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RESEARCH PAPER

Use of additives, scaffolds and extracellular matrix components for improvement of human pancreatic islet outcomes in vitro: A systematic review

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ABSTRACT

Pancreatic islet transplantation is an established treatment to restore insulin independence in type 1 diabetic patients. Its success rates have increased lately based on improvements in immunosuppressive therapies and on islet isolation and culture. It is known that the quality and quantity of viable transplanted islets are crucial for the achievement of insulin independence and some studies have shown that a significant number of islets are lost during culture time. Thus, in an effort to improve islet yield during culture period, researchers have tested a variety of additives in culture media as well as alternative culture devices, such as scaffolds. However, due to the use of different categories of additives or devices, it is difficult to draw a conclusion on the benefits of these strategies. Therefore, the aim of this systematic review was to summarize the results of studies that described the use of medium additives, scaffolds or extracellular matrix (ECM) components during human pancreatic islets culture. PubMed and Embase repositories were searched. Of 5083 articles retrieved, a total of 37 articles fulfilled the eligibility criteria and were included in the review. After data extraction, articles were grouped as follows: 1) "antiapoptotic/anti-inflammatory/antioxidant," 2) "hormone," 3) "sulphonylureas," 4) "serum supplements," and 5) "scaffolds or ECM components." The effects of the reviewed additives, ECM or scaffolds on islet viability, apoptosis and function (glucose-stimulated insulin secretion - GSIS) were heterogeneous, making any major conclusion hard to sustain. Overall, some "antiapoptotic/anti-inflammatory/antioxidant" additives decreased apoptosis and improved GSIS. Moreover, islet culture with ECM components or scaffolds increased GSIS. More studies are needed to define the real impact of these strategies in improving islet transplantation outcomes.

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additives in cultures; culture of islets; extracellular matrix; human pancreatic islet; islet isolation; scaffolds

Introduction

Pancreatic islet transplantation is an established treatment for patients with type 1 diabetes mellitus (T1DM) that suffer from hypoglycemia unawareness with frequent episodes of hypoglycemia and marked glycemic lability.[1-5] A publication from The Collaborative Islet Transplant Registry (CITR) in 2012 showed a clear improvement in islet transplantation outcomes in the recent era. Insulin independence, 3 y after transplantation, improved from 27% in 1999–2002 (n = 214) to 37% in 2003–2006 (n = 255) and to 44% in the most recent period, 2007–2010 (n = 208).[4] Recently, Brennan *et al.*[6] reported the results from a 12 -year follow-up of 7 subjects initially assigned for the Edmonton protocol in 2000. One patient experienced graft failure only 10.9 y after islet transplantation.

The other 6 patients continued to have sustained C-peptide and improved glycemic control without episodes of severe hypoglycemia after islet transplantation even in this long follow-up, although all of them have lost insulin independence in different timepoints. Hering *et al.*[7] recently published results from the Phase 3 study of Clinical Islet Transplantation (CIT) Consortium. They demonstrated that purified human pancreatic islets transplantation provided good glycemic control, restoration of hypoglycemia awareness, and protection from severe hypoglycemia.

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Also related to CIT, Ricordi *et al.*[8] demonstrated the feasibility of implementing a harmonized process at multiple facilities for the manufacture of a complex cellular product of human islet for transplantation.

An important criteria for the achievement of long-term insulin independence is the total number of viable islets transplanted per Kg of the recipient's weight.[9] It is generally assumed that a combined implant mass of at least 10,000 islet equivalents (IEQ) per kg is required to routinely achieve insulin-independence.[4] Nowadays, most of the islet isolation facilities keep the isolated islets in culture for 24–48 hours prior transplantation, allowing them to recover from the stress generated during the isolation process and also allowing the preparation of the recipient, including the administration of immunosuppressive induction therapy.[10] During this culture period, up to 10–20% of the total islet mass is lost, which may, in turn, compromise the success of the transplant.[11]

Studies have shown that the loss of islets during the culture period is due, in part, to the apoptosis that is triggered along the whole process of procurement (due to brain death catecholamine storm and cold ischemia time) and also during the islet isolation process.[12-15] In this context, protective strategies to preserve islets from damage during culture time have been studied as a way to improve islet transplant outcomes. These strategies include the use of different additives in the islet culture media or novel culture methods, such as scaffolds or extracellular matrix (ECM) components.[16-20] However, the high variability of additives and culture methods tested makes it difficult to draw a conclusion on the subject. So, the aim of this systematic review was to summarize the research findings on the use of medium additives, scaffolds or ECM components to improve viability and function during human pancreatic islet culture.

Results

Literature search

Fig. 1 is a flow diagram showing the strategy used to identify and select studies for inclusion in this systematic review. All studies that analyzed effects of additives added to the human pancreatic islet culture medium on IEQ, viability/apoptosis and/or function (assessed by glucose-stimulated insulin secretion - GSIS) were selected for inclusion. In addition, studies that cultured islets on different scaffolds or ECM components were also selected for inclusion. A total of 5083 possible relevant citations were retrieved by searching the electronic databases, and 5032 of them were excluded following the reading of titles and abstracts. Fifty articles remained to be fully evaluated. However, after careful analysis of the complete texts, another 14 articles were excluded due to use of islet encapsulation or co-culture.

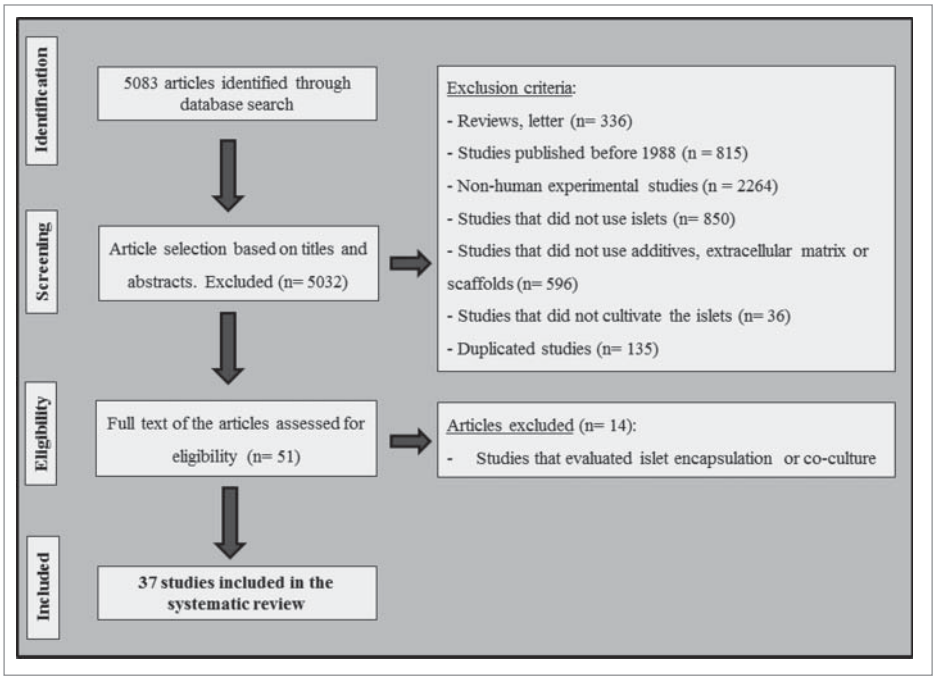

Figure 1. Flowchart illustrating the search strategy used to identify studies for inclusion in the systematic review.

Thirty-seven articles[16-19,21-53] fulfilled the eligibility criteria and were included in the systematic review (Fig. 1). After data extraction, the studies were grouped to better describe and summarize the results, as follows: 1) "antiapoptotic/anti-inflammatory/antioxidant" additives, 2) "hormone" additives, 3) "sulphonylurea" agents, 4) "serum supplements," and 5) "scaffolds or ECM components."

Main characteristics of the eligible studies

Table 1 shows the main characteristics of the 37 studies included in this systematic review. In brief, the number of donors ranged from 2 to 18 and the donor's mean age ranged from 24 y to 70 y among studies. Of note, some studies did not reported donor's characteristics. Purity (%) of the islet preparations were reported by 21 studies, but only in the pre-culture period, and no description was available after the interventions.

All studies included in this systematic review used the Ricordi's semi-automated technique for islet isolation.[54] Twenty-three studies (62.2%) used CRML 1066 as the islet culture media after isolation,[16,17,19,24,25,27,32-35,37-39,41-43,47-53] 4 studies used Miami media,[22,23,29,30] 3 used M199 media,[21,31,44] 6 of them did not report the media used,[18,26,28,36,40,45] and one study used Ham's/F10 media.[46] All studies had an experimental group where modifications

Table 1. Main characteristics of the pancreas donors and purity of the isolated islets described in the included studies.

Groups of additives	N° of donors	Mean age	Gender (%male)	CIT (h)	Pre-culture purity (%)
1st Author (year)[Ref]					
"Antiapoptotic/anti-inflammatory/antioxidant"					
Emamaullee (2008)[32]	—	—	—	—	—
Mancarella (2008)[18]	8	49	50	—	—
McCall (2011)[33]	—	—	—	—	—
Mita (2008)[23]	9	—	—	—	Pure: >90; Impure: 40–60
Moriscot (2007)[24]	—	—	—	—	—
Mwangi (2011)[34]	4	45.8	25		89.2
Nakano (2004)[17]	12	41.8	50	—	70–95
Omori (2010)[25]	—	—	—	—	>70
Pepper (2017)[35]	2	—	—	—	43.8
Scholz (2009)[26]	5	57.6	20	9.52	50–95
Yang (2005)[27]	3	46	80	11	50–95
Zhang (2004)[19]	6	43	—	16	57
"Hormones"					
Farilla (2003)[21]	3	—	—	—	>90
Liu (2009)[28]	—	—	—	—	—
Miki (2014)[22]	—	—	—	—	—
Sakuma (2009)[29]	—	—	—	—	—
Toso (2010)[16]	14	53	21.4	10	—
Yamamoto (2010)[30]	14	48	64.3	11.5	—
"Sulphonylureas"					
Del Guerra (2005)[31]	18	51	55.6	—	—
Maedler (2005)[47]	7	38–70	—	—	>75
"Serum"					
Avgoustiniatos (2012)[48]	—	—	—	—	>70
Bucher (2003)[53]	—	—	—	—	85
Kerr-Conte (2010)[49]	—	—	—	—	50–80
Lee (2008)[50]	—	—	—	—	>50
Matsumoto (2003)[51]	—	47.4	20	35.8	62
Nacher (2013)[52]	15	—	—	—	—
"Scaffolds or ECM components"					
Benti-Barnes (2008)[36]	8	—	—	—	>70
Buitinga (2013)[37]	4	—	—	—	—
Daoud (2010)[38]	—	—	—	<8	>80
Daoud (2011)[39]	—	—	—	<8	>80
Kitzmann (2014)[40]	—	—	—	—	low purity
Maillard (2011)[41]	8	24–61	—	5.5–9	—
Matsushima (2016)[42]	—	—	—	—	—
Marchioli (2015)[43]	—	—	—	—	—
Murray (2009)[44]	—	—	—	—	—
Papas (2005)[45]	3	—	—	—	>90
Zhang (2012)[46]	—	—	—	—	~90

CIT: Cold ischemia time (in hours); ECM: extracellular matrix.

were added to the culture and a control group without modifications added to the culture. The culture time varied from 16 hours to 10 d. The seeding density ranged from 30 IEQ/cm^2 to 5000 IEQ/cm^2, and the volume density varied from 300 IEQ/mL to 1500 IEQ/mL.

Table 2 shows the interventions made to the islet culture, including type of modification, concentrations of additives, ECM components or substances used in scaffolds, and the number of replications per each study. Twelve studies analyzed "antiapoptotic/anti-inflammatory/antioxidant" additives, 6 studies analyzed "hormones," 2 study investigated "sulphonylurea" agents, 6 studies analyzed "serum supplements," and 11 studies investigated the use of "scaffolds or ECM components" in islet culture. Concentrations of these substances

Table 2. Additives, scaffolds and ECM components used in included studies and their concentrations.

Groups of additives	Component	Concentration	n experimental
1st Author (year)[Ref]			
"Antiapoptotic/anti-inflammatory/ antioxidant"			
Emamaullee (2008)[32]	EP1013	1mg/mL	3
Mancarella (2008)[18]	IAC	10μM/L	8
Mc Call (2011)[33]	IDN-6556	100μM	3
Mita (2008)[23]	Sirolimus	30ng/mL	3
Moriscot (2007)[24]	MnTMPyP	25μM/L	3
Mwangi (2011)[34]	GDNF	100ng/mL	4
Nakano (2004)[17]	Z-DEVD-FMK	25 and100μM/L	—
Omori (2010)[25]	SD-282	0.1μM and 0.3μM	3
Pepper (2017)[35]	F573	100μM	2
Scholz (2009)[26]	GW3965	1μM/L	10
Yang (2005)[27]	LSF	20, 50 e 100μM/L	3
Zhang (2004)[19]	Polyphenol (green tea extract)	0, 30, 60, 125, 250 and 500μg/mL	—
"Hormones"			
Farilla (2003)[21]	GLP-1	10nM	3
Liu (2009)[28]	β-E2 and α-E2 (17α-estradiol)	10^{-8}M	5
Miki (2014)[22]	Exendin-4	10nM	3
Sakuma (2009)[29]	PACAP	10^{-12}M/L	—
Toso (2010)[16]	Liraglutide	1μM/L	—
Yamamoto (2010)[30]	Prolactin	500μg/L	—
"Sulphonylureas"			
Del Guerra (2005)[31]	Glimepiride, glibenclamide and chlorprapamide	10μM, 10μM and 600μM; respectively	10
Maedler (2005)[47]	Repaglimide, nateglimide and glibenclamide	0.01 or 1μM, 10 or 1000μM, 0.1 or 1 or 10 or 100nM; respectively	3
"Serum"			
Avgoustiniatos (2012)[48]	FBS and HSA	10% and 0.5%; respectively	10
Bucher (2003)[53]	FCS, HSA and human AB serum	10%, 0.625% and 2.5%; respectively	5
Kerr-Conte (2010)[49]	HSA and human AB serum	0.625% and 2.5%; respectively	9–15
Lee (2008)[50]	HSA and human AB serum	0.5% and 10%; respectively	4
Matsumoto (2003)[51]	FBS and HA	10% and 1.4%; respectively	—
Nacher (2013)[52]	HSA and HS	—	—
"Scaffolds or extracellular matrix"			
Bentsi-Barnes (2008)[36]	Gas-permeable membranes	—	1–7
Buitinga (2013)[37]	PEOT/PBT	—	3
Daoud (2010)[38]	Collagen I/IV, fibronectin and laminin	6.25μg/cm^2	3
Daoud (2011)[39]	Collagen I gel with or without ECM components, micro-fabricated scaffold	4mg/mL and 100 g/mL; respectively	3
Kitzmann (2014)[40]	Silicone rubber membrane	—	5–6
Maillard (2011)[41]	Fibrinogen, thrombin and PDC	20mg/mL, 10mU/mL and 10%; respectively	7–8
Matsushima (2016)[42]	Fibroblasts	—	5–6
Marchioli (2015)[43]	Alginate/gelatingel	—	—
Murray (2009)[44]	Pancreatic duct-derived epithelial cells	—	6
Papas (2008)[45]	Silicone rubber membrane	—	3
Zhang (2012)[46]	CM and FPCM	—	3

EP1013: N-benzyloxycabonyl-Val Asp-fluoromethyl ketone [zVD-FMK]; IAC: bis (1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl) decantionate; IDN-6556: caspase inhibitor; MnTMPyP: SOD mimeticmanganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP); GDNF: glial cell line-derived neurotrophic factor; Z-DEVD-FMK: Z-Asp (OMe)-Glu (OMe)-Val-Asp (OMe)-fluoromethylketone; SD-282: indole-5-carboxamide ATP-competitive inhibitor of p38α MAPK; F573: pan-caspase inhibitor; GW3965: synthetic nonsteroidal liver X receptor (LXR) agonist; LSF: lysofylline; GLP-1: glucagon-like peptide-1; PACAP: pituitary adenylate cyclase-activating peptide; FBS: fetal bovine serum; FCS: fetal calf serum; HSA: human serum albumin; HA: human albumin; HS: human serum; PEOT: poly(ethylene oxide terephthalate); PBT: poly(butylenes terephthalate); ECM: extracellular matrix; PDC: perfluorodecalin; CM: collagen matrix; FPCM: human fibroblast-populated collagen matrix.

Table 3. Methods used to evaluate the outcomes of interest.

	Methods		
1st Author (year)[Ref]	Viability assesment	Apoptosis assessment	Insulin secretion
Emamaullee (2008)[32]	Sytogreen/ethidium bromide	—	Static incubation/RIA
Mancarella (2008)[18]	—	—	Static incubation/IRMA
Mc Call (2011)[33]	Sytogreen/ethidium bromide	TUNEL/DAPI	—
Mita (2008)[23]	FACS using NG, TMRE and 7AAD	—	Perifusion/ELISA
Moriscot (2007)[24]	FACS using Live/Dead kit	—	Static incubation/RIA
Mwangi (2011)[34]	—	TUNEL/DAPI	Static incubation/ELISA
Nakano (2004)[17]	—	DNA fragment using ELISA	Static incubation/ELISA
Omori (2010)[25]	—	TUNEL	Perifusion/ELISA
Pepper (2017)[35]	Sytogreen/ethidium bromide	TUNEL/DAPI	Static incubation/ELISA
Scholz (2009)[26]	ApoGlow Kit/XTT Assay/CellTiter-Glo	Apo-ONE caspase assay/ELISA	Perifusion/ELISA
Yang (2005)[27]	—	Apo Percentage apoptosis assay kit	Static incubation/ELISA
Zhang (2004)[19]	FDA/PI	—	Static incubation/ELISA
Farilla (2003)[21]	—	DAPI	Static incubation/RIA
Liu (2009)[28]	—	Hoechst	—
Miki (2014)[22]	FACS using NG, TMRE and 7AAD	—	Perifusion/ELISA
Sakuma (2009)[29]	FACS using NG, TMRE and 7AAD	—	Perifusion/ELISA
Toso C (2010)[16]	SYTO_13 Kit/ethidium bromide	TUNEL	Static incubation/ELISA
Yamamoto T (2010)[30]	FACS using NG, TMRE and 7AAD	—	—
Del Guerra (2005)[31]	—	TUNEL/ELISA	Static incubation/IRMA
Maedler (2005)[47]	—	TUNEL	RIA
Avgoustiniatos (2012)[48]	OCR	—	—
Bucher (2003)[53]	—	Cell Death Detection ELISA	Static incubation / -
Kerr-Conte (2010)[49]	Tripan-blue	Cell Death Detection Kit	Static incubation/RIA
Lee (2008)[50]	—	—	Static incubation/Immulite immunometric assay
Matsumoto (2013)[51]	AO/PI	—	Static incubation/ELISA
Nacher (2013)[52]	—	TUNEL/DAPI	Static incubation/ELISA
Bentsi-Barnes (2008)[36]	—	—	Perifusion/ELISA
Buitinga (2013)[37]	—	—	Static incubation/ELISA
Daoud (2010)[38]	—	—	Static incubation/ELISA
Daoud (2011)[39]	—	—	Static incubation/ELISA
Kitzmann (2014)[40]	OCR/FDA/PI	—	—
Maillard (2011)[41]	FDA/ethidium bromide	Caspase 3/ELISA	Static incubation/ELISA
Matsushima (2016)[42]	AM/PI	—	Static incubation/ELISA
Marchioli (2015)[43]	—	—	Static incubation/ELISA
Murray (2009)[44]	—	—	Static incubation/ELISA
Papas (2008)[45]	OCR	—	—
Zhang (2012)[46]	Viability/cytotoxicity assay kit	—	Static incubation/ELISA

FACS: Fluorescence-activated cell sorting; NG: Newport green; TMRE: Tetramethyl rhodamine ethylester; 7AAD: 7-Aminoactinomycin D; FDA: Fluorescein diacetate; PtdIns: Propidium iodide; OCR: oxygen consumption rate; AO: acridine orange; AM: calcein-acetoxymethyl; TUNEL: Terminal deoxynucleotidyl transferase dUTP nickend labeling; DAPI: 4′,6-diamidino-2-phenylindole; ELISA: enzyme-linked immunosorbent assay; RIA: Radioimmunoassay; IRMA: Immunoradiometric assay.

varied among studies, and the number of experimental replications per study ranged from 2 to 15.

The methods used to assess viability, apoptosis and function (GSIS) varied widely among studies, as depicted in Table 3. Twenty out of 37 studies evaluated islet viability,[16,19,22-24,26,29,30,32-35,40-42,45,46,48,49,51] but only 3 of them applied the standard technique used to evaluate islet viability for transplantation [fluorescein diacetate (FDA) and propidium iodide (PI)].[19,34,40] Apoptosis evaluation was reported in 16 studies, with Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL),[16,25,31,33-35,47,52] and ELISA[17,26,31,41,53] being the most used techniques. The majority of studies used ELISA for GSIS evaluation.[16,17,19,22,23,25-27,29,34-39,41-44,46,51,52] Some studies analyzed GSIS using radioimmunoassays (RIA)[21,24,32,47,49] or immunoradiometric assays (IRMA).[18,31] Islet equivalents (IEQ) were calculated using diphenylthiocarbozone dye (Dithizone).[16,17,25]

Results of studies that evaluated "antiapoptotic/anti-inflammatory/antioxidant" additives

Results of the studies that evaluated the effect of "anti-apoptotic/anti-inflammatory/antioxidant" additives added to the culture media on islet viability, apoptosis, GSIS or IEQs are summarized in Table 4. A total of 12 studies analyzed the effects of this group of additives on the islet outcomes of interest. Among them, 7 studies analyzed islet viability. Three of them showed an increased in viability using the pan-caspase inhibitor F573,[35] the caspase inhibitors EP1013[32] and IDN-

Table 4. Summary of the effects of the additives, scaffolds and ECM components on islet outcomes of interest.

Groups of additives	N of studies	Results
		Viability
"Antiapoptotic/anti-inflammatory/antioxidant"	7	↑ 3 studies found increased viability in treated groups (EP1013;[32] IDN-6556;[33] F573[35]) ↔ 4 studies found no differences between groups.[19,23,24,26]
"Hormones"	3	↑ 2 studies found increased viability in treated groups (exendin-4;[22] PACAP[29]) ↔ 1 study found no differences between groups[16]
"Serum"	3	↑ 2 studies found increased viability in treated groups (FBS *vs* HSA;[48] AB serum *vs* HSA[49]) ↔ 1 study found no differences between groups[51]
"Scaffolds or ECM components"	6	↑ 4 studies found increased viability in treated groups (SRM;[40] collagen I, IV, fibronectin and laminin;[38] fibroblasts;[42] CM and FPCM[46]) ↔ 2 studies found no differences between groups[41,45]
		Apoptosis
"Antiapoptotic/anti-inflammatory/antioxidant"	7	↔ 2 studies found no differences between groups[25,26] ↓ 5 studies found decreased apoptosis in treated groups (Z-DEVD-FMK;[17] LSF;[27] IDN-6556;[33] F573;[35] GDNF[34])
"Hormones"	3	↓ 2 studies found decreased apoptosis in treated groups (GLP-1;[21] β-E2 or α-E2 estradiol[28]) ↔ 1 study found no difference between groups[16]
"Sulphonylureas"	2	↔ 1 study found no difference between groups[31] ↑ 1 study found increased apoptosis in treated groups (repaglimide, nateglimide and glibenclamide[47])
"Serum"	3	↓ 3 studies found decreased apoptosis in treated group (AB serum *vs* HSA,[49] and *vs* FCS;[53] HS *vs* HSA[52])
"Scaffolds or ECM components"	1	↓ 1 study found decreased apoptosis in treated groups (fibrinogen, thrombin and PDC[41])
		GSIS
"Antiapoptotic/anti-inflammatory/antioxidant"	11	↑ 7 studies found increased insulin secretion in treated groups (Z-DEVD-FMK;[17] IAC;[18] Sirolimus;[23] GW3965;[26] LSF;[27] F573;[35] GDNF[34]) ↔ 3 studies found no differences between groups[19,25,32] ↓ 1 study found decreased insulin secretion in treated group (MnTMPyP[24])
"Hormones"	4	↑ 2 studies found increased insulin secretion in treated groups (GLP-1;[21] exendina-4[22]) ↔ 2 studies found no differences between groups[16,29]
"Sulphonylureas"	2	↓ 1 study found decreased insulin secretion in treated group (glimepiride, glibenclamide and chlorprapamide[31]) ↑ 1 study found increased insulin secretion in treated group (Glibenclamide[47])
"Serum"	5	↑ 3 studies found increased insulin secretion in treated groups (AB serum *vs* HSA,[49,53] and *vs* FCS;[53] HS *vs* HSA[52]) ↔ 2 studies found no differences between groups[50,51]
"Scaffolds or ECM components"	9	↑ 6 studies found increased insulin secretion in treated groups (gas-permeable membrane;[36] collagen I gel with or without ECM components, micro-fabricated scaffold;[39] fibrinogen, thrombin and PDC;[41] fibroblasts;[42] pancreatic duct-derived epithelial cells;[44] CM and FPCM[46]) ↔ 2 studies found no differences between groups[37,43] ↓ 1 study found decreased insulin secretion in treated group (collagen I, IV, fibronectin and laminin[38])
		IEQ
"Antiapoptotic/anti-inflammatory/antioxidant"	2	↑ 1 study found increased IEQ in treated group (Z-DEVD-FMK[17]) ↔ 1 study found no differences between groups[25]
"Hormone"	1	↑ 1 study found increased IEQ in treated group (liraglutide[16])

↔: No difference in outcome between treated and non-treated islets; ↑: increase of the outcome in treated islets; ↓: decrease of the outcome in the treated islets. ECM: extracellular matrix components; GSIS: glucose stimulation insulin secretion; IEQ: islets equivalents. EP1013: N-benzyloxycabonyl-Val Asp-fluoromethyl ketone [zVD-FMK]; IDN-6556: caspase inhibitor; F573: pan-caspase inhibitor; PACAP: pituitary adenylate cyclase-activating peptide; FBS: fetal bovine serum; HSA: human serum albumin; SRM: silicone rubber membrane; CM: collagen matrix; FPCM: human fibro-blast-populated collagen matrix; Z-DEVD-FMK: Z-Asp (OMe)-Glu (OMe)-Val-Asp (OMe)-fluoromethylketone; LSF: lysofylline; GDNF: glial cell line-derived neurotrophic factor; FCS: fetal calf serum; GLP-1: glucagon-like peptide-1; HS: human serum; PDC: perfluorodecalin; IAC: bis (1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl) decantionate; GW3965: synthetic nonsteroidal liver X receptor (LXR) agonist; MnTMPyP: SOD mimetic manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin.

6556[33] additives. Four studies did not observe any difference in viability between additive-treated and non-treated islets. The additives tested in these 4 studies were: polyphenol (green tea extract);[19] sirolimus;[23] SOD mimetic manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP),[24] and synthetic non-steroidal liver X receptor (LXR) agonist (GW3965).[26]

Regarding apoptosis, P38 inhibitor SD-282[25] and GW3965[26] additives had no significant effect on this outcome, while caspase-3 inhibitor Z-DEVD-FMK,[17] lysofylline (LSF),[27] IDN-6556,[33] F573,[35] and glial cell line-derived neurotrophic factor (GDNF)[34] additives were associated with decreased apoptosis rates compared with control condition.

Eleven of the 12 studies in the "antiapoptotic/anti-inflammatory/antioxidant" group evaluated GSIS. Islets treated with Z-DEVD-FMK,[17] radical scavenger IAC,[18] sirolimus,[23] GW3965,[26] LSF,[27] GDNF[34] and F573[35] additives had increased GSIS compared with non-treated islets. In contrast, one study showed that the MnTMPyP[24] additive added to the culture media decreased GSIS in comparison to the control group. Three other studies found no significant effects of polyphenol (green tea extract),[19] SD-282[25] and EP1013[32] additives on GSIS.

Nakano *et al.*[17] reported that Z-DEVD-FMK also was associated with an increase in IEQ in relation to the non-treated group, which was in agreement with a protective effect of this additive against apoptosis. The other study that evaluated IEQ showed no effect of SD-282 on this outcome.[25]

Based on above-mentioned studies, it is possible to suggest that some additives with "antiapoptotic/anti-inflammatory/antioxidant" effects added to the islet culture media have the potential to improve GSIS and decrease apoptosis (Table 4).

Results of studies that evaluated "hormones" additives

Six studies reported the effects of hormones added to the culture medium on islet outcomes (Table 4). Viability was evaluated in 3 studies. Exendin-4[22] and pituitary adenylate cyclase-activating peptide (PACAP)[29] additives were associated with increased viability compared with control conditions. In contrast, liraglutide[16] did not modify islet viability. Moreover, glucagon-like peptide-1 (GLP-1)[21] and estradiol[28] were able to decrease apoptosis rates compared with non-treated islets. Liraglutide added to the culture medium did not alter apoptosis rates.[16]

Four studies analyzed the effects of culture medium additives on GSIS (Table 4). Islet treated with GLP-1[21] or Exendin-4[22] had an improvement in GSIS compared with non-treated islets. Liraglutide[16] and PACAP[29] had no significant effect on this outcome. However, liraglutide in the culture medium of islets was associated with higher IEQ than the control condition.[16]

Results of studies that evaluated "sulphonylureas" agents

Only 2 studies by Del Guerra *et al.*[31] and Maedler *et al.*[47] added "sulphonylurea" agents to the culture media. Del Guerra *et al.*[31] showed that glimepiride, glibenclamide, and chlorpropamide had no effect on apoptosis rate but decreased GSIS. Maedler *et al.*[47] reported that repaglimide, nateglimide and glibenclamide caused an increase in islet apoptosis, but only glibenclamide was able to increase GSIS. Viability was not assessed in these studies.

Results of studies that evaluated "serum supplements"

Six studies analyzed "serum supplements" added to the islet culture media: 3 evaluated viability, 3 evaluated apoptosis and 5 evaluated GSIS. Regarding viability, 2 studies found an increase in viability when using fetal bovine serum (FBS)[48] or AB serum[49] while one study found no difference between islet cultured with FBS or human albumin.[51] The 3 studies that analyzed apoptosis showed that AB serum,[49,53] and human serum[52] decreased apoptosis. These 3 supplements were also able to improve GSIS.[49,52,53] Two other studies reported no difference on GSIS when comparing human albumin *vs.* AB serum[50] or FBS *vs.* human albumin.[51]

Results of studies that evaluated "scaffolds or extracellular matrix components"

Eleven studies were included in the group "scaffolds or ECM components." Of them, 6 evaluated viability, one analyzed apoptosis, and 9 evaluated GSIS. In relation to viability, 4 studies demonstrated an improvement in this outcome when culturing islets with collagen I/IV, fibronectin and laminin,[38] collagen human fibroblast-populated collagen matrix (FPCM),[46] on a silicon rubber membrane[40] or on a fibroblast matrix.[42] Two other studies found no differences in viability between groups.[41,45] Only one study in this group evaluated apoptosis, showing a reduction in this outcome when using a perfluorodecalin (PDC)-enriched fibrin matrix.[41]

Regarding GSIS, 6 studies demonstrated an improvement in insulin secretion when islets were cultured on gas-permeable membranes,[36] collagen I with or without ECM components or micro-fabricated scaffold with ECM,[39] PDC-enriched fibrin matrix,[41] fibroblasts,[42] pancreatic duct-derived epithelial cells,[44] and collagen or FPCM.[46] Two studies were not able to find any differences between experimental groups,[37,43] and one study observed a decrease in GSIS when islets

were cultured on collagen I/IV, fibronectin and laminin matrixes.[38]

Discussion

Since the initial era of islet isolation and transplantation, many advances have been achieved in respect to the islet isolation process and its standardization, and also a better knowledge of the handling and implantation (transplantation) of the islets was acquired. Despite these improvements, islet loss during isolation, culture period and right after implantation still represent a barrier for a widespread utilization of this therapy. There is evidence to link early graft loss following islet transplantation to isolation-induced β-cell apoptosis.[15] It has been established, *in vitro*, that apoptosis participates in the death of freshly isolated islets cultured under standard conditions and it might be related, in part, to anoikis and lack of growth factors.[55-58]

The ability to maintain isolated islets in culture have been essential for the improvements of islet transplantation outcomes.[59] Factors that may augment or even preserve β-cell mass are of particular interest in the field of islet transplantation because, not unfrequently, the number of viable islets isolated from one pancreas is not sufficient to perform the transplant.[3,60]

As shown in this systematic review, many additives, ECM components and scaffolds have been investigated as potential agents to increase or preserve islet mass before and after transplantation.[61-68] For a better analysis in this systematic review, we classified the additives, ECM components and scaffolds used during culture of human islets in groups according to its main mechanism of action. Regarding "antiapoptotic/anti-inflammatory/antioxidant" additives, 3 studies[32,33,35] were able to show an improvement in viability, 5 studies[17,27,33-35] demonstrated a reduction in apoptosis rate and 7 studies showed an increase in GSIS.[17,18,23,26,27,34,35]

The research group from University of Alberta (Edmonton, AB, Canada) has shown, in 3 different studies,[32,33,35] the improvement of viability when adding anti-apoptotic additives in culture media of human islets. Especially during culture time, it seems that the use of caspase inhibitor additives has the ability to distress human islets leading to substantial reduction in cell death; thereby, improving viability and reducing islet mass required for transplantation. Nakano *et al.*[17] showed that caspase-3 has a crucial role in apoptosis of human islets immediately after isolation and that its inhibitor ameliorates the function of isolated islets. Moreover, the caspase-3 inhibitor Z-DEVD-FMK prevented apoptosis in a dose-dependent manner and also improved islet yield.[17] According to these findings, Yang *et al.*[27] showed that *in vitro* short-term treatment with LSF enhanced human islet metabolism and β-cell insulin secretion, also reducing apoptosis as compared with the control group. These effects were associated with promotion of mitochondrial metabolism since mitochondrial function regulates β-cell insulin secretion and controls the end point of apoptosis. Unexpectedly, this occurred through inhibition of TNF, which induces apoptosis in β-cells through suppression of caspase-8 pathway but not through caspase-3, contradicting the results of Nakano *et al.* In addition to an antiapoptotic effect, studies that used other "antiapoptotic/anti-inflammatory/antioxidant" additives in the human islets culture also have shown an improvement in GSIS.[17,18,23,26,27,34,35] However, the study that used sirolimus in islet culture showed no improvement in viability or GSIS.[23] Sirolimus is an immunosuppressive drug that inhibits IL-2 pro-inflammatory cytokine and, consequently, inhibits the activation and proliferation of T lymphocytes through mTOR.[23] This result should be expected by the authors since sirolimus is anti-proliferative and decreases insulin secretion.[69]

In relation to "hormones," some of them seem to enhance islet "health" Sakuma *et al.*[29] observed a significant increase of viability (4.2%) in islets cultured with PACAP. Miki *et al.*[22] verified that exendin-4 increased islet viability up to 1.85 fold in relation to the control group. However, it is uncertain to what extent these increases in viability are clinically relevant. It is known that viability above 80% is a release criterion to perform islet transplantation and that the majority of the isolations reach viability post-culture above 80% with the standard culture media. So, we still do not have the answer if, above 80%, small increments in viability may impact outcomes.

Besides viability, some "hormone" additives enhanced GSIS and reduced apoptosis. We ought to highlight results from studies using GLP-1 analogs, a 30-amino-acid peptide hormone secreted from the L-cells of the intestinal epithelium in

response to meals. Its analogous (exendin-4 and liraglutide) were approved as a therapy for type 2 diabetes, since they enhance glucose-stimulated postprandial insulin release, and inhibit inadequate glucagon secretion and gastrointestinal motility.[70,71] Lately, anti-inflammatory, antiapoptotic and cytoprotective properties of the GLP-1 analogues have been revealed, opening new therapeutic perspectives for this class of drugs.[72-75]

Farilla *et al.*[21] demonstrated that GLP-1 analogues delayed the morphological changes that occurs in human islets in culture, as indicated by a longer-lasting preservation of their 3D structure. GLP-1 analogs also promoted an increase in expression of the antiapoptotic protein Bcl-2 and a downregulation of the active form of caspase-3.[21] Moreover, these authors verified that GLP1-treated islets contained more insulin and were capable of a greater glucose-dependent insulin secretion.[21] Miki *et al.*[22] showed that exendin-4 supplementation in the culture media significantly reduced pro-inflammatory cytokine/chemokine production from human islet preparations and improved β-cell survival through increased Erk2 phosphorylation, which may be helpful for possible β-cell proliferation after islet transplantation.

Regarding "sulphonylurea" additives, 2 studies were found.[31,47] These oral hypoglycemic agents reduce blood glucose levels by stimulating insulin release from β-cells.[76] Their actions occur through ATP-sensitive potassium (K-ATP) channel, fundamental to the control of β-cell function.[76,77] Based on their mechanism of action, it is expected that the use of sulfonylureas in islet culture media would improve, at least, insulin secretion, as seen in Maedler *et al.*[47] study but not by Del Guerra *et al.*[31]

On the topic of "serum supplements," the use of AB serum,[49,53] and human serum[52] decreased apoptosis and increased GSIS when compared with HSA, the most widely used serum supplement. AB serum has its rational based on serum derived from AB blood donors, making it less immunoreactive. AB serum[49] and FBS[48] improved viability compared with HSA, also suggesting that the quality of clinical islet preparations might be improved when culture is performed in media supplemented with serum instead of albumin.

Scaffolds and ECM components had positive effects on islets in many studies, especially related to viability,[38,40,42,46] and function.[34,37,39,40,42,44] In general, this group of cell culture modifications transmits a variety of chemical and mechanical signals to the islets, mediating key aspects of cellular physiology, such as adhesion, migration, proliferation, differentiation, and death.[78] Probably for these reasons the "scaffolds and ECM components" group showed a good performance on the evaluated outcomes.

An emerging strategy to improve islet viability and function and, thus, graft survival, involves the co-culture of pancreatic islets with mesenchymal stem cells (MSCs). This topic was not included in this study because our group has recently published a systematic review and meta-analysis on it.[79] Souza *et al.*[79] evaluated 20 studies of co-culture of human islets with MSCs, showing that the co-culture with MSCs improved both islet viability and GSIS compared with islets cultured alone. Thus, this co-culture system has the potential for protecting islets from injury after isolation and during culture period.

The results of this systematic review should be interpreted in the context of some limitations. First, the fact that studies included in the systematic review were experimental studies. Second, most additives and scaffolds/ECM components were tested only once, not been replicated in other studies, making difficult to draw firm conclusions. Third, despite the increased number of human isolation facilities inaugurated around the world in the last decade, allowing studies in human islets, most of the studies with additives, ECM components and scaffolds were performed in murine islets, which was not the scope of our systematic review. These facts limited the number of included studies. Fourth, small variation in the composition of the standard culture media used in different clinical centers might have influenced the results of the analyzed studies; however, this information was not described in the articles.

This systematic review results allowed us to draw a better picture on the effect of additives and scaffolds/ECM components in culture of human islets. Overall some "antiapoptotic/anti-inflammatory/antioxidant" additives appear to offer an increment in islets outcomes after culture period by improving GSIS and reducing apoptosis. Moreover, culture of islets on scaffolds or ECM components is able to improve GSIS. More studies, especially with human islets, are needed to define the real impact of these therapeutic strategies in improving islet transplantation, as well as the combination of more than one approach.

Material and methods

Selection criteria and search strategy

PubMed and Embase repositories were searched to identify all articles that analyzed the effect of additives added to the culture medium of human pancreatic islets on IEQ, viability, apoptosis and/or GSIS outcomes. In addition, studies that cultured islets on different scaffolds or with ECM components were also selected for inclusion. The following medical subject headings (MeSH) were used for this search: ("Cell Culture Techniques" OR "Primary Cell Culture" OR "Batch Cell Culture Techniques") OR ("Culture Media" OR "Culture Media, Conditioned" OR "Culture Media, Serum-Free") OR ("Tissue Scaffolds") OR ("Extracellular Matrix" OR "Extracellular Matrix Proteins") AND ("Islets of Langerhans Transplantation" OR "Islets of Langerhans"). The search was restricted to human islet studies and it was completed on January, 2017. All articles identified were also searched manually to detect other relevant citations. This systematic literature search was designed and described in agreement with current guidelines.[80]

Study selection and data extraction

Eligibility evaluation was made by 2 pairs of independent investigators (A.C.B. and N.E.L.; A.P.B. and J. R.), through title and abstracts reviews. When abstracts did not provide sufficient data, the full text of the paper was retrieved for analysis. Disagreements were resolved by discussion between the investigators and, when required, a third reviewer (DC) was consulted. Articles were excluded from the systematic review if: 1) were published before 1988 (data of publication of Ricordi's semi-automated technique);[54] 2) did not use human pancreatic islets; or 3) were review articles, letter or abstracts without description of results (Fig. 1). If data were duplicated and had been published more than once, the most complete study was chosen.

Information of interest from each study was independently extracted by 2 investigators (N.E.L. and L.A.B.) using a standardized extraction form and consensus was sought in all extracted items. When consensus could not be achieved, differences in data extraction were decided by reading the original publication. The data extracted from each study were as follows: (1) general characteristics of the studies, including name of first author and publication year; (2) brain-dead donor characteristics, such as age, gender, cold ischemia time (CIT) of the pancreas; (3) pre-culture islet isolation characteristics as purity and total IEQ; (4) additives or scaffolds/ECM components used and their concentrations (5) post-culture outcomes of interest: number of IEQ, purity, viability, apoptosis and function (assessed by GSIS).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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