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Review Article

Perspective in 3D mesenchymal stromal cells as tools for potential diabetes treatment

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Abstract

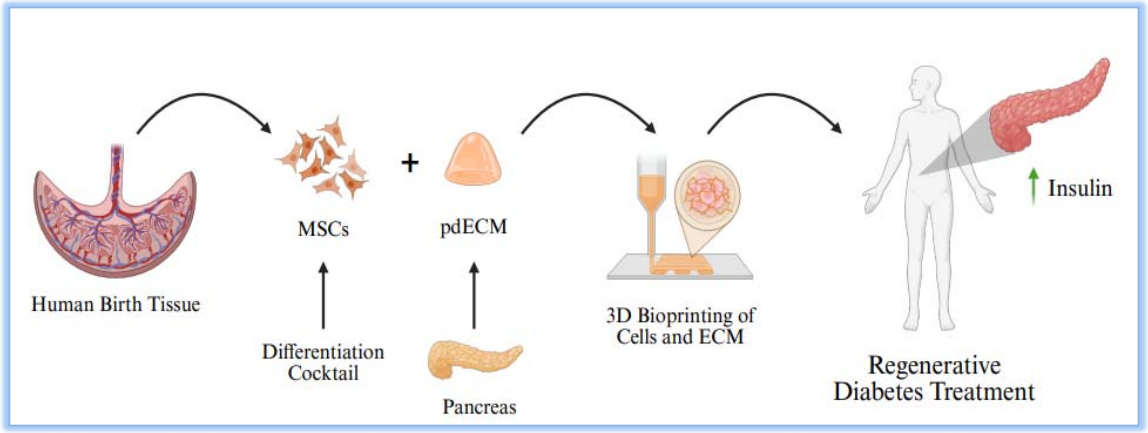
Background and purpose: Diabetes represents one of the major global health issues, and the number of cases is expected to increase dramatically in the coming years. In the new century, many strategies have been developed to meet the urge for new therapies through technological innovations. Innovative pharmacological treatments, such as electronic insulin pumps, represent one of the gold standards for diabetes, even if cell replacement therapy is preferred in the most severe cases. Mesenchymal stromal cells (MSCs) can be differentiated into insulin-producing cells or islet-like cell clusters. Consequently, understanding the role of the extracellular matrix and three-dimensional (3D) structure in guiding MSC differentiation is crucial for advancing regenerative therapies.

Experimental approach: The literature was examined using Google Scholar, PubMed, and Scopus, analyzing the pertinence with the focus of this manuscript.

Findings: MSCs derived from birth tissues are characterized by a high level of plasticity and represent the best candidates for regenerative treatments. Together with MSCs, 3D approaches can be integrated to obtain complex structures supporting many biological requirements, namely the possibility of reproducing better physiological conditions, as well as supporting vascular network development. Furthermore, the incorporation of extracellular matrix components like laminin and collagen IV into hydrogels has been shown to enhance the insulin secretion and survival of insulin-producing cells.

Conclusions and implications: In this review, we present an overview of the different strategies applied as of today in regenerative medicine, by unraveling the different cellular and matrix characteristics that can be crucial for future and potential diabetes treatment. Ultimately, the convergence of MSC biology with advanced gbioartificial pancreas to treat diabetes.

Keywords: 3D culture; β cell differentiation; Diabetes; Extracellular matrix; Hydrogels; Mesenchymal Stromal cells.



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1. INTRODUCTION

In the last decade, diabetes cases increased dramatically (1). This disease can be divided into three different types, namely type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes. These are characterized by hyperglycemia due to a lack of insulin production from β cells and/or insulin resistance. Notably, in T1D, the pathological landscape implies the destruction of insulin-producing β-cells caused by an autoimmune reaction (2). In T2D, both defective insulin secretion and decreased tissue sensitivity to insulin occur. This metabolic imbalance is usually related to risk factors such as overweight, lack of physical activity, history of other diseases, and age (3). Gestational diabetes is a condition that usually arises during pregnancy, caused by insulin resistance of the tissues of the mother, caused by the release of placental hormones (4). The consequences of diabetes imply long-term damage that can occur to different organs, such as the eyes, kidneys, and heart (5), thus representing a serious economic burden worldwide (6).

The number of cases is rising not only among adult patients but also among children. This number is expected to substantially increase in future decades, according to the SEARCH study (7).

Currently, available pharmacological treatments rely mainly on insulin and metformin administration. Although side effects are relatively rare, these therapies imply long-term treatment and do not represent a cure. We previously described the growing interest in G-protein-coupled receptors (GPCRs) as targets to better manage and prevent diabetes, particularly in children (8). Multi-peptide drugs are being developed to act simultaneously on multiple receptors and better address several molecular processes involved in the pathophysiology of diabetes. Novel *in vitro* models would be highly beneficial to understand these mechanisms and to develop new therapies. Animal models such as rodents still represent the best choice for *in vivo* studies of diabetes, despite ethical limitations. However, recent technological developments in biomaterials are rapidly improving the opportunities to study the many different *in-vitro* aspects of diabetes. As a surrogate of β-cells, researchers often used the rat insulinoma cell line, INS-1. In particular, INS-1 832/13 responds to glucose oscillations in a wide range (2.8-16.7 mM), comparable to primary rodent β-cells (9) although not as dynamically as human β-cells (10). The effective use of stem cells for autotransplantation has been recently reported for MSCs derived from bone marrow, for the treatment of T1D in humans: autologous stem cell therapy was safe, effective, and partially reversed autoimmunity characteristic of T1D (11). Despite this successful example, stem cell therapy for diabetes treatment is quite far from being applied in humans as a safe and consolidated therapy; thus, new strategies in this sense need to be developed. It is becoming increasingly appreciated how 3D cell culture may fill the gap between 2D cell culture and animal models and provide experimental models that better resemble the *in vivo* cell environment, remaining more versatile and affordable than animal experimentation (12). At the same time, the ability to reproduce the original microenvironment is also fundamental for replacement therapies. Replacement therapies for diabetes treatment consist of the infusion of functional insulin-producing cells,

generated from stem cell differentiation or by isolation from a donor (13). Moreover, extensive efforts have been dedicated to creating pseudo-islets by developing scaffolds. Artificial and decellularized tissues have been tested to provide a suitable structure that better supports β-cells and other endocrine cells. 3D cultures imply different approaches, such as spheroids and organoids (12). For example, 3D INS-1 cell spheroids display better *in vivo* features than 2D cultures; Ntamo *et al.* confirmed by toluidine staining an increase in active cellular turnover and increased metabolic activity in the 3D group, which is strongly correlated to insulin and amylin production in response to glucose (14).

Current diabetes treatments are not fully effective in the replacement of β-cell function, and by artificially developing living functional tissues, it could be possible to address the demand for replacement therapy of the pancreas, in part or as a whole (15). With this perspective, 3D bioprinting is an advanced technology that enables the creation of 3D bioartificial constructs capable of restoring the intricate structure and proper functionality of complex tissues (16). Consequently, bioprinting is rapidly gaining acceptance worldwide as a promising approach embraced by doctors and researchers to enhance the quality of life for patients affected by various diseases and conditions, as 3D bioprinting of skin for burned skin replacement, bone bioprinting for replacement of lost or damaged bone tissue, but also for cancer research by 3D bioprinting models to improve the understanding of cancer development (17). Satisfactory results implying the bioprinting of cells for diabetes treatment have been achieved with the implantation of 3D-pancreatic islets, embedded in pancreatic extracellular matrix (pdECM) and hyaluronic acid methacrylate, into diabetic mice, leading to increased insulin secretion and stable blood glucose levels (18). Although in literature, different examples are reported of functional 3D organs (19) and especially the pancreas (20), reconstructing the exact architecture and cellular composition of the native tissue represents an unmet challenge. For this reason, one of the foremost requirements for 3D is the hydrogel or matrix, a substance that typically consists of biocompatible materials and bioactive components, such as growth factors and signaling molecules (21). The literature proposed in this manuscript was extracted from Google Scholar, PubMed, and Scopus, utilizing the following search terms: diabetes, 3D bioprinting, MSCs differentiation, matrix, hydrogel, and pdECM. This review presents the different strategies that can be applied for the generation of a 3D pancreatic tissue to increase the knowledge of potential diabetes treatment, focusing on the differentiation potential of MSCs and the required matrix characteristics.

2. Challenges in β-cells differentiation

B-cell dysfunction for inadequate glucose-sensing and deficits in β-cell mass are identified as the leading causes of diabetes onset. Since β-cells' regenerative capacity is almost null during adulthood, new strategies point to differentiating β-cells from other cellular sources (22). Three types of stem cells can be identified: embryonic, adult, and induced pluripotent stem cells (iPSCs). Excluding the embryonic source for ethical problems and iPSCs for risk of teratoma development, MSCs are the most promising source of plastic cells.

Mesenchymal stromal cells are adult and multipotent cells that can be extracted from different tissues within the body. These cells have been reported to retain highly immunomodulatory properties and participate in physiological tissue regeneration and repair. MSCs are characterized by the possibility to differentiate in vitro into osteoblasts, adipocytes, and chondrocytes (23). A literature survey revealed that many attempts have been reported to push their differentiation outside the mesodermal lineage. For this reason, considering their well-established multipotency in terms of stemness, whether these should be considered multipotent or pluripotent remains debated (24). Moreover, MSCs offer the possibility to differentiate cells derived directly from the patient, excluding the risk of rejection (25). Successful differentiation into β-like cells has been reported for MSCs extracted from dental pulp, umbilical cord, menstrual blood

(25), bone marrow (26), adipose tissue, urine, Wharton's jelly, placenta, amniotic fluid, cord blood, and pancreas (27). MSCs derived from birth tissues represent the best choice for β-cell regeneration due to the great immunomodulatory capacity and the higher degree of plasticity in comparison to MSCs derived from adult tissues (27). These sources offer many advantages, including the absence of ethical implications and non-invasive procedures for their collection. Particularly, Wharton's jelly-derived-MSCs have been reported to show anti-inflammatory effects following intravenous infusion in T2D patients, improving β-cells function and glycated hemoglobin levels (27). MSCs are also reported overcome the β-cell destruction caused by immunological attack of autoreactive T cells, characteristic of T1D (28). In the last two decades, β-cell replacement by intrahepatic islet transplantation has been an option for T1D treatment, although this procedure requires lifelong treatment with immunosuppressive drugs. Being an autoimmune disease, the additional challenge of the transplantation approach for T1D is to avoid not only the immune rejection directed to the donor's cells but also the autoimmune effects toward these cells (29). Although immune rejection is not critical in the case of MSCs usage for autotransplantation, it is still not clear if the autotransplant could eliminate in the long term the immune reaction characteristic of T1D. The lack of knowledge about the molecular mechanisms by which MSCs contribute to T1D treatment (also considering variables such as the number of MSCs infused, frequency of injection, and best infusion route) does not permit a full translation of the treatment to a large-scale (28).

Regarding MSC differentiation as tools for diabetes treatment, protocols for β-cell differentiation from MSCs involve a first differentiation step into pancreatic progenitors and a second step for β-cell maturation. These phases imply different culture conditions, such as the use of a high glucose medium and different factors such as nicotinamide, exendin-4, and glucagon-like peptide 1 (GLP1). Although many groups reported the attempt for β-cell differentiation, very few reported functional glucose-stimulated insulin response and homogeneity in terms of pancreatic marker expression, suggesting that the different protocols proposed led to the differentiation of a non-physiological population of pancreatic endocrine cells. Different pathways are reported to regulate MSC differentiation into β cells, such as bone morphogenetic protein (BMP), Wnt, Nodal, Notch, retinoic acid, epidermal growth factor (EGF), and fibroblast growth factor (FGF) (27). Substrates related to EGF, such as phosphoinositide 3-kinase (PI3-kinase) and hepatocyte growth factor (HGF) signalling pathways, have been demonstrated to strongly influence not only differentiation but also proliferation and cell survival of pancreatic lineage cells (27). In the context of the signalling pathway, reagents that promote β-cell differentiation or proliferation play an important role in potential diabetes treatment. Exendin-4, a GLP1 analog approved as a therapy for patients affected by T2D (30), has been demonstrated to induce β-cell proliferation in EGF-receptor (EGFR) knock-out mice. GLP1 exerts its therapeutic action through the interaction with GLP1-receptor (GLP1R), a member of the GPCR receptor family, acting through increasing cAMP levels inside the cell and activation of PI3-kinase as a downstream effector. Although the precise mechanism of action of GLP1 that leads to β-cell proliferation remains unclear, it seems that GLP1R relies on EGFR to promote proliferation (31). As reported by Svendsen *et al*. (32), intra-islet signaling is another factor influencing the β-cell secretory responses, and particularly the fine-tuned balance between the glucagon and GLP1 receptors is pivotal to maintaining adequate insulin secretion. Undoubtedly, unraveling the signaling pathway that controls such a transition could lead to the development of new innovative therapies for diabetes treatment.

From a transcriptional point of view, it is established *in vitro* that the co-expression of critical transcription factors such as PDX1 and NKX6.1 is crucial for β-cells establishment, even if the exact molecular mechanism of glucose-stimulated insulin secretion (GSIS) in cells influenced by these factors remains

undefined (27). Xu *et al.* demonstrated by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and immunocytochemistry that MSCs in 3D culture express higher PDX1 and insulin compared to the 2D control group, suggesting the impact of the extracellular matrix on their differentiation (33). The stiffness of the matrix is another factor that could influence MSC differentiation, inducing the expression of lineage-specific markers depending on the cultivation of soft or hard matrices. This is reasonable because during their physiological development, β-cells are sensitive to the external stimuli of blood shear stresses as they are part of Langerhans islets, which are highly vascularized (34). Although in the literature, no specific information is reported regarding the influence of shear stress and matrix stiffness on MSCs differentiation to β-cell lineage, it is reasonable that these aspects could play an important role in this process.

The lack of adequate vascularization represents another obstacle related to islet transplantation (35). In fact, after the transplantation, the absence of a vascular system not only limits the oxygenation, nutrient diffusion, and clearance of waste materials but also impairs the sensitivity of pancreatic islets to glucose fluctuation and thus insulin release (36). MSCs secrete pro-angiogenic factors (*i.e.,* FGF-2, vascular endothelial growth factor, and interleukin-6) (37,38) and could thus promote tissue vascularization upon incorporation in 3D constructs for islet transplantation. In diabetic rats, MSCs have been demonstrated to promote vessel development and support the survival of transplanted islets (39). Moreover, to improve long-term engraftment after transplantation, Kim *et al.* co-cultured islets together with human umbilical vein endothelial cells (HUVECs), given the previous evidence of positive effects of endothelial cells on the paracrine functions of islets. The co-cultures *in vitro* improved both responsiveness to glucose and the amount of secreted insulin, also reducing the necrosis rate (40), suggesting that the co-culture with these cells could be a winning strategy for improving 3D β-cell culture conditions.

Human islet-like organoids from induced pluripotent stem cells have been generated and represent an important field of research (41). Despite these later advances, the functional maturity of cadaveric islets is still outperforming all attempts made so far to differentiate β-cells i*n vitro.* Could improved hydrogels and 3D culture fill the gap?

3. 3D matrices and hydrogels for β-cells and pseudo-islets cells

β-cell activity depends on the ECM and the different cell types present in the islet of Langerhans: i.e., glucagon-producing α cells, somatostatin-producing δ cells, pancreatic peptide-producing cells, ghrelin-producing ε cells, and endothelial cells, which represent the tissue interface with the blood flow. Evidently, the achievement of fully functional β-cells implies the recreation of appropriate spatial distribution and communications among these cell populations. An artificial recreation of this architecture appears still distant. However, several groups developed different matrices to support the islets' survival and functionality in 3D culture, as summarized in Table 1.

The ECM defines the architecture of the organ by interacting with adhesion molecules present on the cell membrane, such as integrins, which, together with cell-cell interactions (connexins, cadherins, N-CAM), produce intracellular signaling that adds to paracrine cues (18,41-49). Specifically, for the pancreas, its ECM is composed of almost 30% collagen, particularly type IV and VI (50) in addition to fibronectins, elastins, and laminins-211 and -511 (51). β-Cell interaction with collagen is mediated by β1 integrin subunit combined with α6 or α3 subunits (52). In INS-1 cells, the interaction between collagen IV-α6 β1-integrin was reported to be instrumental in organizing the cytoskeleton and the exocytotic machinery (52,53).

Table 1. Summarizes different 3D approaches reported in the literature for the culturing of islets/β-cell like, with the type of matrix and their effects, and the final result.

Type of matrix	Cell type	Matrix effects	Result	Reference No.
pdECM	HUVECs, human-derived and rat-derived pancreatic islets	Higher level of GSIS compared to other biomaterials (i.e., collagen and alginate)	Co-culture in vitro and 3D bioprinting for an applicable transplant size that can represent an allogenic source of islets	(40)
Hyaluronic acid methacrylate-pdECM	Rat-derived pancreatic islets	HAMA-pdECM promoted the expression of insulin and glucagon	3D-printed islet organoids implanted in diabetic mice induced a mild immune response, also promoting vascularization	(18)
Alginate-pdECM and alginate-fibrinogen	Porcine pancreatic islets, HUVECs and human MSCs	Promotion of the secretion of insulin upon stimulation and of the vascularization	3D bioprinting of hydrogel scaffolds characterized by high shape fidelity and open porosity	(43)
ECM-alginate	Human pancreatic islets	Increase in islets' functionality for islets encapsulated compared to the unencapsulated islets	Manipulation of the hydrogel to improve mechanical strength to increase the success rate of encapsulated islet transplantation	(44)
Alginate-human adipose ECM	Murine pancreatic beta islet cells (MIN6 β cells)	ECM improved the viability of encapsulated MIN6 β cells compared to the alginate-only control	AEC microcapsules can be exploited as a platform to support islet cells for potential T1D treatment	(45)
3D silk matrix functionalized with ECM-derived motifs	Human-derived and mouse-derivedislets	No influence of silk matrix upon insulin secretion during glucose stimulation	Develop a functionalized matrix to support ex vivo expansion of endocrine cells	(46)
Functionalized spider silk	Human-derived and mouse-derived pancreatic islets	Human islets can adhere to silk foam, maintaining their function after long-term culture, developing vascularization and promote the formation of new insulin-positive islet-like clusters	Following islet transplantation into the anterior chamber of the mouse's eye, the matrix was preserved without causing side effects and islets in spider silk foam show improved engraftment	(47)
Type 1 collagen	Mouse dermal fibroblasts and mice-derived pancreatic islets	A collagen matrix improved islet survival and function	Fibroblast-populated collagen matrix improved long-term isograft function in chemically induced diabetic mice	(48)
Collagen-tannic acid	INS1E cells	ColTa spheroids improved insulin secretion in response to glucose compared to 2D culture	High-throughput method for cell-laden microsphere production	(49)

pdECM, Pancreatic extracellular matrix

The details of the interactions established by the different components of the ECM and islet cells have been reviewed by Llacua *et al*. (54). During islets' enzymatic isolations, ECM natural components are disrupted, affecting cell viability and transplant outcomes (55). While cell-cell interaction is re-established relatively rapidly, the restoration of the ECM is slower and requires endothelial cells. Hydrogels obtained from different tissues, including the pancreas, have been tested to surrogate the peri-insular basement membrane (56-59). On the other hand, protocols that differentiate progenitor cells into multiple cell types, based on the differentiation of progenitor cells in hand, are expected to secrete their own ECM

and thus better recapitulate the original. In-vitro differentiation will unlikely recreate the architecture of the islet with endothelial cells surrounding the endocrine component and secreting the basal membrane interposed between the acinar. 3D printing of a structure that spatially guides differentiation in space may allow us to achieve this goal in the future.

To date, hydrogels developed from pdECM appear advantageous, not only compared to 2D cultures on treated plastic (59), but also compared to collagen alone, demonstrating better control of insulin secretion in response to the extracellular glucose level (40). The effect of ECM on MSC differentiation towards insulin-producing cells remains to be characterized, whereas, in vivo, the importance of ECM in controlling self-renewal and proliferation in the staminal niche has been established. Particularly, integrins have been demonstrated to control stem cell differentiation (60). The availability of human pancreatic samples is limited. Most studies took advantage of porcine-derived pdECM. Particularly, porcine pdECM represents a promising alternative for its capacity to replicate tissue-specific composition, maintaining the proteomic characteristics and other bioactive components that are naturally present in the native matrix (59). The literature reports different pdECM extraction protocols from the porcine pancreas with common main steps (40,61,62). Briefly, the pancreas is minced and rinsed in deionized water. Then, a decellularization step is undertaken, mainly by utilizing detergents such as Triton® X-100 or sodium dodecyl sulfate (SDS). The matrix is then rinsed with PBS to clear waste materials and detergents. The resulting matrix is subsequently frozen, lyophilized, pulverized, and digested with pepsin in an acidic solution to finally form a hydrogel at 37 °C after neutralization and salt balancing. Pepsin treatment is not only aimed at increasing pdECM solubilization but also allows efficient binding of cells to the matrix, for the cleavage of collagen at telopeptides (63). The whole extraction procedure should be conducted in a cold room to reduce native pancreatic protease activity. At the end of this process, spectrometric analysis of the dsDNA amount allows us to check for successful pdECM decellularization. Cell viability assays, such as the MTT assay, must be conducted to check the toxicity of the matrix due to reagents leftover from the decellularization steps, which could interfere with cellular viability. Dimethyl-methylene blue assay and hydroxyproline assay, showing glycosaminoglycans (GAGs) and collagen presence, respectively, must be performed to assess the pdECM general composition.

One example of positive pdECM components effects comes from Weber *et al.*, who reported greater GSIS in β-cell culture when polyethylene glycol (PEG)-based hydrogel was supplemented with laminin and type IV collagen, reaching a 350% greater release with laminin, and a 75% increase in the presence of type IV collagen (64). Previous studies also reported that collagen type IV promotes insulin production and cell survival of human islets (65). Laminins, heterotrimeric glycoproteins, have been demonstrated to induce the expression of islet transcription factors, such as PDX1, but also to activate Akt and extracellular-signal-regulated kinase (ERK), important factors for the induction of β-cell differentiation from precursor cells (54). Particularly, when laminin was exploited in combination with collagen IV, three specific laminin sequences, RGD, LRE, and PDSGR, had a positive impact on islet functionality and viability by reducing islet cytokine-mediated cell death (55). The ECM not only provides structural support, spatial organization, and related signals but also preserves and presents signaling molecules, also sensitive to their synthesis and degradation exerted by enzymes released from embedded cells, whose survival is ensured by the correct viscoelasticity of the matrix itself (66).

4. CONCLUSION

Undoubtedly, the allotransplantation of pancreatic islets represents the safest approach for the worst cases of diabetes patients, albeit many limitations characterize this treatment as poor graft survival and the need for multiple donors. Different factors contribute to the failure of engraftmen after islet

transplantation, notably the lack of adequate vascularization, immunological responses, and the disruption of pancreatic ECM-specific factors due to the enzymatic dissociation. The winning strategy for 3D pancreatic tissue is represented by the utilization of patient-derived cells and to reproduce the best pancreatic cellular population, and a supportive cellular compartment is also required. With this perspective, 3D constructs not only represent a tool for studying and delineating molecular mechanisms related to diabetes insurgence but also offer the chance to develop possible implantable devices as a potential treatment. Recently, there has been significant progress in cell encapsulation and 3D culture methods for diabetes therapeutic approaches. Islet cells have shown higher activity and insulin secretion in constructed models. However, several challenges remain for the development of implantable bioartificial pancreases. MSCs in this sense could offer an untapped source for the potential generation of β cells, although huge efforts still need to be made to reach functional maturation. Challenges related to nutrient transport, vascular network formation, matrix biocompatibility, and implanted bioartificial organs' stress resistance can be addressed with more advanced 3D techniques, particularly with modern 3D bioprinters. Ultimately, 3D cultures offer a solution to the shortage of donor organs and various medical challenges in the field of complex organ engineering and manufacturing. These results suggest that soon the treatment of diabetes could be transferred from the pharmacological approach to the generation of an artificial biological pancreas as a potential tool for islet transplantation.

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6. Conflict of interest statement

All authors declared no conflict of interest in this study.

7. Authors' contributions

A. Dallatana and D. Coato contributed to the conceptualization of the study and drafted the manuscript. G. Innamorati contributed to the manuscript editing. L. Cremonesi and L. Giacomello contributed to the manuscript review. The finalized article was read and approved by all authors.

8. REFERENCES

1 Klonoff DC. The increasing incidence of diabetes in the 21st century. J Diabetes Sci Technol. 2009;3(1):1-2.
DOI: 10.1177/193229680900300101.
2. Dilworth L, Facey A, Omoruyi F. Diabetes mellitus and its metabolic complications: the role of adipose tissues. Int J Mol Sci. 2021;22(14):1-18.
DOI: 10.3390/IJMS22147644.
3. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, *et al*. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):1-34.
DOI: 10.3390/IJMS21176275.
4. Quintanilla Rodriguez BS, Vadakekut ES, Mahdy H. Gestational diabetes. 2023;1-29.
PMID: 31424780.
5. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010; 33(Suppl1):S62-S69.
DOI: 10.2337/DC10-S062.
6. O'Connell JM, Manson SM. Understanding the economic costs of diabetes and prediabetes and what we may learn about reducing the health and economic burden of these conditions. Diabetes Care. 2019;42(9):1609-1611.
DOI: 10.2337/DCI19-0017.
7. Tönnies T, Brinks R, Isom S, Dabelea D, Divers J, Mayer-Davis EJ, *et al*. Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2060: the SEARCH for diabetes in youth study. Diabetes Care. 2023;46(2):313-320.
DOI: 10.2337/DC22-0945.
8. Dallatana A, Cremonesi L, Trombetta M, Fracasso G, Nocini R, Giacomello L, *et al*. G Protein-coupled receptors and the rise of type 2 diabetes in children. Biomedicines. 2023;11(6):1576,1-16.
DOI: 10.3390/BIOMEDICINES11061576.
9. Hectors TLM, Vanparys C, Pereira-Fernandes A, Martens GA, Blust R. Evaluation of the INS-1 832/13 cell line as a beta-cell based screening system to assess pollutant effects on beta-cell function. PLoS One. 2013;8(3):1-10.
DOI: 10.1371/JOURNAL.PONE.0060030.
10. Green AD, Vasu S, Flatt PR. Cellular models for beta-cell function and diabetes gene therapy. Acta Physiol (Oxf). 2018;222(3):1-45.
DOI: 10.1111/APHA.13012.
11. Jawale S. Stem cell therapy for type 1 diabetes with transplantation of stem cells into the Omental pouch,

peritoneum, and blood, experimental study. Ann Med Surg. 2022;81,1-13.
DOI: 10.1016/J.AMSU.2022.104468.
12. Jensen C, Teng Y. Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci. 2020;7(33):1-15.
DOI: 10.3389/FMOLB.2020.00033.
13. Shilleh AH, Russ HA. Cell replacement therapy for type 1 diabetes patients: potential mechanisms leading to stem-cell-derived pancreatic β-cell loss upon transplant. Cells. 2023;12(5):698,1-19.
DOI: 10.3390/CELLS12050698.
14. Ntamo Y, Samodien E, Burger J, Muller N, Muller CJF, Chellan N. *In vitro* characterization of insulin−producing β-cell spheroids. Front Cell Dev Biol. 2021;8:623889,1-12.
DOI: 10.3389/FCELL.2020.623889.
15. Kumar SA, Delgado M, Mendez VE, Joddar B. Applications of stem cells and bioprinting for potential treatment of diabetes. World J Stem Cells. 2019;11(1):13-32.
DOI: 10.4252/WJSC.V11.I1.13.
16. Agarwal S, Saha S, Balla VK, Pal A, Barui A, Bodhak S. Current developments in 3D bioprinting for tissue and organ regeneration review. Front Mech Eng. 2020;6:589171,1-22.
DOI: 10.3389/FMECH.2020.589171.
17. Matai I, Kaur G, Seyedsalehi A, McClinton A, Laurencin CT. Progress in 3D bioprinting technology for tissue/organ regenerative engineering. Biomaterials. 2020;226:119536,1-32.
DOI: 10.1016/J.BIOMATERIALS.2019.119536.
18. Wang D, Guo Y, Zhu J, Liu F, Xue Y, Huang Y, *et al*. Hyaluronic acid methacrylate/pancreatic extracellular matrix as a potential 3D printing bioink for constructing islet organoids. Acta Biomater. 2023;165:86-101.
DOI: 10.1016/J.ACTBIO.2022.06.036.
19. Panja N, Maji S, Choudhuri S, Ali KA, Hossain CM. 3D bioprinting of human hollow organs. AAPS PharmSciTech. 2022;23(5):1-18.
DOI: 10.1208/S12249-022-02279-9.
20. Xu Y, Song D, Wang X. 3D Bioprinting for pancreas engineering/manufacturing. Polymers. 2022;14(23):5143,1-19.
DOI: 10.3390/POLYM14235143.
21. Chen EP, Toksoy Z, Davis BA, Geibel JP. 3D bioprinting of vascularized tissues for *in vitro* and *in vivo* applications. Front Bioeng Biotechnol. 2021;9:664188,1-22.
DOI: 10.3389/FBIOE.2021.664188.
22. Saisho Y. β-cell dysfunction: its critical role in prevention and management of type 2 diabetes. World J Diabetes. 2015;6(1):109-124.
DOI: 10.4239/WJD.V6.I1.109.
23. Zhou J, Shi Y. Mesenchymal stem/stromal cells (MSCs): origin, immune regulation, and clinical applications. Cell Mol Immunol. 2023;20:555-557.
DOI: 10.1038/s41423-023-01034-9.
24. Horwitz EM, Andreef M, Frassoni F. Mesenchymal stromal cells. Curr Opin Hematol. 2006;13(6): 419-425.
DOI: 10.1097/01.MOH.0000245697.54887.6F.
25. Mo Y, Wang Z, Gao J, Yan Y, Ren H, Zhang F, *et al*. Comparative study of three types of mesenchymal stem cell to differentiate into pancreatic β-like cells *in vitro*. Exp Ther Med. 2021;22(3):936,1-11.
DOI: 10.3892/ETM.2021.10368.
26. Sariboyaci AE, Uysal O, Gunes S. Differentiation of human bone marrow-derived mesenchymal stem cells into functional pancreatic beta cells. Med Sci | Int Med J. 2021;10(3):951-958.
DOI: 10.5455/MEDSCIENCE.2021.07.228.
27. Pavathuparambil Abdul Manaph N, Sivanathan KN, Nitschke J, Zhou XF, Coates PT, Drogemuller CJ. An overview on small molecule-induced differentiation of mesenchymal stem cells into beta cells for diabetic therapy. Stem Cell Res Ther. 2019 10:1. 2019;10:293:1-18.
DOI: 10.1186/S13287-019-1396-5.
28. Jayasinghe M, Prathiraja O, Perera PB, Jena R, Silva MS, Weerawarna PSH, *et al*. The role of mesenchymal stem cells in the treatment of type 1 diabetes. Cureus. 2022;14(7):1-13.
DOI: 10.7759/CUREUS.27337.
29. Wan XX, Zhang DY, Khan MA, Zheng SY, Hu XM, Zhang Q, *et al*. Stem cell transplantation in the treatment of type 1 diabetes mellitus: from insulin replacement to beta-cell replacement. Front Endocrinol (Lausanne). 2022;13:859638,1-13.
DOI: 10.3389/FENDO.2022.859638.
30. Thapa G, Kumari A, Dasgupta D, Bandyopadhy S, Sarkar N, Roy K, *et al*. Insight into the mechanism of action of anti-diabetic drugs. In: Kazemi I. How synthetic drugs work: insights into molecular pharmacology of classic and new pharmaceuticals. Academic Press. 2023. pp. 95-122.
DOI: 10.1016/B978-0-323-99855-0.00005-1.
31. Fusco J, Xiao X, Prasadan K, Sheng Q, Chen C, Ming YC, *et al*. GLP-1/Exendin-4 induces β-cell proliferation *via* the epidermal growth factor receptor. Sci Rep. 2017;7(1):9100,1-6.
DOI: 10.1038/S41598-017-09898-4.
32. Svendsen B, Larsen O, Gabe MBN, Christiansen CB, Rosenkilde MM, Drucker DJ, *et al*. Insulin secretion depends on intra-islet glucagon signaling. Cell Rep. 2018;25(5):1127-1134.
DOI: 10.1016/j.celrep.2018.10.018.
33. Xu B, Fan D, Zhao Y, Li J, Wang Z, Wang J, *et al*. Three-dimensional culture promotes the differentiation of human dental pulp mesenchymal stem cells into insulin-producing cells for improving the diabetes therapy. Front Pharmacol. 2020;10:509287,1-11.
DOI: 10.3389/FPHAR.2019.01576.
34. Galli A, Algerta M, Marciani P, Schulte C, Lenardi C, Milani P, *et al*. Shaping pancreatic β-cell differentiation and functioning: the influence of mechanotransduction. Cells. 2020;9(2):413,1-24.
DOI: 10.3390/CELLS9020413.
35. Cayabyab F, Nih LR, Yoshihara E. Advances in pancreatic islet transplantation sites for the treatment of diabetes. Front Endocrinol (Lausanne). 2021;12:732431,1-21.
DOI: 10.3389/FENDO.2021.732431.

36. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AMJ. A prevascularized subcutaneous device-less site for islet and cellular transplantation. Nat Biotechno. 2015;33(5):518-523. DOI: 10.1038/nbt.3211.
37. Tao H, Han Z, Han ZC, Li Z. Proangiogenic features of mesenchymal stem cells and their therapeutic applications. Stem Cells Int. 2016;2016:1-11. DOI: 10.1155/2016/1314709.
38. Watt SM, Gullo F, Van Der Garde M, Markeson D, Camicia R, Khoo CP, *et al*. The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential. Br Med Bull. 2013; 108(1):25-53. DOI: 10.1093/BMB/LDT031.
39. Figliuzzi M, Cornolti R, Perico N, Rota C, Morigi M, Remuzzi G, *et al*. Bone marrow-derived mesenchymal stem cells improve islet graft fu nction in diabetic rats. Transplant Proc. 2009;41(5):1797-1800. DOI: 10.1016/J.TRANSPROCEED.2008.11.015.
40. Kim J, Shim IK, Hwang DG, Lee YN, Kim M, Kim H, *et al*. 3D cell printing of islet-laden pancreatic tissue-derived extracellular matrix bioink constructs for enhancing pancreatic functions. J Mater Chem B. 2019;7(10):1773-1781. DOI: 10.1039/C8TB02787K.
41. Wieland FC, van Blitterswijk CA, van Apeldoorn A, LaPointe VLS. The functional importance of the cellular and extracellular composition of the islets of Langerhans. J Immunol Regen Med. 2021;13:100048,1-8. DOI: 10.1016/J.REGEN.2021.100048.
42. Thomas D, O'Brien T, Pandit A. Toward customized extracellular niche engineering: progress in cell-entrapment technologies. Adv Mater. 2018;30(1):1703948,1-20. DOI: 10.1002/ADMA.201703948.
43. Idaszek J, Volpi M, Paradiso A, Nguyen Quoc M, Górecka Ż, Klak M, *et al*. Alginate-based tissue-specific bioinks for multi-material 3D-bioprinting of pancreatic islets and blood vessels:a step towards vascularized pancreas grafts. Bioprinting. 2021;24:e00163,1-12. DOI: 10.1016/J.BPRINT.2021.E00163.
44. Enck K, Tamburrini R, Deborah C, Gazia C, Jost A, Khalil F, *et al*. Effect of alginate matrix engineered to mimic the pancreatic microenvironment on encapsulated islet function. Biotechnol Bioeng. 2021;118(3):1177-1185. DOI: 10.1002/BIT.27641.
45. Wang JK, Cheam NMJ, Irvine SA, Tan NS, Venkatraman S, Tay CY. Interpenetrating network of alginate–human adipose extracellular matrix hydrogel for islet cells encapsulation. Macromol Rapid Commun. 2020;41(21):2000275. DOI: 10.1002/MARC.202000275,1-6.
46. Shalaly ND, Ria M, Johansson U, Åvall K, Berggren PO, Hedhammar M. Silk matrices promote formation of insulin-secreting islet-like clusters. Biomaterials. 2016;90:50-61. DOI: 10.1016/J.BIOMATERIALS.2016.03.006.
47. Johansson U, Ria M, Åvall K, Shalaly ND, Zaitsev S V., Berggren PO, *et al*. Pancreatic islet survival and engraftment is promoted by culture on functionalized spider silk matrices. PLoS One. 2015;10(6):e0130169,1-21. DOI: 10.1371/JOURNAL.PONE.0130169.
48. Jalili RB, Moeen Rezakhanlou A, Hosseini-Tabatabaei A, Ao Z, Warnock GL, Ghahary A. Fibroblast populated collagen matrix promotes islet survival and reduces the number of islets required for diabetes reversal. J Cell Physiol. 2011;226(7):1813-1819. DOI: 10.1002/JCP.22515.
49. Clua-Ferré L, De Chiara F, Rodríguez-Comas J, Comelles J, Martinez E, Godeau AL, *et al*. Collagen-tannic acid spheroids for β-Cell encapsulation fabricated using a 3D bioprinter. Adv Mater Technol. 2022;7(7):2101696,1-12. DOI: 10.1002/ADMT.202101696.
50. Townsend SE, Gannon M. Extracellular matrix-associated factors play critical roles in regulating pancreatic β-cell proliferation and survival. Endocrinology. 2019;160(8):1885-1894. DOI: 10.1210/EN.2019-00206.
51. Ferrara B, Pignatelli C, Cossutta M, Citro A, Courty J, Piemonti L. The extracellular matrix in pancreatic cancer: description of a complex network and promising therapeutic options. Cancer. 2021;13(17):4442,1-32. DOI: 10.3390/CANCERS13174442.
52. Bosco D, Meda P, Halban PA, Rouiller DG. Importance of cell-matrix interactions in rat islet beta-cell secretion *in vitro*: role of alpha6beta1 integrin. Diabetes. 2000;49(2):233-243. DOI: 10.2337/DIABETES.49.2.233.
53. Barillaro M, Schuurman M, Wang R. Collagen IV-β1-integrin influences INS-1 cell insulin secretion *via* enhanced snare protein expression. Front Cell Dev Biol. 2022;10:894422,1-18. DOI: 10.3389/FCELL.2022.894422.
54. Llacua LA, Faas MM, de Vos P. Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. Diabetologia. 2018;61(6):1261-1272. DOI: 10.1007/S00125-017-4524-8.
55. Smink AM, de Vos P. Therapeutic strategies for modulating the extracellular matrix to improve pancreatic islet function and survival after transplantation. Curr Diab Rep. 2018; 18(7):39,1-7. DOI: 10.1007/S11892-018-1014-4.
56. Jiang K, Chaimov D, Patel SN, Liang JP, Wiggins SC, Samojlik MM, *et al*. 3-D physiomimetic extracellular matrix hydrogels provide a supportive microenvironment for rodent and human islet culture. Biomaterials. 2019;198:37-48. DOI: 10.1016/J.BIOMATERIALS.2018.08.057.
57. Krishtul S, Skitel Moshe M, Kovrigina I, Baruch L, Machluf M. ECM-based bioactive microencapsulation significantly improves islet function and graft performance. Acta Biomater. 2023;171:249-260. DOI: 10.1016/J.ACTBIO.2023.09.009.

58. Wang X, Jin L, Liu W, Stingelin L, Zhang P, Tan Z. Construction of engineered 3D islet micro-tissue using porcine decellularized ECM for the treatment of diabetes. Biomater Sci. 2023;11(16):5517-5532. DOI: 10.1039/D3BM00346A.
59. Tremmel DM, Sackett SD, Feeney AK, Mitchell SA, Schaid MD, Polyak E, *et al*. A human pancreatic ECM hydrogel optimized for 3-D modeling of the islet microenvironment. Sci Rep. 2022;12(1):1-14. DOI: 10.1038/s41598-022-11085-z.
60. Irvine SA, Venkatraman SS. Bioprinting and differentiation of stem cells. Molecules. 2016;21(9):1188. DOI: 10.3390/MOLECULES21091188,1-23.
61. Gaetani R, Aude S, Demaddalena LL, Strassle H, Dzieciatkowska M, Wortham M, *et al*. Evaluation of different decellularization protocols on the generation of pancreas-derived hydrogels. Tissue Eng Part C Methods. 2018;24(12):697-708. DOI: 10.1089/TEN.TEC.2018.0180.
62. Kim J, Kim M, Hwang DG, Shim IK, Kim SC, Jang J. Pancreatic tissue-derived extracellular matrix bioink for printing 3D cell-laden pancreatic tissue constructs. J Vis Exp. 2019;154:1-7. DOI: 10.3791/60434.
63. Pouliot RA, Young BM, Link PA, Park HE, Kahn AR, Shankar K, *et al*. Porcine lung-derived extracellular matrix hydrogel properties are dependent on pepsin digestion time. Tissue Eng Part C Methods. 2020;26(6):332-346. DOI: 10.1089/TEN.TEC.2020.0042.
64. Weber LM, Hayda KN, Anseth KS. Cell-matrix interactions improve beta-cell survival and insulin secretion in three-dimensional culture. Tissue Eng Part A. 2008;14(12):1959-1968. DOI: 10.1089/TEN.TEA.2007.0238.
65. Kaido T, Yebra M, Cirulli V, Montgomery AM. Regulation of human β-cell adhesion, motility, and insulin secretion by collagen IV and its receptor α1β1. J Biol Chem. 2004;279(51):53762-53769. DOI: 10.1074/jbc.M411202200.
66. Shahjalal HM, Abdal Dayem A, Lim KM, Jeon T Il, Cho SG. Generation of pancreatic β cells for treatment of diabetes: advances and challenges. Stem Cell Res Ther. 2018;9(1):355,1-19. DOI: 10.1186/S13287-018-1099-3.