate oxidation of enzymes and structural proteins as well as DNA fragmentation and apoptosis [49–55]. This has significant implications for human islet secretory capacity [56,57] and for islet post-transplant function [58,59]. The present study clearly demonstrates that individually added COL-4, L-521 and NID-1 reduced the chemokine and the ROS production in hypoxic islets. The reduction of pro-inflammatory mediators as well as the increased morphological integrity and viability of islets appeared to be slightly more pronounced when the reduced concentration of COL-4, L-521 and NID-1 was applied. Surprisingly, when COL-4, L-521 and NID-1 had been combined at the higher concentration, the inhibitory effect of individually applied IBMP on human islet chemokine and ROS production as well as on islet loss and cell death was completely diminished. By contrast, when the concentration of the combined IBMP was reduced, chemokine and ROS production as well as islet loss decreased markedly whilst the proportion of viable cells raised to the level of individually used COL-4, L-521 and NID-1.

The decrease in chemokine production that was mediated in the present experiments by individual IBMP, does not only concern pro-inflammatory chemokines such as TNF- α , IL-1 β , IL-6 or MCP-1, but also pro-angiogenic factors such as IL-8 and VEGF-A. IL-8 plays an ambivalent role in islet transplantation as it is involved, together with MCP-1, in pro-inflammatory pathways in transplanted islets contributing to early loss of islet viability and posttransplant function [58]. In addition, IL-8 belongs to the group of pro-angiogenic chemokines that can stimulate neovascularisation in isolated islets [60]. Even more concerning is the IBMP-mediated reduction of VEGF-A which is decisive factor for revascularisation of islets [61,62]. Apart from its role as pro-angiogenic factor, VEGF seems to have an islet-protective effect that is independent of revascularisation and contributes to human islet survival under adverse conditions [63]. Like other chemokines VEGF-A is regulated by TNF- α , which plays a central and dominant role within the complex chemokine network [46,64]. Since ROS have been identified as essential signalling molecules for TNF- α activities, any reduction in ROS production affects the TNF- α -induced expression of downstream cytokines [45,46,64–67] including VEGF-A [68–71]. Our data confirm the tight and significant correlation between these pro-oxidant agents and all chemokines assessed. In fact, experiments in rat mesangial cells revealed that Fibronectin or the addition of COL-IV combined with Laminin influence mitochondrial function and decrease apoptosis in cells exposed to an

adverse environment [72,73]. Those experiments, which could be replicated in isolated rat islets [12], are in contradiction to the data we have collected in human islets. These indicate that the binding of Annexin-V remained on a relatively high level irrespective which IBMP concentration was applied. Our statistical analysis revealed a strong correlation between TNF- α and apoptosis, whilst no correlation could be detected between this cytokine and islet viability, thus underlining the dominant impact of chemokine-controlled pro-apoptotic pathways on islets subjected to hypoxic stress. In support of our observations, it was previously demonstrated, that an inactivation of ROS by antioxidants can inhibit TNF- α mediated necrotic cell death, but is inefficient to reduce TNF- α -triggered apoptosis [74]. These previous findings, combined with our present observations, suggest that chemokines can induce apoptotic cell death in islets without involving ROS as mediators or effectors [52,75].

To date, the vast majority of previous approaches to restore the IBM for human islet cells assessed the effect of individual IBMP [24–27] and did not consider that IBM supra-structures, composed of L-511, COL-4 and NID-1 [76–78], represent the most relevant structural and functional constructs of the native human islet microenvironment [9,79]. In this context it is important to underline, that L-511, as the only Laminin isoform present in the human IBM [80], could be replaced by L-521 without experiencing any disadvantageous effects on islet morphological and functional integrity. Nevertheless, one of the very few islet studies, that have assessed combinations of IBMP, supported the findings of our present experiments. Microencapsulated human islets suffered from a dose-dependent toxicity of IBMP when presumably incompatible IBMP combinations had been incorporated into the encapsulation material [81,82]. Similarly, silk-encapsulated mouse islets did not benefit from a combination of Laminin and COL-4 when compared to individually incorporated IBMP [83]. These findings can be specifically attributed to the ratio between different IBMP when integrated in microcapsules [84] and may be even more important than the absolute concentration of IBMP such as assessed for COL-6 [85].

These previous experiments suggest that the most suitable ratio between different IBMP appears to be crucial for assembling biocompatible scaffolds and microencapsulation devices focussing on optimisation of posttransplant islet survival [86]. It is therefore remarkable that IBMP implementation has not been considered so far to improve vascularisation of immunoprotective devices to overcome the inadequate supply with oxygen and nutrients as the overall limiting factor for long-term transplant function of encapsulated islets [87]. This is even more striking as Laminin, COL-4 and NID-1, which had already been assessed for rodent islet encapsulation [88], are the major components of the IBM around human islets which may offer the option to stimulate angiogenesis or attract vessels to attach to devices [77].

Very recently, Santini-González et al. described in detail the biochemical conditions that are essential for self-assembly of Laminin, Nidogen and COL-4 at the surface of rat and human islets thereby providing a useful protocol for the re-establishment of the IBM in vitro [89]. It was observed that a network of several IBMP can only be assembled at the islet cell surface when Laminin is present as an anchor point for binding of additional IBMP. However, while this study is scientifically very interesting, the Laminin and Nidogen had been sourced from murine tumor tissue, which is known to have a significant batch-to-batch variability, that makes translating this precise method into a clinical setting rather difficult [90,91]. In addition, two of the three components, that are used for IBM re-assembly, are provided only as an already linked combination what impedes to attribute the individual roles of Laminin or Nidogen within the re-assembly process as described [89].

Despite the importance of these attempts for engineering a bio-compatible environment for islet engraftment, these experiments do not explore the complexity of the natural intra- and peri-islet extracellular matrix. In a recent and comprehensive proteome study a total of 67 matrisome and matrisome-associated proteins could be identified within a total of 613 proteins detected in pancreases from human organ donors. Of those, 10 proteins were found in basement membranes [92]. In decellularized human pancreases 120 proteins, that are related or associated to the human pancreatic matrisome, could be detected [93]. An even higher number of 185 proteins could be most recently identified and attributed to the human pancreatic extracellular matrix [94]. Unfortunately, the peri-islet environment or IBMP were not detailly analysed in these studies. For that reason we have to refer to experiments with mouse islets that identified around 120 proteins as members of the matrisome or as matrisome-associated proteins [95].

Comparing the effects of individually applied compounds, the present study clearly revealed that NID-1 expresses the largest protective capacity amongst all IBMP assessed in our study. This is in agreement with the widely accepted specificity of IBMP where Laminin and COL-4 isoforms provide mainly mechanical structural support whereas Fibronectin, Perlecan and Nidogen primarily mediate cellular signaling via growth factors or chemokines [96–98].

The relevance of our study to clinical islet transplantation was estimated using extrapolation as a tool to predict the potential of culture media supplementation with IBMP. Focussing on NID-1 treatment of cultured human islets, the simulation revealed a substantial increase of conversion of suboptimal islet preparations during culture by over 90% to transplantation. When the total number of marginal donor pancreases processed during the observation period were considered, we calculate that the proportion of preparations eligible for clinical islet allotransplantation could be enhanced from a proportion of actual 0% to 20%. These observations may not only be important to increase the utilisation rate of retrieved donor pancreases but also for islet post-transplant function. Analysis in a large number of islet transplant recipients performed by the CITR demonstrated a clear positive correlation between fasting C-peptide levels in the patients and islet quality in terms of viability and yield [3].

5. Conclusion

To the best of our knowledge, the present study is the first one to investigate the protection of human islets exposed to hypoxia by means of several IBMP, used as single or combined culture media supplements. When dissolved individually, COL-4, L-521 and NID-1 significantly improved islet yield, viability and *in vitro* function, finally resulting in a two-fold increase of islet overall survival in hypoxic atmosphere. In contrast, combining these IBMP enhanced the production of numerous chemokines and ROS diminishing all positive effects of individually added IBMP. The harmful impact of combining IBMP was concentration-dependent and could be avoided by reducing the IBMP concentration in the culture media. As shown by our predictive extrapolation, a substantial proportion of islet preparations currently not being transplanted, could potentially be converted from suboptimal into transplantable preparations, thereby significantly increasing the utilisation rate of donor pancreases for islet transplantation. Overall, this study confirms that the supplementation of culture media with IBMP protects hypoxic human islets from chemokine- and ROS-mediated inflammation. Nevertheless, pro-apoptotic pathways remained to be active in IBMP-treated islets on a significant level, without involving ROS as mediators of chemokine activities. Further studies are required to identify presumably incompatible IBMP combinations.

Funding

Isolation of human islets for research was supported by the Oxford NIHR Biomedical Research Centre Theme and a Juvenile Diabetes Research Foundation (JDRF) award to P.R.V.J. (31-2008-617). The views expressed are those of the authors and not necessarily those of the NHS, NIHR or the Department of Health. Members of the Oxford islet isolation team are funded by the Diabetes Research and Wellness Foundation (DRWF). The study was supported by grants from the European Union's Horizon 2020 (645991).

Availability of data

Data are available from the authors on request.

Declaration of Competing Interest

The authors of this manuscript have no conflict of interest to disclose as defined by *Acta Biomaterialia*.

CRediT authorship contribution statement

Daniel Brandhorst: Visualization, Investigation, Data curation, Writing – original draft. **Heide Brandhorst:** Visualization, Investigation, Data curation, Writing – original draft. **Shannon Lee Layland:** Resources, Writing – review & editing. **Samuel Acreman:** Investigation. **Katja Schenke-Layland:** Resources, Writing – review & editing. **Paul R.V. Johnson:** Investigation, Writing – review & editing.

Acknowledgements

The authors would like to thank all past and present members of the Oxford Human Islet Isolation Team for isolating human islets for research purposes in the DRWF Human Islet Isolation Facility in Oxford.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.actbio.2021.10.013](https://doi.org/10.1016/j.actbio.2021.10.013).

References

- [1] B.J. Hering, W.R. Clarke, N.D. Bridges, T.L. Eggerman, R. Alejandro, M.D. Bellin, K. Chaloner, C.W. Czarniecki, J.S. Goldstein, L.G. Hunsicker, D.B. Kaufman, O. Korsgren, C.P. Larsen, X. Luo, J.F. Markmann, A. Naji, J. Oberholzer, A.M. Posselt, M.R. Rickels, C. Ricordi, M.A. Robien, P.A. Senior, A.M.J. Shapiro, P.G. Stock, N.A. Turgeon, Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia, *Diabetes Care* 39 (7) (2016) 1230–1240.
- [2] S. Moassesfar, U. Masharani, L.A. Frassetto, G.L. Szot, M. Tavakol, P.G. Stock, A.M. Posselt, A comparative analysis of the safety, efficacy, and cost of islet versus pancreas transplantation in nonuremic patients with type 1 diabetes, *Am. J. Transplant.* 16 (2) (2016) 518–526.
- [3] Collaborative Islet Transplant Registry (CITR), Tenth annual report, *CITR* 10 (1) (2017) 1–28.
- [4] P.A. Bateman, K.J. Devereux-Cooke, J.D. Johnson, H. Brandhorst, D. Brandhorst, D.W. Gray, S.E. Cross, S.J. Hughes, P.R.V. Johnson, Degradation of laminin and laminin-511 in the human peri-islet extracellular matrix is targeted by neutral protease and thermolysin, but not collagenase, *Transplantation* 96 (S6) (2013) S18.
- [5] S.E. Cross, R.H. Vaughan, A.J. Willcox, A.J. McBride, A.A. Abraham, B. Han, J.D. Johnson, E. Maillard, P.A. Bateman, R.D. Ramrath, P. Rorsman, K.E. Kadler, M.J. Dunne, S.J. Hughes, P.R.V. Johnson, Key matrix proteins within the pancreatic islet basement membrane are differentially digested during human islet isolation, *Am. J. Transplant.* 17 (2) (2017) 451–461.
- [6] M.A. Schwartz, M.D. Schaller, M.H. Ginsberg, Integrins: emerging paradigms of signal transduction, *Annu. Rev. Cell Dev. Biol.* 11 (1995) 549–599.
- [7] R.N. Wang, S. Paraskevas, L. Rosenberg, Characterization of integrin expression in islets isolated from hamster, canine, porcine, and human pancreas, *J. Histochem. Cytochem.* 47 (4) (1999) 499–506.

- [8] F. Ris, E. Hammar, D. Bosco, C. Pilloud, K. Maedler, M.Y. Donath, J. Oberholzer, E. Zeender, P. Morel, D. Rouiller, P.A. Halban, Impact of integrin-matrix matching and inhibition of apoptosis on the survival of purified human beta-cells in vitro, *Diabetologia* 45 (6) (2002) 841–850.
- [9] M. Kragl, E. Lammert, Basement membrane in pancreatic islet function, *Adv. Exp. Med. Biol.* 654 (2010) 217–234.
- [10] R.N. Wang, J.M. Li, K. Lyte, N.K. Yashpal, F. Fellows, C.G. Goodyer, Role for beta 1 integrin and its associated alpha 3, alpha 5, and alpha 6 subunits in development of the human fetal pancreas, *Diabetes* 54 (7) (2005) 2080–2089.
- [11] G. Parnaud, E. Hammar, D.G. Rouiller, M. Armanet, P.A. Halban, D. Bosco, Blockade of beta1 integrin-laminin-5 interaction affects spreading and insulin secretion of rat beta-cells attached on extracellular matrix, *Diabetes* 55 (5) (2006) 1413–1420.
- [12] G.G. Pinkse, W.P. Bouwman, R. Jiawan-Lalai, O.T. Terpstra, J.A. Bruijn, E. de Heer, Integrin signaling via RGD peptides and anti-beta1 antibodies confers resistance to apoptosis in islets of Langerhans, *Diabetes* 55 (2) (2006) 312–317.
- [13] M. Krishnamurthy, J. Li, G.F. Fellows, L. Rosenberg, C.G. Goodyer, R. Wang, Integrin [alpha]3, but not [beta]1, regulates islet cell survival and function via PI3K/Akt signaling pathways, *Endocrinology* 152 (2) (2011) 424–435.
- [14] S. Abdelli, J. Ansire, R. Roduit, T. Borsello, I. Matsumoto, T. Sawada, N. Allaman-Pillet, H. Henry, J.S. Beckmann, B.J. Hering, C. Bonny, Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure, *Diabetes* 53 (11) (2004) 2815–2823.
- [15] R. Bottino, A.N. Balamurugan, H. Tse, C. Thirunavukkarasu, X. Ge, J. Profocich, M. Milton, A. Ziegenfuss, M. Trucco, J.D. Piganelli, Response of human islets to isolation stress and the effect of antioxidant treatment, *Diabetes* 53 (10) (2004) 2559–2568.
- [16] P.D. Campbell, A. Weinberg, J. Chee, L. Mariana, R. Ayala, W.J. Hawthorne, P.J. O'Connell, T. Loudovaris, M.J. Cowley, T.W. Kay, S.T. Grey, H.E. Thomas, Expression of pro- and antiapoptotic molecules of the bcl-2 family in human islets postisolation, *Cell Transplant* 21 (1) (2012) 49–60.
- [17] M.J. Cowley, A. Weinberg, N.W. Zammit, S.N. Walters, W.J. Hawthorne, T. Loudovaris, H. Thomas, T. Kay, J.E. Gunton, S.I. Alexander, W. Kaplan, J. Chapman, P.J. O'Connell, S.T. Grey, Human islets express a marked proinflammatory molecular signature prior to transplantation, *Cell Transplant* 21 (9) (2012) 2063–2078.
- [18] S. Negi, A. Jetha, R. Aikin, C. Hasilo, R. Sladek, S. Paraskevas, Analysis of beta-cell gene expression reveals inflammatory signaling and evidence of dedifferentiation following human islet isolation and culture, *PLoS One* 7 (1) (2012) e30415.
- [19] H.F. Irving-Rodgers, F.J. Choong, K. Hummitzsch, C.R. Parish, R.J. Rodgers, C.J. Simeonovic, Pancreatic islet basement membrane loss and remodeling after mouse islet isolation and transplantation: impact for allograft rejection, *Cell Transplant* 23 (1) (2014) 59–72.
- [20] L. Rosenberg, R. Wang, S. Paraskevas, D. Maysinger, Structural and functional changes resulting from islet isolation lead to islet cell death, *Surgery* 126 (2) (1999) 393–398.
- [21] J.C. Stendahl, D.B. Kaufman, S.I. Stupp, Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation, *Cell Transplant* 18 (1) (2009) 1–12.
- [22] S. Paraskevas, D. Maysinger, R. Wang, T.P. Duguid, L. Rosenberg, Cell loss in isolated human islets occurs by apoptosis, *Pancreas* 20 (3) (2000) 270–276.
- [23] M. Giuliani, W. Moritz, E. Bodmer, D. Dindo, P. Kugelmeier, R. Lehmann, M. Gassmann, P. Groscurth, M. Weber, Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia, *Cell Transplant* 14 (1) (2005) 67–76.
- [24] C. Lucas-Clerc, C. Massart, J.P. Campion, B. Launois, M. Nicol, Long-term culture of human pancreatic islets in an extracellular matrix: morphological and metabolic effects, *Mol. Cell. Endocrinol.* 94 (1) (1993) 9–20.
- [25] T. Kaido, M. Yebra, V. Cirulli, C. Rhodes, G. Diaferia, A.M. Montgomery, Impact of defined matrix interactions on insulin production by cultured human beta-cells: effect on insulin content, secretion, and gene transcription, *Diabetes* 55 (10) (2006) 2723–2729.
- [26] N. Navarro-Alvarez, J.D. Rivas-Carrillo, A. Soto-Gutierrez, T. Yuasa, T. Okitsu, H. Noguchi, S. Matsumoto, J. Takei, N. Tanaka, N. Kobayashi, Reestablishment of microenvironment is necessary to maintain in vitro and in vivo human islet function, *Cell Transplant* 17 (1–2) (2008) 111–119.
- [27] J. Daoud, M. Petropavlovskaya, L. Rosenberg, M. Tabrizian, The effect of extracellular matrix components on the preservation of human islet function in vitro, *Biomaterials* 31 (7) (2010) 1676–1682.
- [28] D. Brandhorst, H. Brandhorst, N. Mullooly, S. Acreman, P.R. Johnson, High seeding density induces local hypoxia and triggers a proinflammatory response in isolated human islets, *Cell Transplant* 25 (8) (2016) 1539–1546.
- [29] K.E. Smith, A.C. Kelly, C.G. Min, C.S. Weber, F.M. McCarthy, L.V. Steyn, V. Badarinarayana, J.B. Stanton, J.P. Kitzmann, P. Strop, A.C. Gruessner, R.M. Lynch, S.W. Limesand, K.K. Papas, Acute ischemia induced by high-density culture increases cytokine expression and diminishes the function and viability of highly purified human islets of Langerhans, *Transplantation* 101 (11) (2017) 2705–2712.
- [30] A. Zbinden, S.L. Layland, M. Urbanczyk, D.A. Carvajal Berrio, J. Marzi, M. Zauner, A. Hammerschmidt, E.M. Brauchle, G. Sudrow, S. Fink, M. Templin, S. Liebscher, G. Klein, A. Deb, G.P. Duffy, G.M. Crooks, J.A. Eble, H.K.A. Mikkola, A. Nsaif, M. Seifert, K. Schenke-Layland, Nidogen-1 mitigates ischemia and promotes tissue survival and regeneration, *Adv. Sci. n/a* (n/a) (2020) 2002500.
- [31] C. Ricordi, J.S. Goldstein, A.N. Balamurugan, G.L. Szot, T. Kin, C. Liu, C.W. Czarniecki, B. Barbaro, N.D. Bridges, J. Cano, W.R. Clarke, T.L. Eggerman, L.G. Hunsicker, D.B. Kaufman, A. Khan, D.E. Lafontant, E. Linetsky, X. Luo, J.F. Markmann, A. Naji, O. Korsgren, J. Oberholzer, N.A. Turgeon, D. Brandhorst, X. Chen, A.S. Friberg, J. Lei, L.J. Wang, J.J. Wilhelm, J. Willits, X. Zhang, B.J. Hering, A.M. Posselt, P.G. Stock, A.M. Shapiro, X. Chen, National institutes of health-sponsored clinical islet transplantation consortium phase 3 trial: manufacture of a complex cellular product at eight processing facilities, *Diabetes* 65 (11) (2016) 3418–3428.
- [32] C. Ricordi, D.W. Gray, B.J. Hering, D.B. Kaufman, G.L. Warnock, N.M. Kneteman, S.P. Lake, N.J. London, C. Socci, R. Alejandro, Y. Zeng, D.W. Scharp, G. Viviani, L. Falqui, A. Tzakis, R.G. Bretzel, K. Federlin, G. Pozza, R.F.L. James, R.V. Rajotte, V. Di Carlo, P.J. Morris, D.E. Sutherland, T.E. Starzl, D.H. Mintz, P.E. Lacy, Islet isolation assessment in man and large animals, *Acta Diabetol. Lat.* 27 (3) (1990) 185–195.
- [33] H. Brandhorst, N. Raemisch-Guenther, C. Raemisch, O. Friedrich, S. Huettler, M. Kurfuerst, O. Korsgren, D. Brandhorst, The ratio between collagenase class I and class II influences the efficient islet release from the rat pancreas, *Transplantation* 85 (3) (2008) 456–461.
- [34] N.J. London, H. Contractor, S.P. Lake, G.C. Aucott, P.R. Bell, R.F. James, A fluorometric viability assay for single human and rat islets, *Horm. Metab. Res. Suppl.* 25 (1990) 82–87.
- [35] H. Brandhorst, S. Asif, K. Andersson, B. Theisinger, H.H. Andersson, M. Felldin, A. Foss, K. Salmela, A. Tibell, G. Tufveson, O. Korsgren, D. Brandhorst, A new oxygen carrier for improved long-term storage of human pancreata before islet isolation, *Transplantation* 89 (2) (2010) 155–160.
- [36] K. Meghana, G. Sanjeev, B. Ramesh, Curcumin prevents streptozotocin-induced islet damage by scavenging free radicals: a prophylactic and protective role, *Eur. J. Pharmacol.* 577 (1–3) (2007) 183–191.
- [37] N. Alam, H.L. Goel, M.J. Zarif, J.E. Butterfield, H.M. Perkins, B.G. Sansoucy, T.K. Sawyer, L.R. Languino, The integrin-growth factor receptor duet, *J. Cell. Physiol.* 213 (3) (2007) 649–653.
- [38] D. Brandhorst, H. Brandhorst, T. Linn, Induction and amelioration of environmental stress in isolated islets until transplantation, *Immun. Endoc. Metab. Agents Med. Chem.* 6 (2006) 209–218.
- [39] E.S. Wijelath, S. Rahman, M. Namekata, J. Murray, T. Nishimura, Z. Mostafavi-Pour, Y. Patel, Y. Suda, M.J. Humphries, M. Sobel, Heparin-II domain of fibronectin is a vascular endothelial growth factor-binding domain: enhancement of VEGF biological activity by a singular growth factor/matrix protein synergism, *Circ. Res.* 99 (8) (2006) 853–860.
- [40] M. Ishijima, N. Suzuki, K. Hozumi, T. Matsunobu, K. Kosaki, H. Kaneko, J.R. Hassell, E. Arikawa-Hirasawa, Y. Yamada, Perlecan modulates VEGF signaling and is essential for vascularization in endochondral bone formation, *Matrix Biol.* 31 (4) (2012) 234–245.
- [41] J. Ishihara, A. Ishihara, K. Fukunaga, K. Sasaki, M.J.V. White, P.S. Briquez, J.A. Hubbell, Laminin heparin-binding peptides bind to several growth factors and enhance diabetic wound healing, *Nat. Commun.* 9 (1) (2018) 2163.
- [42] V.J. Auer, E. Janas, V. Ninichuk, E. Eppler, T.S. Weiss, S. Kirchner, A.M. Otto, M.J. Stangl, Extracellular factors and immunosuppressive drugs influencing insulin secretion of murine islets, *Clin. Exp. Immunol.* 170 (2) (2012) 238–247.
- [43] G.M. Beattie, G. Leibowitz, A.D. Lopez, F. Levine, A. Hayek, Protection from cell death in cultured human fetal pancreatic cells, *Cell Transplant* 9 (3) (2000) 431–438.
- [44] G.M. Beattie, A.M. Montgomery, A.D. Lopez, E. Hao, B. Perez, M.L. Just, J.R. Lakey, M.E. Hart, A. Hayek, A novel approach to increase human islet cell mass while preserving beta-cell function, *Diabetes* 51 (12) (2002) 3435–3439.
- [45] K. Schulze-Osthoff, R. Beyaert, V. Vandevoorde, G. Haegeman, W. Fiers, Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF, *EMBO J.* 12 (8) (1993) 3095–3104.
- [46] V.J. Thannickal, B.L. Fanburg, Reactive oxygen species in cell signaling, *Am. J. Physiol. Lung Cell Mol. Physiol.* 279 (6) (2000) L1005–L1028.
- [47] D. Yang, S.G. Elner, Z.M. Bian, G.O. Till, H.R. Petty, V.M. Elner, Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells, *Exp. Eye Res.* 85 (4) (2007) 462–472.
- [48] M.P. Murphy, How mitochondria produce reactive oxygen species, *Biochem. J.* 417 (1) (2009) 1–13.
- [49] E.R. Stadtman, R.L. Levine, Free radical-mediated oxidation of free amino acids and amino acid residues in proteins, *Amino Acids* 25 (3–4) (2003) 207–218.
- [50] C.A. Delaney, D. Pavlovic, A. Hoorens, D.G. Pipeleers, D.L. Eizirik, Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells, *Endocrinology* 138 (6) (1997) 2610–2614.
- [51] J.M. Cook-Mills, Reactive oxygen species regulation of immune function, *Mol. Immunol.* 39 (9) (2002) 497–498.
- [52] J. Saldeen, Cytokines induce both necrosis and apoptosis via a common Bcl-2-inhibitable pathway in rat insulin-producing cells, *Endocrinology* 141 (6) (2000) 2003–2010.
- [53] L.G. Grunnet, R. Aikin, M.F. Tonnesen, S. Paraskevas, L. Blaabjerg, J. Størling, L. Rosenberg, N. Billestrup, D. Maysinger, T. Mandrup-Poulsen, Proinflammatory cytokines activate the intrinsic apoptotic pathway in beta-cells, *Diabetes* 58 (8) (2009) 1807–1815.
- [54] W. Fiers, R. Beyaert, W. Declercq, P. Vandenabeele, More than one way to die: apoptosis, necrosis and reactive oxygen damage, *Oncogene* 18 (54) (1999) 7719–7730.
- [55] H.U. Simon, A. Haj-Yehia, F. Levi-Schaffer, Role of reactive oxygen species (ROS) in apoptosis induction, *Apoptosis* 5 (5) (2000) 415–418.
- [56] D.L. Eizirik, S. Sandler, N. Welsh, M. Cetkovic-Cvrlje, A. Nieman, D.A. Geller, D.G. Pipeleers, K. Bendtzen, C. Hellerstrom, Cytokines suppress human islet

- function irrespective of their effects on nitric oxide generation, *J. Clin. Invest.* 93 (5) (1994) 1968–1974.
- [57] B. Armann, M.S. Hanson, E. Hatch, A. Steffen, L.A. Fernandez, Quantification of basal and stimulated ROS levels as predictors of islet potency and function, *Am. J. Transplant.* 7 (1) (2007) 38–47.
- [58] R. Melzi, A. Mercalli, V. Sordi, E. Cantarelli, R. Nano, P. Maffi, G. Sitia, L.G. Guidotti, A. Secchi, E. Bonifacio, L. Piemonti, Role of CCL2/MCP-1 in islet transplantation, *Cell Transplant.* 19 (8) (2010) 1031–1046.
- [59] L. Piemonti, B.E. Leone, R. Nano, A. Sacconi, P. Monti, P. Maffi, G. Bianchi, A. Sica, G. Peri, R. Melzi, L. Aldrighetti, A. Secchi, V. Di Carlo, P. Allavena, F. Bertuzzi, Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation, *Diabetes* 51 (1) (2002) 55–65.
- [60] B. Movahedi, C. Gysemans, D. Jacobs-Tulleneers-Thevisen, C. Mathieu, D. Pipeleers, Pancreatic duct cells in human islet cell preparations are a source of angiogenic cytokines interleukin-8 and vascular endothelial growth factor, *Diabetes* 57 (8) (2008) 2128–2136.
- [61] Y. Lai, D. Schneider, A. Kiszun, I. Hauck-Schmalenberger, G. Breier, D. Brandhorst, H. Brandhorst, M. Iken, M.D. Brendel, R.G. Bretzel, T. Linn, Vascular endothelial growth factor increases functional beta-cell mass by improvement of angiogenesis of isolated human and murine pancreatic islets, *Transplantation* 79 (11) (2005) 1530–1536.
- [62] M. Brissava, A. Shostak, M. Shiota, P.O. Wiebe, G. Poffenberger, J. Kantz, Z. Chen, C. Carr, W.G. Jerome, J. Chen, H.S. Baldwin, W. Nicholson, D.M. Bader, T. Jetton, M. Gannon, A.C. Powers, Pancreatic islet production of vascular endothelial growth factor- α is essential for islet vascularization, revascularization, and function, *Diabetes* 55 (11) (2006) 2974–2985.
- [63] S.E. Cross, S.K. Richards, A. Clark, A.V. Benest, D.O. Bates, P.W. Mathieson, P.R. Johnson, S.J. Harper, R.M. Smith, Vascular endothelial growth factor as a survival factor for human islets: effect of immunosuppressive drugs, *Diabetologia* 50 (7) (2007) 1423–1432.
- [64] A.J. Puren, G. Fantuzzi, Y. Gu, M.S. Su, C.A. Dinarello, Interleukin-18 (IFN γ -inducing factor) induces IL-8 and IL-1 β via TNF α production from non-CD14 $^{+}$ human blood mononuclear cells, *J. Clin. Invest.* 101 (3) (1998) 711–721.
- [65] A.K. Garg, B.B. Aggarwal, Reactive oxygen intermediates in TNF signaling, *Mol. Immunol.* 39 (9) (2002) 509–517.
- [66] E. Naik, V.M. Dixit, Mitochondrial reactive oxygen species drive proinflammatory cytokine production, *J. Exp. Med.* 208 (3) (2011) 417–420.
- [67] S.J. Forrester, D.S. Kikuchi, M.S. Hernandez, Q. Xu, K.K. Griending, Reactive oxygen species in metabolic and inflammatory signaling, *Circ. Res.* 122 (6) (2018) 877–902.
- [68] S. Yoshida, A. Yoshida, T. Ishibashi, Induction of IL-8, MCP-1, and bFGF by TNF- α in retinal glial cells: implications for retinal neovascularization during post-ischemic inflammation, *Graefes Arch. Clin. Exp. Ophthalmol.* 242 (5) (2004) 409–413.
- [69] E.M. Paleolog, S. Young, A.C. Stark, R.V. McCloskey, M. Feldmann, R.N. Maini, Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor α and interleukin-1 in rheumatoid arthritis, *Arthritis Rheum* 41 (7) (1998) 1258–1265.
- [70] D. Sun, S. Matsune, J. Ohori, T. Fukuiwa, M. Ushikai, Y. Kurono, TNF- α and endotoxin increase hypoxia-induced VEGF production by cultured human nasal fibroblasts in synergistic fashion, *Auris. Nasus. Larynx* 32 (3) (2005) 243–249.
- [71] M.R. Shin, S.K. Kang, Y.S. Kim, S.Y. Lee, S.C. Hong, E.C. Kim, TNF- α and LPS activate angiogenesis via VEGF and SIRT1 signalling in human dental pulp cells, *Int. Endod. J.* 48 (7) (2015) 705–716.
- [72] A. Mooney, K. Jackson, R. Bacon, C. Streuli, G. Edwards, J. Bassuk, J. Savill, Type IV collagen and laminin regulate glomerular mesangial cell susceptibility to apoptosis via β (1) integrin-mediated survival signals, *Am. J. Pathol.* 155 (2) (1999) 599–606.
- [73] D. Wu, X. Chen, D. Guo, Q. Hong, B. Fu, R. Ding, L. Yu, K. Hou, Z. Feng, X. Zhang, J. Wang, Knockdown of fibronectin induces mitochondria-dependent apoptosis in rat mesangial cells, *J. Am. Soc. Nephrol.* 16 (3) (2005) 646–657.
- [74] S. Sakon, X. Xue, M. Takekawa, T. Sasazuki, T. Okazaki, Y. Kojima, J.H. Piao, H. Yagita, K. Okumura, T. Doi, H. Nakano, NF- κ B inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death, *EMBO J.* 22 (15) (2003) 3898–3909.
- [75] A. Barbu, N. Welsh, J. Saldeen, Cytokine-induced apoptosis and necrosis are preceded by disruption of the mitochondrial membrane potential (Deltapsi(m)) in pancreatic RINm5F cells: prevention by Bcl-2, *Mol. Cell. Endocrinol.* 190 (1–2) (2002) 75–82.
- [76] D.R. Eyre, M.A. Weis, J.J. Wu, Advances in collagen cross-link analysis, *Methods* 45 (1) (2008) 65–74.
- [77] I. Virtanen, M. Banerjee, J. Palgi, O. Korsgren, A. Lukinius, L.E. Thornell, Y. Kikkawa, K. Sekiguchi, M. Hukkanen, Y.T. Kontinen, T. Otonkoski, Blood vessels of human islets of Langerhans are surrounded by a double basement membrane, *Diabetologia* 51 (7) (2008) 1181–1191.
- [78] M. Aumailley, H. Wiedemann, K. Mann, R. Timpl, Binding of nidogen and the laminin-nidogen complex to basement membrane collagen type IV, *Eur. J. Biochem.* 184 (1) (1989) 241–248.
- [79] P. Bruckner, Suprastructures of extracellular matrices: paradigms of functions controlled by aggregates rather than molecules, *Cell Tissue Res* 339 (1) (2010) 7–18.
- [80] T. Otonkoski, M. Banerjee, O. Korsgren, L.E. Thornell, I. Virtanen, Unique basement membrane structure of human pancreatic islets: implications for beta-cell growth and differentiation, *Diabetes Obes. Metab.* 10 (Suppl 4) (2008) 119–127.
- [81] A. Llacua, B.J. de Haan, S.A. Smink, P. de Vos, Extracellular matrix components supporting human islet function in alginate-based immunoprotective microcapsules for treatment of diabetes, *J. Biomed. Mater. Res. A* 104 (7) (2016) 1788–1796.
- [82] L.A. Llacua, B.J. de Haan, P. de Vos, Laminin and collagen IV inclusion in immunisolating microcapsules reduces cytokine-mediated cell death in human pancreatic islets, *J. Tissue Eng. Regen. Med.* 12 (2) (2018) 460–467.
- [83] N.E. Davis, L.N. Beenken-Rothkopf, A. Mirsoian, N. Kojic, D.L. Kaplan, A.E. Barron, M.J. Fontaine, Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk hydrogel, *Biomaterials* 33 (28) (2012) 6691–6697.
- [84] L.M. Weber, K.N. Hayda, K.S. Anseth, Cell-matrix interactions improve beta-cell survival and insulin secretion in three-dimensional culture, *Tissue Eng. Part A* 14 (12) (2008) 1959–1968.
- [85] L.A. Llacua, A. Hoek, B.J. de Haan, P. de Vos, Collagen type VI interaction improves human islet survival in immunisolating microcapsules for treatment of diabetes, *Islets* 10 (2) (2018) 60–68.
- [86] A.M. Smink, P. de Vos, Therapeutic strategies for modulating the extracellular matrix to improve pancreatic islet function and survival after transplantation, *Curr. Diab. Rep.* 18 (7) (2018) 39.
- [87] D.T. Bowers, W. Song, L.H. Wang, M. Ma, Engineering the vasculature for islet transplantation, *Acta Biomater.* 95 (2019) 131–151.
- [88] L.M. Weber, K.S. Anseth, Hydrogel encapsulation environments functionalized with extracellular matrix interactions increase islet insulin secretion, *Matrix Biol.* 27 (8) (2008) 667–673.
- [89] J. Santini-Gonzalez, J.A. Simonovich, R. Castro-Gutierrez, Y. Gonzalez-Vargas, N.J. Abuid, C.L. Stabler, H.A. Russ, E.A. Phelps, In vitro generation of peri-islet basement membrane-like structures, *Biomaterials* 273 (2021) 120808.
- [90] J.Y. Cheng, M. Raghunath, J. Whitelock, L. Poole-Warren, Matrix components and scaffolds for sustained islet function, *Tissue Eng. Part B Rev.* 17 (4) (2011) 235–247.
- [91] E.A. Aisenbrey, W.L. Murphy, Synthetic alternatives to Matrigel, *Nat. Rev. Mater.* 5 (7) (2020) 539–551.
- [92] A. Asthana, R. Tamburrini, D. Chaimov, C. Gazia, S.J. Walker, M. Van Dyke, A. Tomei, S. Lablanche, J. Robertson, E.C. Opara, S. Soker, G. Orlando, Comprehensive characterization of the human pancreatic proteome for bioengineering applications, *Biomaterials* 270 (2021) 120613.
- [93] S.D. Sackett, D.M. Tremmel, F. Ma, A.K. Feeney, R.M. Maguire, M.E. Brown, Y. Zhou, X. Li, C. O'Brien, L. Li, W.J. Burlingham, J.S. Odorico, Extracellular matrix scaffold and hydrogel derived from decellularized and delipidized human pancreas, *Sci. Rep.* 8 (1) (2018) 10452.
- [94] Z. Li, D.M. Tremmel, F. Ma, Q. Yu, M. Ma, D.G. Delafield, Y. Shi, B. Wang, S.A. Mitchell, A.K. Feeney, V.S. Jain, S.D. Sackett, J.S. Odorico, L. Li, Proteome-wide and matrisome-specific alterations during human pancreas development and maturation, *Nat. Commun.* 12 (1) (2021) 1020.
- [95] A. Naba, K.R. Clauser, D.R. Mani, S.A. Carr, R.O. Hynes, Quantitative proteomic profiling of the extracellular matrix of pancreatic islets during the angiogenic switch and insulinoma progression, *Sci. Rep.* 7 (2017) 40495.
- [96] R.O. Hynes, The extracellular matrix: not just pretty fibrils, *Science* 326 (5957) (2009) 1216–1219.
- [97] R. Jayadev, D.R. Sherwood, Basement membranes, *Curr. Biol.* 27 (6) (2017) R207–R211.
- [98] S. Zhou, S. Chen, Y.A. Pei, M. Pei, Nidogen: a matrix protein with potential roles in musculoskeletal tissue regeneration, *Genes. Dis.* 9 (2021) 1–10.