UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE MEDICINA
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LUCIANA XAVIER PEREIRA

AVALIAÇÃO DA PROLIFERAÇÃO DO PARÊNQUIMA PANCREÁTICO NO IMPLANTE DE MATRIZ SINTÉTICA DE POLIÉTER-POLIURETANO EM CAMUNDONGOS

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AVALIAÇÃO DA PROLIFERAÇÃO DO PARÊNQUIMA PANCREÁTICO NO IMPLANTE DE MATRIZ SINTÉTICA DE POLIÉTER-POLIURETANO EM CAMUNDONGOS

LUCIANA XAVIER PEREIRA

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Realizou-se, no dia 02 de agosto de 2017, às 09:00 horas, Sala Nelo Rangel, bloco K3, ICB, UFMG, da Universidade Federal de Minas Gerais, a defesa de tese, intitulada *AVALIAÇÃO DA PROLIFERAÇÃO DO PARÊNQUIMA PANCREÁTICO NO IMPLANTE DE MATRIZ SINTÉTICA DE POLIÉTER-POLIURETANO EM CAMUNDONGOS*, apresentada por LUCIANA XAVIER PEREIRA, número de registro 2013709271, graduada no curso de CIÊNCIAS BIOLÓGICAS, como requisito parcial para a obtenção do grau de Doutor em PATOLOGIA, à seguinte Comissão Examinadora: Prof(a). Paula Peixoto Campos Lopes - Orientador (UFMG), Prof(a). Ricardo Goncalves (UFMG), Prof(a). Nubia Braga Pereira (UFMG), Prof(a). Sandra Aparecida Lima de Moura (UFOP), Prof(a). Janice Sepúlveda Reis (IEP - Santa Casa de BH).

A Comissão considerou a tese:

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“*O real não está na saída nem na chegada: ele se dispõe para a gente é no meio da travessia*”. Guimarães Rosa

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Resumo

A habilidade de regeneração do pâncreas já foi demonstrada em alguns estudos, porém muitas vezes não é suficiente para reparar lesões decorrentes de pancreatite e diabetes. Assim, modelos animais de lesão são utilizados para estudos de regeneração, afim de encontrar novas estratégias para induzir a proliferação de células do pâncreas. Abordagens alternativas na área de biomateriais e terapia celular têm sido estudadas. Porém, uma plataforma que recrie um microambiente biológico com precisão ainda não foi desenvolvida, dificultando a aplicação clínica na terapêutica da pancreatite e no diabetes tipo 1. Implantamos uma matriz sintética de poliéter-poliuretano em interface com o pâncreas de camundongo para avaliarmos a cinética de indução do novo parênquima pancreático intra-implante. Na matriz removida cirurgicamente 15, 30 e 45 dias pós implante, os componentes do pâncreas (ácinos, ductos e ilhotas), vasos sanguíneos e marcadores inflamatórios foram analisados. O número de ilhotas e ácinos foram similares, e o número de ductos e vasos foram maiores no interior da matriz comparando com o pâncreas nativo. Células positivas para insulina mostraram-se organizadas em estruturas como ilhotas e observou-se células ductais também positivas para insulina. Além disso, parâmetros inflamatórios (atividade de MPO e NAG e níveis de TNF-α e CCL2) foram maiores no dia 15 comparando com os dias 30 e 45 pós implante. Para verificar se este parênquima pancreático proliferado no implante apresentava funções metabólicas, implantamos a plataforma em animais diabéticos e posteriormente inoculamos com células tronco mesenquimais derivadas de tecido adiposo humano (hASCs), para melhorar a resposta. Avaliamos parâmetros locais (inflamação, angiogênese e proliferação de células endócrinas intra-implante) e parâmetros metabólicos de animais diabéticos. No contexto sistêmico, observamos que animais diabéticos que receberam hASCs intra-implante melhoraram o metabolismo glicêmico, produção de peptídeo C e peso corporal. Localmente, os implantes de animais diabéticos que receberam o inóculo de hASCs, mostraram menor conteúdo de citocinas inflamatórias, aumento da vascularização e melhor proliferação de células endócrinas. Nossos resultados mostraram que a matriz foi capaz de atuar como plataforma biológica para crescimento de um novo parênquima pancreático. Além disso, por ser facilmente acessível, pode ser explorado como modelo de proliferação de parênquima pancreático, sendo relevante no entendimento de processos celulares que ocorrem na formação do novo tecido.

Abstract

The pancreas regenerative ability has been demonstrated in some studies, but it is no often enough to repair pancreatitis and diabetes lesions. Therefore, animal models of injury have been used for regeneration studies in order to find new strategies to induce pancreas cells proliferation. Alternative approaches in the biomaterials and cell therapy areas have been studied, however, a platform that reconstructs an accurately biological microenvironment has not yet been developed, making clinical application difficult. We implanted a polyether-polyurethane synthetic matrix in interface to the mice pancreas to evaluate the kinetics of induction of the new intra-implant pancreatic parenchyma. Matrix were surgically removed at 15, 30 and 45 days post implant and the components of the pancreas (acini, ducts and islets), blood vessels and inflammatory markers were analyzed. The number of islets and acini were similar, and the number of ducts and vessels were higher inside matrix comparing to the native pancreas. Insulin-positive cells were organized into structures like islets and some ductal cells were positive for insulin. In addition, inflammatory parameters (activity of MPO and NAG and levels of TNF-α and CCL2) were higher at day 15 comparing to days 30 and 45 post-implant. To verify if this proliferated pancreatic parenchyma inside implant had metabolic functions, we implanted the scaffold in diabetic animals and later we inoculated it with mesenchymal stem cells derived from human adipose tissue (hASCs), to improve the response. We evaluated local parameters intra-implant (inflammation, angiogenesis and endocrine cells proliferation) and metabolic parameters of diabetic animals. In the systemic context, we observed that diabetic animals receiving hASCs intra-implant improved glycemic metabolism, C-peptide production and body weight. Locally, diabetic animal implants that received the inoculum of hASCs showed lower inflammatory cytokine content, increased vascularization, and improved endocrine cell proliferation. Our results showed that the matrix was capable of acting as a biological platform for the growth of a new pancreatic parenchyma. Furthermore, because it is easily accessible, it could be explored as a model of pancreatic parenchyma proliferation, being relevant in the understanding of cellular processes that occur in the formation of new tissue.

Lista de abreviaturas e símbolos

ATP – Trifosfato de adenosina, do inglês adenosine triphosphate
AUC – Área sob a curva – do inglês *area under curve*
ASCs – Células tronco adiposas - do inglês *adipose stem cells*
CAPI – Centro de Aquisição e processamento de imagens
CCK - Colecistocinina
CDC47 – Anticorpo marcador de proliferação celular
CEBIO – Centro de Bioterismo
CEUA – Comissão de Ética no Uso de Animais
c-MET - Receptor do fator de crescimento de hepatócito
CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico
CT – Controle
Diab – Diabético
DM - Diabetes *mellitus*
DNA - Ácido desoxirribonucleico, do inglês *deoxyribonucleic acid*
FAPEMIG - Fundação de Amparo à Pesquisa de Minas Gerais
GAD65 - Descarboxilase do ácido glutâmico
GLUT2 – Transportador de glicose, do inglês *glucose transporter 2*
hASCs – Células tronco adiposas humanas, do inglês *human adipose stem*
HGF - Fator de crescimento de hepatócito, do inglês *hepatocyte growth factor receptor*)
H&E- Hematoxilina-eosina
HuNu – Anticorpo anti núcleo humano, do inglês *human nuclei*
IAA - Anticorpos antiinsulina
IA - Tirosinafosfatases
ICB- Instituto de Ciências Biológicas
IDF - Federação Internacional do Diabetes, do inglês *International Diabetes Federation*
IHC – Imunohistoquímica, do inglês *immunohistochemistry*
mCT – Microtomografia computadorizada, do inglês *computed microtomography*
MPO – Mieloperoxidase
MSCs – Células tronco mesenquimais, do inglês *mesenchymal stem cells*
NAD+ - Nicotinamida adenina dinucleotídeo
NAG - N-acetyl-β-D-glucosaminidase
OGTT – do inglês *oral glucose tolerance test*
PARP - Enzima poli ADP ribose polimerase
PBS- Tampão fosfato salina, do inglês *Phosphate buffered saline*
Pdx1 - *Pancreatic and duodenal homeobox 1*
PTF1A - *Pancreas specific transcription factor, 1a*
Sox9 - SRY(sex determining region Y)-box 9
STZ – Estreptozotocina, do inglês *streptozotocin*
TNFα - Fator de Necrose Tumoral, do inglês *Tumor Necrosis Factor -Alpha*
TUNEL - do inglês *Terminal deoxynucleotidyltransferase-mediated dUPT nick endlabelling*
T1D – Diabetes *mellitus* tipo 1, do inglês type 1 diabetes *mellitus*
UFMG – Universidade Federal de Minas Gerais
VEGF - Fator de crescimento endotelial, do inglês *Vascular endothelial growth fator*
ZNT8 - Transportador de zinco 8

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1. Introdução

O pâncreas é um órgão complexo composto por um compartimento endócrino e outro exócrino. Lesões ao compartimento exócrino ocorrem na pancreatite, aguda e crônica, e causam várias complicações clínicas. Na pancreatite aguda, altos níveis de amilase e lipase estão presentes na circulação, mas normalmente a lesão se resolve em dias ou semanas (Wang 2009; Forsmark et al. 2016; Manohar et al. 2017). Ao contrário, na pancreatite crônica, graves alterações inflamatórias e fibróticas estão presentes (Lew et al. 2017; Manohar et al. 2017). A morte de células β no compartimento endócrino causam o diabetes, que é caracterizado pela hiperglicemia e pode estar relacionado a complicações micro e macrovasculares (Van Belle et al. 2011; Paneni et al. 2013; Skyler et al. 2017; Katsarou et al. 2017), sendo considerada uma doença de epidemia global por afetar milhões de pessoas no mundo (World Health Organization 2016).

No diabetes tipo 1, a terapia com insulina exógena foi essencial para o aumento da sobrevida dos pacientes, entretanto, a incidência de diabetes tipo 1 vem aumentando 3% anualmente em todo o mundo. Atualmente são 542.000 casos em pessoas com menos de 15 anos (Maahs et al. 2010; International Diabetes Federation 2015). Além disso, a hipoglicemia severa causada pela terapia insulínica afeta muitos pacientes, o que levou ao desenvolvimento de técnicas de transplante de pâncreas ou de ilhotas (Robertson 2015). Estudos na área de engenharia de tecidos e terapia celular têm buscado aprimorar as técnicas de transplante de ilhotas e aplicação de células tronco afim de obter novas alternativas terapêuticas (Langer & Tirrell 2004; Godfrey et al. 2012; Amer et al. 2014). Porém, ainda não se desenvolveu uma plataforma ideal que

consiga sustentar de forma viável e prolongada o transplante de células e/ou a formação de um novo tecido.

Modelos animais para o estudo de lesões no compartimento exócrino (administração de ceruleína, ligação de ducto) e no compartimento endócrino (administração de estreptozotocina, pancreatectomia subtotal) têm sido descritos, fornecendo informações valiosas sobre o mecanismo de regeneração pancreática (Murtaugh & Keefe 2015). Entretanto, a capacidade regenerativa do pâncreas nem sempre é suficiente para as necessidades corporais de enzimas digestivas e hormônios, levando à lesões crônicas e ao agravamento do diabetes (Nir et al. 2007; Menge et al. 2012). Existe, portanto, necessidade de desenvolvimento de novas estratégias para melhorar a capacidade regenerativa e/ou proliferativa do pâncreas.

Nosso grupo utiliza uma matriz sintética de poliéter–poliuretano como um modelo de implante de material no subcutâneo ou na cavidade intraperitoneal de roedores, para analisar a interação hospedeiro-corpo estranho. Em trabalhos anteriormente publicados pelo nosso grupo de pesquisa, foi demostrado que na ausência de adição exógena de fatores de crescimento ou componentes extracelulares, os implantes são infiltrados por um tecido proliferativo fibrovascular contendo também células parenquimatosas de órgãos próximos ao implante (Mendes et al 2007; Castro et al 2012). Estas observações proveram a ideia de uma plataforma biohíbrida que integrasse o biomaterial ao microambiente tecidual. Nossa hipótese é que o implante dessa matriz sintética em interface com pâncreas poderia induzir a proliferação de parênquima pancreático em animais normoglicêmicos e hiperglicêmicos. Além disso, poderia também servir como plataforma para receber células tronco

mesenquimais, permitindo o estudo de aplicação dessas células na terapêutica do diabetes. Ao contrário dos outros modelos *in vivo* utilizados para investigar a capacidade de regeneração pancreática, nosso modelo é minimamente invasivo, não requer lesão prévia no pâncreas nem adição de fatores de crescimento exógenos à plataforma e permite um estudo mais detalhado da proliferação pancreática. O entendimento dos processos celulares envolvidos nesta resposta é central no desenvolvimento das estratégias de engenharia de tecido que utilizarão o parênquima pancreático recém-formado.

2. Revisão Bibliográfica

2.1 Pâncreas

O pâncreas é uma glândula acessória ao tubo digestivo, dividido em lóbulos e envolto em tecido conectivo. Trata-se de órgão composto de um componente exócrino e um componente endócrino. Seu compartimento exócrino, que ocupa mais de 80% do órgão, é constituído por glândula acinosa composta, com rede ramificada de ductos e células epiteliais secretoras que se organizam de forma circular com um lúmen central, formando os ácinos. As células acinares produzem e secretam pró enzimas, cerca de 20 tipos diferentes, que são transportadas através de ductos para atuarem na degradação de proteínas, carboidratos e lipídios que chegam ao duodeno. O compartimento endócrino consiste de agregados circulares de células endócrinas, as ilhotas de Langerhans, dispostas em meio ao parênquima exócrino. As ilhotas são constituídas de diferentes tipos celulares que secretam hormônios na corrente sanguínea, sendo as principais as células-β produtoras de insulina e células-α produtoras de glucagon, além das células- δ produtoras de somatostatina, células PP produtoras de polipeptídio pancreático e células-ε produtoras de grelina (Brasileiro Filho 2016; Shih et al. 2013).

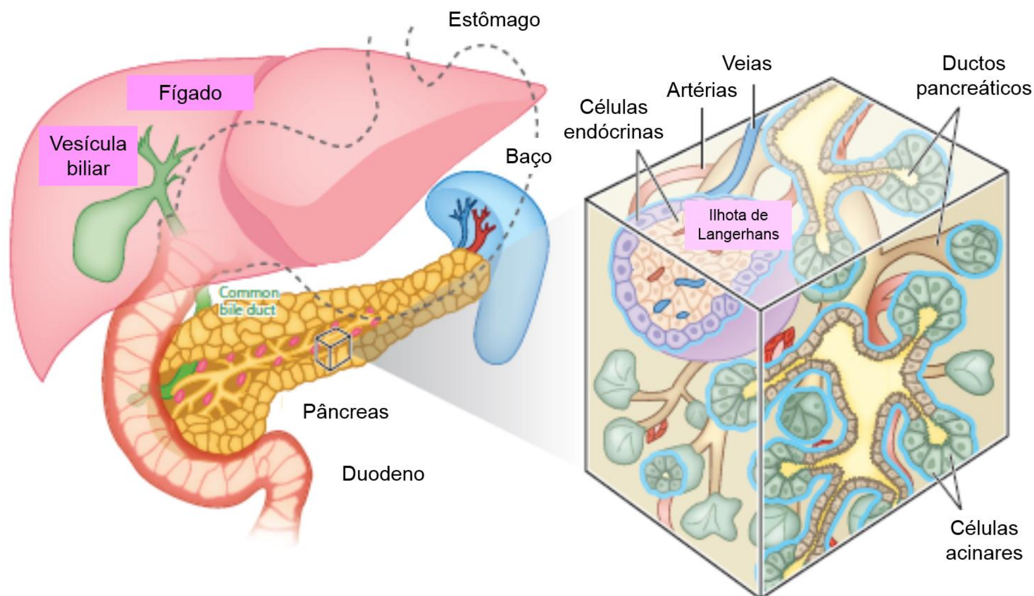

Figura 1. Anatomia geral do pâncreas. O pâncreas é um órgão retroperitoneal, situado posterior ao estômago, estendendo-se do baço ao duodeno. Para fins de ilustração, o estômago é delineado com uma linha tracejada e o duodeno é mostrado como uma visão de corte. As células acinares exócrinas secretam enzimas, as quais são transportadas através de uma rede ductal até o duodeno, para a degradação de proteínas, carboidratos e lipídios. O pâncreas endócrino consiste em grupos de células (ilhotas de Langerhans) contendo cinco tipos de células endócrinas que secretam hormônios (células α, β, δ, PP e ε). A organização da ilhota na figura é típica de camundongos, com células β centralmente localizadas. As ilhotas pancreáticas são cercadas por uma rede capilar densa através da qual os hormônios são liberados para a corrente sanguínea. Fonte e adaptado de Shih et al 2013.

A estrutura anatômica do pâncreas apresenta algumas diferenças entres as espécies. Em camundongos, o pâncreas adulto é dito mesentérico, pois diferente do pâncreas humano compacto e retroperitoneal, seus lóbulos estão dispersos em meio a gordura mesentérica, tecido conectivo e agregados linfoides, se estendendo entre o duodeno e o baço. Esta disposição não permite clara divisão anatômica como no pâncreas humano, em cabeça, corpo e cauda. Há outras diferenças entre pâncreas de camundongo e humano, como pode ser observado na tabela 1 (Dintzis & Liggitt 2012).

Tabela 1 – Pâncreas (adaptado de Dintzis & Liggitt 2012)

Característica	Camundongo	Humano
Anatomia	Mesentérico	Compacto
Tamanho do lóbulo	Pequeno	Grande
Tamanho da ilhota (diâmetro)	116±80µm	50±29µm
Localização da ilhota	Desigualmente distribuídas pelo órgão; interlobular	Igualmente distribuídas; intralobular
Células da ilhota	Predomina células β (±75%); centrais. Células alfa (±18%); células alfa e gama: periféricas	Células β menos dominantes (±55%); mais células alfa (±37%); células da ilhota mais espalhadas e aleatoriamente distribuídas ao longo dos vasos.
Ductos pancreáticos	O ducto primário se une ao ducto biliar antes da entrada no duodeno; múltiplos ductos acessórios são comuns.	O ducto primário se une ao ducto biliar em papilas duodenais maiores; Um ducto acessório único é comum.
Microvasculatura	Em ilhotas, a lâmina basal acompanha apenas vasos	Em ilhotas, a lâmina basal acompanha células e vasos endócrinos (membrana basal "dupla")

O pâncreas se desenvolve a partir do intestino primitivo, derivado do endoderma, e todas as células que o compõe originam de células progenitoras multipotentes. Ao longo do desenvolvimento no período embrionário, alguns fatores de transcrição são expressos e outros inibidos, levando a diferenciação celular específica. O fator Pdx1 é mais específico para células β, o Sox9 para células do ducto e Ptf1a para células acinares (Shih et al. 2013; Stanger and Hebrok 2013). O controle desses fatores de transcrição ocorrem por diferentes vias, como a via de sinalização Notch, envolvida na definição de linhagens celulares endócrinas e exócrinas (Afelik and Jensen 2013; X. Li et al. 2015) e a via Wnt/β-catenina, relacionada a proliferação de células acinares (Heiser et al. 2006; Wells et al. 2007).

2.2 Plasticidade do pâncreas

Após o completo desenvolvimento do pâncreas, acreditava-se haver uma estabilidade no número de células do órgão, que poderia passar por pequenas variações. Porém, recentes estudos tem mostrado uma dinâmica na massa celular pancreática, por uma maior demanda metabólica ou restauração pós-lesão (Cigliola et al. 2016).

Ferramentas genéticas e modelos animais de lesão pancreática têm possibilitado conhecer essa capacidade regenerativa do pâncreas adulto. Fatores e vias envolvidos no desenvolvimento embrionário podem ser reativados pós lesão, iniciando um processo regenerativo (Puri & Hebrok 2010; Keefe et al. 2012; Stanger & Hebrok 2013; Murtaugh & Keefe 2015).

Entre os modelos de lesão pancreática em camundongos e ratos para estudar a regeneração no pâncreas adulto, há os que geram lesões na porção exócrina. Um deles é o modelo de pancreatite aguda induzida por ceruleína, um análogo da colecistocinina (CCK) um hormônio que estimula a secreção de enzimas acinares em altas doses, causando necrose e induzindo inflamação (Su et al. 2006). Outro modelo é a ligação parcial de ducto que gera danos à células acinares por obstruir a drenagem das secreções exócrinas (De Groef et al. 2015). A pancreatectomia parcial é um modelo de lesão de ambas as porções, exócrina e endócrina, a qual remove cerca de 50-90% da massa do pâncreas (Bonner-Weir et al. 1993; Peshavaria et al. 2006). Há também outros métodos de lesão da porção endócrina, como a morte de células β pelo uso de estreptozotocina (Goud et al. 2015). Através destes modelos e uso de ferramentas genéticas, pesquisadores conseguiram identificar algumas vias e origens celulares da regeneração pancreática. A regeneração de células

acinares pode ocorrer pela proliferação de células acinares residuais (Desai et al. 2007) e entre as vias que podem estar envolvidas nessa regeneração estão a via Notch (Siveke et al. 2008), a via Hedgehog (Fendrich et al. 2008) e a via do HGF/c-MET (fator de crescimento de hepatócito e seu receptor) (Gaziova et al. 2016). A regeneração de células β pode ter origem a partir de outras células endócrinas (Thorel et al. 2010) ou de células acinares (Pan et al. 2013), ou de células de ductos (Peshavaria et al. 2006) ou por duplicação própria (Dor et al. 2004; Teta et al. 2007; Meier et al. 2008) ou ainda a partir de células progenitoras (Xu et al. 2008; Kim & Lee 2016).

Estes estudos demonstraram a grande plasticidade de células pancreáticas, já que células maduras já diferenciadas foram capazes de se reprogramarem frente a algum estímulo e assumirem características de tipos celulares diferentes. Além disso, possíveis células progenitoras também podem permanecer quiescentes em meio ao tecido adulto e ativarem vias de diferenciação quando preciso, afim de manter a função do órgão.

Portanto, mais estudos baseados em modelos experimentais são necessários para se entender os possíveis mecanismos de proliferação celular do pâncreas. Identificar os sinais e fatores que controlam essa mudança adaptativa do pâncreas é importante para aprimorar sua capacidade plástica intrínseca, a qual é potencialmente promissora no desenvolvimento de novas estratégias terapêuticas para doenças relacionadas ao órgão, como o diabetes.

2.3 Diabetes

2.3.1 Classificação e fisiopatologia

Diabetes *mellitus* (DM) é uma doença crônica em que o organismo perde o controle glicêmico fisiológico, apresentando alterações metabólicas que irão resultar na característica comum da doença, a hiperglicemia. O excesso de glicose no sangue pode afetar a saúde do organismo como um todo, levando ao desenvolvimento de alterações macrovasculares (cardiopatias, doença arterial periférica) e microvasculares (retinopatia, nefropatia, neuropatias) (Van Belle et al. 2011; Paneni et al. 2013; Skyler et al. 2017; Katsarou et al. 2017). A perda do controle da glicose sanguínea pode derivar de diferentes causas, portanto, a etiologia é usada para definir as quatro principais classificações para o DM: DM tipo 1, DM tipo 2, outros tipos específicos de DM e DM gestacional (American Diabetes Association 2017).

No DM tipo 1 as células β pancreáticas são destruídas, células estas responsáveis por sintetizar e secretar o hormônio insulina controlando os níveis de glicose no sangue. Neste caso, a perda de células β ocorre principalmente por reações autoimunes. Porém, pode haver casos não relacionados à autoimunidade, consistindo na forma idiopática de DM tipo 1. Já no DM tipo 2 a ação da insulina é deficiente, uma vez que o organismo desenvolve uma resistência ao hormônio. Os outros tipos específicos de DM podem estar relacionados por exemplo a doenças relacionadas ao pâncreas exócrino, alterações genéticas das células β, consequências secundárias ao uso de medicamentos. O DM gestacional ocorre quando a hiperglicemia é diagnosticada durante a gravidez (American Diabetes Association 2017).

O diagnóstico principal é o alto índice glicêmico no sangue (≥126 mg/dl em jejum e ≥200 mg/dl 2h após a ingestão de 75g de glicose) e sinais clínicos e sintomas como poliúria, polidipsia, perda de peso, visão embaçada. Tais sintomas e sinais são mais sutis no diabetes tipo 2, levando a um diagnóstico mais tardio. Diabetes tipo 1 é mais comum em crianças e adultos jovens enquanto diabetes tipo 2 predomina em adultos. Porém, ambos os tipos podem ocorrer em qualquer faixa etária. Um aumento de casos de diabetes tipo 2 tem sido observado entre a população jovem e diabetes autoimune pode ser desenvolvida por adultos de forma lenta e progressiva, neste caso denominada como diabetes autoimune latente do adulto (LADA – do inglês "latent autoimmune diabetes in adults"). Assim, outros testes são importantes para definição, como a detecção de autoanticorpos para diabetes autoimune (Largay 2012; American Diabetes Association 2017).

2.3.2 Epidemiologia e Etiopatogênese do diabetes mellitus tipo 1

A Federação Internacional do Diabetes (IDF) estimou mais de 400 milhões de pessoas com diabetes em todo o mundo, número que poderá chegar aos 642 milhões em 2040 (International Diabetes Federation 2015). O DM tipo 1 atinge principalmente crianças e adolescentes e em 2015 foram estimados 542 mil casos na faixa etária abaixo de 15 anos de idade, no mundo, com uma tendência significativa de crescimento na média de 3% a cada ano (Maahs et al. 2010; International Diabetes Federation 2015). O Brasil é o terceiro país com maior número de crianças com DM tipo 1, apresentando mais de 30.000 casos em 2015. Uma avaliação dos gastos com saúde relacionados ao diabetes foram expressivamente altos em 2015 e a expectativa é aumentar

até 2040, estando o Brasil entre os 5 países com maiores gastos (International Diabetes Federation 2015).

Na maioria dos casos, DM tipo 1 é desencadeado por uma reação autoimune, decorrente da apresentação de autoantígenos de células β por células dendríticas e células B, desencadeando respostas de células T CD8+ e CD4+ específicas. A exposição aos autoantígenos também leva as células B desenvolverem uma resposta humoral, com produção de autoanticorpos contra antígenos específicos da ilhota, como anticorpos antiinsulina (IAA), contra a descarboxilase do ácido glutâmico (anti-GAD65), contra as tirosinafosfatases IA-2 e IA-2B e contra transportador de zinco 8 (ZNT8) (Katsarou et al. 2017). O evento desencadeador dessa reação autoimune contra células β é ainda desconhecido, mas estudos têm demonstrado a existência de fatores de risco genéticos, ambientais e epigenéticos (Wang et al. 2017).

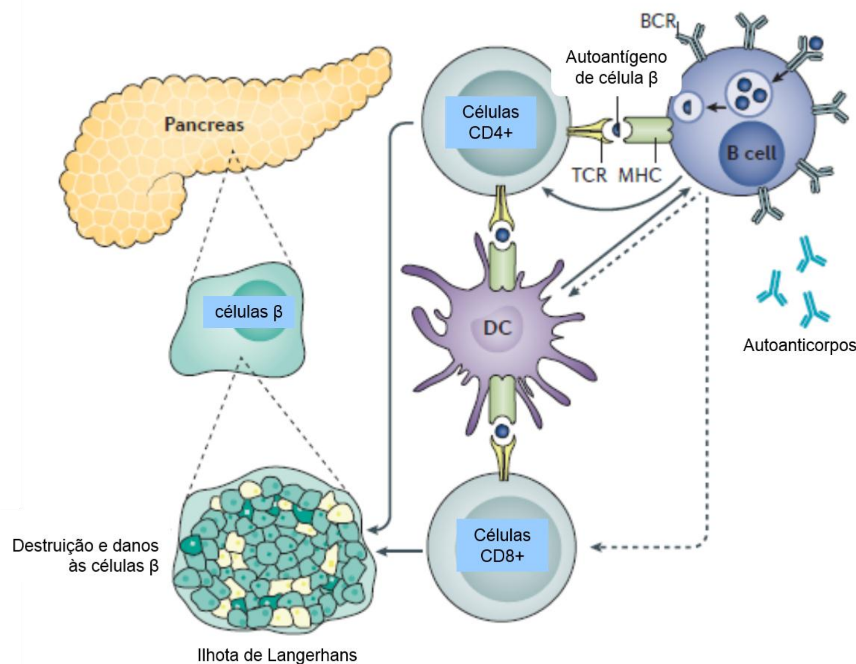

Figura 2. Patogênese do diabetes tipo 1. Células B ativadas interagem com células T (CD4+ e CD8+) e com células dendríticas. Células B e células dendríticas apresentam autoantígenos de células β, ativando células T específicas para células β. Além disso, células B também produzem autoanticorpos de ilhotas pela exposição aos antígenos. Fonte e adaptado de Katsarou et al 2017.

2.4Terapias para DM tipo 1

O DM tipo 1 não tem cura e sua terapia consiste no controle contínuo da glicemia, baseado na administração diária de insulina exógena, associado a um estilo de vida saudável. O controle glicêmico é importante para se prolongar a vida do paciente, evitando o desenvolvimento de complicações macro e microvasculares (The Diabetes Control and Complications Trial Research Group 1993).

Alcançar esse controle é um desafio para o paciente, pois requer adaptações de acordo com seu próprio organismo, buscando evitar tanto a hiperglicemia como a hipoglicemia. A hipoglicemia grave é um efeito colateral do tratamento com insulina, podendo ocorrer em média 2 episódios de hipoglicemia por semana (Frier 2014). Como consequência desses episódios hipoglicêmicos, o paciente pode sofrer desde alterações leves como fraqueza e mudança de humor, até alterações mais graves como perda de consciência que pode ocasionar acidentes e coma. A alta recorrência desses eventos pode também alterar a capacidade cognitiva dos pacientes (Frier 2014; Martín-Timón & del Cañizo-Gómez 2015). Portanto, um adequado e regulado controle glicêmico é importante para a qualidade de vida do paciente. Estudos têm mostrado que quando existe um controle restrito no tratamento, com intervenções de ações educacionais, análogos de insulina e tecnologias que permitem o controle contínuo da glicose associadas a bombas de infusão de insulina, ocorre redução de número e gravidade de episódios de hipoglicemia entre os pacientes (Choudhary et al. 2015; Ajjan et al. 2016). Porém, ainda há casos de hipoglicemia severa entre pacientes que não respondem as

intervenções, sendo indicado então o transplante de pâncreas ou ilhotas (Choudhary et al. 2015).

O transplante de pâncreas foi inicialmente descrito na década de 60 (Kelly et al. 1967). As vantagens do transplante abrangem independência da insulina, a não ocorrência de hipoglicemia e melhora das complicações macro e microvasculares. Apesar das vantagens, a metodologia do transplante de órgãos apresenta por si algumas desvantagens, como o alto risco cirúrgico, o risco de rejeição, a falha do enxerto, a necessidade de administração de imunossupressores, além da falta de doadores (Boggi et al. 2012; Gruessner & Gruessner 2013).

O transplante de ilhotas pancreáticas apresenta a característica se ser cirurgicamente menos invasivo e foi primeiramente descrito com sucesso em controlar a hiperglicemia em ratos, estudo realizado por Ballinger & Lacy em 1972 (Ballinger & Lacy 1972). O sucesso dessa metodologia em humanos ocorreu a partir de 2000, quando Shapiro e colaboradores definiram um novo protocolo para o transplante de ilhotas, conhecido como Protocolo de Edmonton por ter sido desenvolvido na Universidade de Alberta na cidade de Edmonton (Canadá) (Shapiro et al. 2000; Shapiro et al. 2006) . Porém, ainda persistem algumas desvantagens, como os efeitos nocivos do uso de imunossupressores, existência de risco de trombose, além da dificuldade em se alcançar ilhotas suficientes e mantê-las viáveis (Moberg et al. 2002; Lee et al. 2007; Calafiore et al. 2014; Khosravi-Maharlooei et al. 2015).

Um ensaio clínico de fase 3 de transplante de ilhotas humanas em pacientes diabéticos tipo 1 com hipoglicemia severa foi realizado em 2016 por Hering e colaboradores. Os resultados desse ensaio clínico foram positivos,

com cerca de 70% dos pacientes mostrando controle glicêmico 2 anos após transplante de ilhotas, hemoglobina glicada regular e resolução da hipoglicemia severa (Hering et al. 2016).

A padronização da produção das ilhotas humanas purificadas alogênicas utilizadas no ensaio clínico descrito acima também foi posteriormente publicada (Ricordi et al. 2016). Juntamente com os dados clínicos, os autores esperam que estes estudos facilitem o licenciamento pela "Food and Drug Administration", para a implementação de fato do transplante de ilhotas pancreáticas humanas alogênicas como terapia clínica e não apenas experimental. Essa implementação licenciada tornaria o transplante de ilhotas humanas mais acessível aos pacientes pela cobertura de planos de saúde, além de facilitar estudos mais avançados de ilhotas de segunda geração, como ilhotas xenogênicas e diferenciadas de células tronco e estudos de encapsulação (Ricordi et al. 2016).

De acordo com Jin and Kim, comparando os resultados dos dois tipos de transplantes (pâncreas total e ilhotas), a taxa de independência da insulina no transplante de pâncreas é maior, mas as duas metodologias se igualam na capacidade de resolução da hipoglicemia severa. Além disso, existem algumas vantagens em relação as ilhotas: a) as taxas de transplante de ilhotas sozinhas e simultâneas ilhotas/rim são equivalentes, enquanto a taxa de transplante simultâneo de pâncreas/rim é maior que a de pâncreas sozinho; b) a mortalidade associada ao transplante de ilhotas é menor, com menor ocorrências de complicações, comparando com o transplante de pâncreas (Jin & Kim 2017). De acordo, um recente estudo retrospectivo, demonstrou que o transplante de ilhotas pode de fato ser uma alternativa ao transplante do

pâncreas total, visto que em seu grupo de estudo os resultados foram semelhantes na porcentagem de independência da insulina e eliminação de hipoglicemia severa, além de que o transplante do órgão foi relacionado a maiores taxas de complicações cirúrgicas (Voglová et al. 2017).

Apesar de ser uma técnica já aplicada na clínica de forma experimental, o sucesso do transplante de ilhotas a longo prazo depende de novos estudos buscando pelo aprimoramento. Entre eles é possível citar o encapsulamento de ilhotas em estruturas como biomateriais pré-transplante, novos locais de implante, associações de outras células e moléculas junto as ilhotas, de forma a diminuir as limitações existentes que induzem a falha do transplante, como a baixa vascularização (falta de nutrientes e oxigênio) e a rejeição do enxerto (Beck et al. 2007; Krishnan et al. 2014). Além disso, novas alternativas têm sido estudadas no âmbito da terapia celular, como a diferenciação de células tronco, visando a substituição das células β destruídas (Godfrey et al. 2012).

2.5 Modelo animal do diabetes induzido pela estreptozotocina (STZ)

O uso de modelos animais para pesquisas do diabetes *mellitus* foi essencial para o entendimento da fisiopatologia e complicações associadas a doença e atualmente tem grande papel nos estudos que buscam por novas alternativas terapêuticas. Existem diversos modelos animais para estudar o DM experimental, sendo um deles o modelo induzido quimicamente por estreptozotocina (STZ). Por apresentar relativo custo benefício, com rápida indução de alterações funcionais e estruturais semelhantes a DM em humanos, este modelo tem sido amplamente utilizado para estudos experimentais do

diabetes e é um dos principais modelos para estudos de transplante de células β (Deeds et al. 2011; Graham & Schuurman 2015).

A STZ é um composto descrito inicialmente como antibacteriano (Sokolski et al. 1959; Vavra et al. 1959), sendo posteriormente descrito como diabetogênico (Rakieten et al. 1963) e antitumoral (Schein et al. 1974). Consiste de uma glicosamina-nitrosureia (2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose), um análogo da glicose, produzido pela bactéria *Streptomyces achromogenes*. É tóxico para células-β, desde que a porção glicose da sua estrutura química permite sua entrada através do receptor GLUT2, levando a alterações celulares irreversíveis (Eleazu et al. 2013; Goud et al. 2015).

Entre os mecanismos de toxicidade da STZ está a metilação do DNA. No interior da célula, a STZ se decompõe de forma espontânea em uma molécula de isocianato, a qual pode levar a carboxilação de proteínas celulares, alterando estrutura e função biológica, e em uma molécula de metildiazohidróxido, que pode gerar grupos metila, que reagem com DNA. Danos no DNA acionam vias de reparo que ativam enzima poli ADP ribose polimerase (PARP) que dependem de NAD+, levando a sua diminuição e consequentemente de ATP, com perda do estoque de energia da célula, o que leva a morte celular. Outro mecanismo é a liberação de radicais de óxido nítrico pela STZ, o qual pode interagir com enzimas mitocondriais, impedindo a produção de ATP. Óxido nítrico também pode afetar canais iônicos impedindo liberação de insulina, além de poder causar danos a nível de DNA e inativar enzimas nucleares, levando à apoptose ou necrose. STZ também leva a liberação de radicais livres como espécies reativas de oxigênio (ROS), que

podem levar a auto oxidação da glicose e glicação de proteínas. Além disso, STZ pode inibir enzimas que têm função de clivar proteínas no citoplasma, levando ao acúmulo de proteínas nocivas, ativação de vias de stress e apoptose (Eleazu et al. 2013; Goud et al. 2015).

Apesar de sua grande aplicação nas pesquisas, as metodologias para indução do diabetes por STZ apresentam diferenças na concentração e número de doses, forma de preparo e administração. Tais parâmetros podem variar de acordo com a linhagem e idade do animal. Isso dificulta a correta comparação entre os diferentes estudos sobre diabetes. Uma das variáveis mais importantes para experimentos de transplante de ilhotas ou regeneração pancreática, é a dosagem. A administração de STZ em baixas doses não é estável, pois pode levar a posterior recuperação espontânea (Eleazu et al. 2013). A administração de STZ em dose alta e única induz a morte de quase o total de células β, eliminando a produção de insulina endógena, sendo possível avaliar com mais clareza a viabilidade e função de células transplantadas ou a regeneração do órgão (Deeds et al. 2011). Desta forma, recentes estudos que avaliam a recuperação de animais induzidos ao diabetes por STZ têm utilizado doses únicas altas entre 150-200mg/kg do peso do animal (Bhang et al. 2013; Jun et al. 2014; Kim et al. 2015; Tripathi et al. 2016; Buitinga et al. 2017).

2.6 Células tronco mesenquimais (MSCs)

Uma das aplicações das MSCs consiste de seu uso na terapia alternativa ao diabetes, pela capacidade de diferenciarem em células produtoras de insulina sob determinado estímulo, capacidade de proliferação, o que eliminaria a dependência de doadores, além da possibilidade de minimização do uso de

imunossupressores (Wen et al. 2011; Godfrey et al. 2012; Bouwens et al. 2013; Nam et al. 2014; Lv et al. 2014; Espes et al. 2017).

As células tronco são definidas como células que apresentam a capacidade de autorreplicação, ou seja, originar novas células com as mesmas características, além de serem capazes de se diferenciarem em outros tipos celulares sob influência de sinais específicos. Entre os diferentes tipos de células tronco, têm-se as células tronco mesenquimais (MSCs – do inglês *mesenchymal stem cells*), definidas pela habilidade de se aderirem ao plástico, se diferenciarem em osteoblastos, condrócitos e adipócitos em cultura, e fenotipicamente expressarem CD105, CD73 e CD90 e não expressarem CD45, CD34, CD14 ou CD11b, CD79α ou CD19 e HLA classe II (Dominici et al. 2006).

As MSCs têm se destacado por apresentar, além da capacidade de diferenciação, propriedades imunossupressoras, podendo atuar na inibição da diferenciação/proliferação de células imunes, além de serem capazes de alterar a secreção de citocinas por estas células (Yañez et al. 2006; De Miguel et al. 2012; Glenn et al. 2014; Xishan et al. 2015; Valencia et al. 2016). Por modular a resposta imune, acredita-se que a utilização das MSCs em transplantes poderia minimizar o risco de rejeição. Além disso, pela sua capacidade em diferenciar em uma diversidade de tipos celulares, as MSCs são uma alternativa para medicina regenerativa (Stoltz et al. 2015; Trounson & McDonald 2015).

As MSCs podem ser isoladas de diferentes tecidos, sendo as principais fontes a medula óssea e tecido adiposo (Murray et al. 2014). Desde a descoberta de células tronco mesenquimais no tecido adiposo (Zuk et al. 2002; Rodriguez et al. 2005), estudos têm demonstrado vantagens de seu uso em

relação as de outras origens. As células tronco derivadas do tecido adiposo (ASCs – do inglês *adipose stem cells*) mostraram melhores efeitos imunomodulatórios, maior capacidade proliferativa e secreção de citocinas, além de derivar de um tecido com maior disponibilidade e acessibilidade (Bassi et al. 2012; Mizuno et al. 2012; C. Li et al. 2015; De Francesco et al. 2015).

Estudos mostraram a ação de MSCs do tecido adiposo na melhora de complicações decorrentes do diabetes, uma vez que animais tratados com tais células apresentaram melhor cicatrização de feridas isquêmicas, possivelmente pelo aumento da angiogênese (Kim et al. 2011).

Além disso, estudos têm utilizado as MSCs em conjunto durante o transplante de ilhotas pancreáticas, e observaram uma melhora na manutenção e funcionalidade do enxerto inoculado com tais células em animais diabéticos (Bhang et al. 2013; Jun et al. 2014).

2.7 Biomaterial

Biomateriais correspondem a qualquer estrutura constituída de produtos naturais e/ou sintéticos que são usados como dispositivos para substituir uma parte ou função de um sistema vivo (Park & Lakes 2007). Eles devem ser biocompatíveis e biotoleráveis, ou seja, ter habilidade de desencadear localmente uma cicatrização normal e integração do tecido, residindo no tecido por longo período com mínima reação inflamatória (Ratner 2016).

Biomateriais como plataformas são uma das bases da regeneração de tecidos, provendo suporte para interação de células e moléculas, tanto em espaço como em tempo (Langer & Tirrell 2004). A busca por novos biomateriais tem sido uma das alternativas aplicáveis no transplante de ilhotas,

protegendo-as contra a rejeição, por funcionar como uma barreira física e também nos estudos de regeneração pancreática, servindo como suporte para diferenciação de células β a partir de células tronco (Amer et al. 2014; Desai & Shea 2016). Recentes trabalhos têm utilizado algum tipo de biomaterial para maximizar o processo de transplante de ilhotas e/ou células tronco (Bhang et al. 2013; Jun et al. 2014; Nam et al. 2014; Marchioli et al. 2015; Sabek et al. 2016).

Entretanto, apesar dos avanços nessa área da pesquisa, ainda não se desenvolveu uma plataforma que replique o completo microambiente biológico de um tecido e assim seja capaz de manter células e tecidos viáveis por longo período. O sucesso de uma plataforma depende da capacidade de proporcionar um microambiente favorável à interação de células e células-matriz (Naderi et al. 2011). A porosidade do biomaterial é uma característica importante, por permitir melhor difusão e comunicação de moléculas e células (Feng et al. 2011). O tamanho e interconectividade desses poros podem influenciar o processo de vascularização dos biomateriais (Bezuidennhout et al. 2002; Somo et al. 2015). De fato, um dos principais obstáculos é conseguir desenvolver uma rede vascular adequada à nutrição e oxigenação local, afim de suprir a necessidade das células e/ou tecido que se desenvolvem no biomaterial (Tilkorn et al. 2010; Novosel et al. 2011; Nishiguchi et al. 2014; Montazeri et al. 2016).

A matriz sintética de poliéter-poliuretano consiste de um biomaterial permeável e poroso que tem sido utilizado pelo nosso grupo de pesquisa com o objetivo de se estudar processos como angiogênese, inflamação e reparo tecidual, associados a diferentes doenças como lúpus e diabetes (Andrade et

al. 1997; Campos et al. 2008; Campos et al. 2011; Oviedo-Socarrás et al. 2014; Socarrás et al. 2014). Este biomaterial pode ser implantado no subcutâneo ou na região intra-peritoneal, ocorrendo pós-implante uma resposta tipo corpo estranho que induz a proliferação de uma matriz celular vascularizada dentro dos poros do biomaterial, permitindo o estudo dos diversos processos citados acima, por ser facilmente acessível. Interessantemente, observou-se também presença de parênquima de órgãos adjacentes intra-implante (Mendes et al. 2007; Castro et al. 2012), sugerindo uma reposta proliferativa de órgãos próximos.

3. Justificativa

Este trabalho se justifica pela falta de uma terapia definitiva para doenças do pâncreas, como o diabetes. Os efeitos colaterais da terapia atual como episódios de hipoglicemia e a alta dependência de insulina gera uma necessidade de mais estudos para se entender os mecanismos envolvidos na plasticidade pancreática frente a lesão, para que novas estratégias terapêuticas possam ser desenvolvidas. Modelos experimentais de lesão permitem o estudo dessa plasticidade, já que o rastreamento celular em humanos ainda não é viável. Nosso modelo propõe uma ideia inovadora, baseada na substituição de um tecido lesado por um tecido do próprio organismo.

4. Objetivos

4.1 Objetivo Geral

Induzir e avaliar a proliferação de parênquima pancreático dentro de matriz sintética de poliéter-poliuretano através de seu implante em interface ao pâncreas de camundongos C57BL/6 diabéticos e normoglicêmicos.

4.2 Objetivos específicos

- Analisar a cinética do crescimento de parênquima pancreático exócrino e endócrino no interior do implante em animais normoglicêmicos, avaliando:

- Área de parênquima pancreático intra-implante.
- Número de ilhotas, ácinos, ductos e vasos sanguíneos por campo, intra-implante e no pâncreas nativo.
- Número de células produtoras de insulina por campo intra-implante.
- Morte celular por apoptose intra-implante.
- Parâmetros inflamatórios intra-implante.
- Parâmetros metabólicos sistêmicos relacionados ao pâncreas.

- Analisar a proliferação de parênquima pancreático no interior da matriz sintética de poliéter-poliuretano em animais diabéticos e normoglicêmicos e a influência da inoculação de células tronco mesenquimais, avaliando:

- Porosidade da matriz sintética.
- Influência de hASCs em animais normoglicêmicos quanto a presença de células produtoras de insulina no implante.

- Influência de hASCs em animais diabéticos quanto a vascularização, inflamação e células produtoras de insulina no implante.
- Influência de hASCs em animais diabéticos quanto aos parâmetros metabólicos sistêmicos e sobrevivência dos animais diabéticos.

5. Aspectos Éticos

O projeto foi aprovado pela Comissão de Ética em Experimentação Animal (CEUA/UFMG) sob protocolo nº 0043/15 (anexo A).

6. Material e métodos, resultados e discussão

Estes tópicos serão apresentados sob a forma de dois artigos científicos elaborados durante o período de doutoramento: artigo 1 - submetido para publicação (comprovante de submissão: anexo B); artigo 2 - publicado.

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Kinetics of pancreatic tissue proliferation in a polymeric platform in mice

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Running head: Pancreatic proliferation in a polymeric platform

Key words: pancreas; animal model; platform; proliferation

Abstract

Background/Objectives: Pancreas regenerative capacity after injury is not always sufficient to comply with the body's requirement of digestive enzymes and hormones. We present an alternative system to induce pancreas parenchyma proliferation (exocrine and endocrine components), rather than regeneration or remodeling in normoglycemic mice.
Methods: Porous discs of polyether-polyurethane were surgically placed adjacent to the native pancreas and removed at days 15, 30 and 45 after implantation. No exogenous growth factors or extracellular matrix components were added to the platform. The synthetic matrix provided a platform that was filled with parenchymal and non-parenchymal pancreas tissue as detected by histological analysis. Immunohistochemistry analysis were performed to identify insulin positive cells in the newly formed tissue. In addition, angiogenic, inflammatory and metabolic parameters were carried out in those mice.
Results: At day 15, the pores of the platform were filled with inflammatory cells, spindled-shaped like fibroblasts, extracellular matrix components, blood vessels and clusters of pancreatic parenchyma (acini, ducts and islet-like structures). At days 30 and 45 the pancreas features remained well organized, its organization resembled that of a native pancreas. Interestingly, besides islet-like structures showed positive cells to insulin, some ductal cells were also positive for insulin immunostaining. No significant differences in serum glucose and c-peptide concentrations during the experimental period were detected.
Conclusions: The plain synthetic porous platform (without addition of exogenous molecules) placed adjacent to the native organ exhibits potential to restore and/or expand exocrine (acini, ducts) and endocrine (β-cell mass) components in pancreatic injuries and in high metabolic demand.

1. Introduction

The pancreas is a complex organ composed of endocrine and exocrine compartments. Injury to the exocrine compartment causes severe clinical complications. In acute pancreatitis, high levels of amylase and lipase are present in the circulation but normally, this condition resolves without incident within days or weeks [1–3]. In contrast, chronic pancreatitis, involve advanced inflammatory and fibrotic alterations[3–5]. Lesion to β-cells in the endocrine compartment causes diabetes, which is characterized by hyperglycemia and is associated to micro and macrovascular alterations, being considered a global epidemic disease [6].

Several animal models have been reported which selectively injury the exocrine component (caerulein administration, duct ligation) or the endocrine compartment (streptozotocin administration, subtotal pancreatectomy), providing valuable insights into mechanisms of pancreas regeneration [1]. Studies with these models showed that acinar cells could regenerate from self-proliferation [7,8]. Whereas, beta cells can have multiple origins as from other endocrine cells [9], duct cells [10], acinar cells [11], self-duplication [12–15] or progenitor cells [16,17]. However, the different repair responses of adult pancreas depends on the nature of damage [18] and it regenerative capacity is not always sufficient to comply with the body's requirement of digestive enzymes and hormones, taking to chronic injury and aggravation of diabetes [9,13,19]. There is, thus a need for the development of strategies to improve pancreas regenerative capacity and/or replacement.

We have used synthetic polyether-polyurethane as an implanted platform to analyze the interaction between the host and the foreign material in the subcutaneous or peritoneal cavity of rodents. In previous publications, we showed that in the absence of exogenous addition of growth factors or extracellular matrix components, the implants become infiltrated by a proliferative fibrovascular tissue containing parenchymal cells of closer organs 8 days after implantation [20,21]. More recently, we reported that this polymeric platform adherent to the native mice pancreas filled with human adipose stem cells (hASCs) was able to induce growth of pancreatic parenchyma decreasing glucose levels in diabetic animals [22]. Thus, in contrast with other in vivo models used to investigate regenerative capacity, our model requires no incision to the animal's internal organs and no addition of exogenous growth factors and/or extracellular matrix components to the platform. As the model does not involve reduction of organ mass or ligation of ducts, it presents an alternative system to induce internal organ proliferation, rather than regeneration or remodeling. We hypothesized that this experimental model could be further exploited to investigate whether the platform would be colonized by exocrine and endocrine pancreatic cells. In order to achieve that we analyzed the kinetics of the cell population inside the platform at different time points post-implantation. Understanding the cellular processes involved in this response is central to the development of tissue engineering strategies using the newly formed pancreatic tissue.

2. Material and Methods

2.1 Animals

The Ethics Committee of Animal Use (CEUA) of the Federal University of Minas Gerais, (protocol number 43/2015) approved all animal procedures. We used 8-week-old male C57BL/6 mice (n=10/group) provided by Centro de Bioterismo (CEBIO) of the Federal University of Minas Gerais (UFMG). All mice were provided with standard chow pellets (NUVILAB CR-1 Brazil) and water *ad libitum* in our pathogen-free facility, and maintained under a 12-hour light/dark cycle.

2.2 Experimental design

Synthetic polyether-polyurethane matrices in disc format, 5 mm thick x 8 mm in diameter (Vitafoam Ltd, Manchester, U.K.), were soaked overnight in 70% ethanol and sterilized by boiling in distilled water for 30 min prior to implantation [23]. All animals were anesthetized with a mixture of ketamine and xylazine (60 mg/kg and 10 mg/kg, respectively). The abdominal hair was shaved and the skin wiped with 70% ethanol. An incision (< 1 cm long) was made in the upper left quadrant of the animals' abdomen. The implant discs were placed directly on the pancreas to create an interface between the synthetic matrix and the biological tissue. The incisions were closed with silk braided absorbable suture. The animals were kept isolated (one animal/cage) and monitored post-surgery for any signs of infection or discomfort.

After 15, 30 and 45 days, the animals were anesthetized with ketamine/xylazine and euthanized. The implant and pancreas were carefully dissected from the adherent tissue, removed, and weighted. In addition, animals blood samples were collected. They were then processed as described below for various assays.

2.3 Animals glycemic metabolism analysis

The blood glucose levels were measured with On Call® Plus Blood Glucose Meter (ACON Laboratories, Inc. San Diego CA, USA). The animals' blood glucose levels were measured weekly throughout the experiment. The blood samples collected during euthanize were centrifuged at 4 ◦C for 10 min at 3000rpm to obtain serum to measure c-peptide level. C-peptide levels were measured using the C-peptide ELISA Kit (DRG Instruments GmbH, Germany) according to manufacturer's protocol.

2.4 Histological and immunohistochemical (IHC) analysis

The implants and pancreas of the animals were removed, and fixed in 10% formol for 24h. After paraffin inclusion, sections with 5-mm thickness were stained with hematoxylin and eosin (H&E), while other sections were processed for immunohistochemical analysis. A total of 30 fields/slide were captured with a digital camera JVC TK-1270/JCB connected to a microscope with a magnification of x400 to analyze the number of pancreatic islet, acini, ducts and blood vessels in the native pancreas and intra platform. Additionally, the percentage of pancreatic area

infiltrating the platform was determined. The morphometric parameters were analyzed by Image Pro Plus 7.0 software.

Immunohistochemistry analysis of insulin producing-cells were performed in deparaffinized sections of the implants. The sections were hydrated, and submitted to subsequent blocking steps: endogenous peroxidase block with 30% H2O2: methanol (1:1) for 15 minutes and 5% BSA for 1 hour at ambient temperature. The slides were then incubated overnight at room temperature with the primary antibody: guinea pig antibody anti-insulin (1:500 - Dako); followed by incubation with Dako kit LINK and HRP–streptavidin (1 hour each). DAB chromagen was used to visualize the peroxidase activity. Sections were then counterstained with hematoxylin. The negative control was performed by omitting the primary antibody and carried out simultaneously.

The presence of cells in apoptosis was examined intra implant by TUNEL (TdT mediated dUTP nick end labeling) in sections (5mm thickness) using a commercial kit (TdTFragEL DNA Fragmentation Detection Kit, Cat QIA33; Calbiochem, San Diego, CA, USA). This allows identification of cells in apoptosis in marked terminal fragments of DNA (portion 39-OH), associated with the characteristically fragmented nuclear DNA. The method was applied according to the manufacturer's instructions. The morphometric parameters described above were analyzed by Image Pro Plus 7.0 software.

2.5 Measurement of cytokine levels produced intra implants

The cytokines TNF-α and CCL2 in the implants were determined by Immunoassay Kits (R and D Systems, USA) according to manufacturer's protocol. The implants were homogenized in PBS pH 7.4 containing 0.05% Tween and centrifuged at 10,000 ×g for 30 min. Following that procedure, 100 µl of the supernatant were used to measure the cytokines. Dilutions of cell-free supernatants were added to ELISA plates coated with a specific murine monoclonal antibody against the cytokine, followed by the addition of a second horseradish peroxidase-conjugated polyclonal antibody, also against the cytokine. After washing to remove any unbound antibody-enzyme reagent, a substrate solution (50 µL of a 1:1 solution of hydrogen peroxide and tetramethylbenzidine 10 mg/ml in DMSO) was added to the wells. Color development was stopped after 20 min incubation with 2 M sulfuric acid (50 µL) and color intensity was measured at 540 nm on a spectrophotometer (Thermoplate). Standards were 0.5-log10 dilutions of recombinant murine cytokines from 7.5 pg/ml to 1000 pg/ml (100 µl). The results were expressed as a picogram of cytokine/mg of wet tissue.

2.6 Determination of myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities intra implant

The activity of neutrophils in the samples was measured by assaying myeloperoxidase (MPO) activity as previously described [24]. The tissues were homogenized in sodium phosphate buffer ($NaPO_4$ 0,05M pH 5,4), and centrifuged at 12000g for 10 min. MPO activity was assayed by measuring the change in

absorbance (optical density; OD) at 450 nm using tetramethylbenzidine (1.6 mM) and H_2O_2 (0.3 mM). The reaction was terminated by adding 50 µl of H_2SO_4 (4M). Results were expressed as a change in OD per g of wet tissue.

The activity of macrophages was quantified by measuring the levels of the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) present in high levels in activated as previously described [25–27]. The samples were homogenized in NaCl solution (0.9% w/v) containing 0.1% v/v Triton X-100 (Promega, Madison, WI, USA) and centrifuged (3000 g; 10 min at 4°C). Samples (100 µl) of the resulting supernatant were incubated for 30 min at 37°C with 100 µl of p-nitrophenyl-N-acetyl-beta-D-glucosaminide (Sigma-Aldrich, St Louis, MO, USA) prepared in citrate/phosphate buffer (0.1 M citric acid, 0.1 M Na_2HPO_4; pH4.5) to yield a final concentration of 2.24mM. The reaction was completed by adding 100µl of 0.2 M glycine buffer (pH 10.6). Hydrolysis of the substrate was determined by measuring the absorption at 400 nm. Results were expressed as nmol/mg of wet tissue.

2.7 Statistical analysis

Quantitative data was presented as mean ± SEM. The assumptions of normality and homoscedasticity were determined for subsequent statistical analysis. Statistical analysis was performed using the GraphPad Prism 5.0 software. Comparisons between three or more groups were made using one-way analysis of variance (ANOVA) followed by the Newman-Keuls correction factor for multiple comparisons as a post-test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Platform-induced pancreatic tissue growth (exocrine and endocrine components)

Implantation of polyether-polyurethane synthetic matrix adjacent to the native pancreas of normoglycemic mice induced proliferation of a fibrovascular tissue containing pancreatic parenchyma. The biomaterial was well tolerated by the animals and no signs of infection or rejection were observed in situ. The newly formed pancreatic tissue occupied 27% of the platform at day 15 after implantation. The area occupied by the new tissue remained unaltered until 45 days after implantation (the last time point examined). Figure 1 (A-E) shows representative images in H&E staining sections of the native pancreas and newly formed pancreatic tissue inside the platform. At day 15 it was possible to observe fibrovascular tissue (with inflammatory infiltrate, spindle-shaped like fibroblasts, proliferating endothelial cells forming microvessels) and clusters of pancreatic-like tissue. At days 30 and 45 post implantation, the pancreatic parenchyma and structures (acini, ducts, blood vessels, and islets) remained well organized (Fig.2 A-E). Interestingly, the number of pancreatic ducts intra implant was higher compared to the native pancreas (Fig.2 C, D and F).

The endocrine component was assessed by immunohistochemistry using antibody to identify insulin-producing cells intra-implant. It was observed that positive cells to insulin inside the platform were organized forming islet-like structures in all-time post-implantation points evaluated (15, 30 and 45 days) (Fig.3

A-B). Interestingly, some ductal cells were also positive for insulin immunostaining (Fig.3 C).

3.2 Vascular analysis in the newly formed pancreatic tissue

The number of blood vessels in the platform-induced pancreatic tissue, as determined by morphometric analysis of H&E staining sections, was higher at all-time points compared with that of native pancreas (Fig. 4 A-C).

3.3 Apoptosis analysis in the newly formed pancreatic tissue

In sections stained with TUNEL, positive cells were not identified in the pancreatic tissue area (Fig.5A). However, in the fibrovascular tissue surrounding the pancreatic parenchyma, positive cells were observed at days 15, 30 and 45. The features, dark-brown TUNEL positive nuclei, apoptotic bodies, cellular shrinkage, and condensed chromatin were clearly identified in the histological sections (Fig.5B-C).

3.4 Inflammation analysis in the newly formed pancreatic tissue

Several measurements of the inflammatory component of the implants (inflammatory enzyme activities and pro-inflammatory cytokines) were performed. As shown in Fig. 6A–B, there was a peak in MPO and NAG activities at day 15, followed by a decrease at day 30 that was maintained until day 45. Similar profile was observed for the cytokine levels. TNF-α and CCL2 levels were higher at 15 days, and decreased in the last two-time points evaluated (Fig.6C-D).

3.5 Glucose metabolism in animals bearing newly formed pancreatic tissue

We assessed whether the newly formed pancreatic tissue would alter glucose metabolism by measuring serum glucose and c-peptide levels of the animals before implantation (time zero) and after implantation (15, 30 and 45 days). There were no significant differences in serum glucose and c-peptide concentrations during the experimental period (Fig.7A-B).

4. Discussion

Pancreatic disorders such as pancreatitis and diabetes are diseases associated with lesions of exocrine and endocrine pancreas and the understanding of tissue regeneration process is important to new therapeutic approaches. The experimental models of pancreas regeneration are important to understand the involvement of native cells in this process, since not all cellular mechanisms involved have been elucidated [1,28]. A number of different approaches using in vitro systems as well as animal models has been established, including administration of damaging chemicals (caerulein) and duct ligation to selectively study exocrine pancreas regeneration, or β-cell ablation (STZ) and subtotal pancreatectomy to study endocrine pancreas regeneration are the most used methods [1]. In addition, several alternative cellular approaches using biomaterials to host steam cells and/or β-cells have been developed to overcome the limitations of current pancreatic dysfunction's treatment. In a number of these reports, the goal has been to increase the speed of regenerative process as well as to determine the source of new islet cells [29].

The in vivo experimental model used here to induce pancreatic tissue growth presents advantages over the existing models to investigate pancreas regeneration. The implantation of polyether-polyurethane matrix adjacent to the animal´s native pancreas requires no incision to the organ (total or subtotal pancreatectomy). No addition of exogenous growth factors and/or extracellular matrix components to the platform was necessary. Moreover, no administration of systemic damaging drugs or any other interference on the native organ took place.

Yet, the stimulus provided by the platform was able to induce migration of pancreatic cells which colonized the biomaterial. More importantly, the newly formed pancreatic tissue exhibited both exocrine (acini and ducts) and endocrine (insulin-producing cells, islet-like structures) components of the organ.

It has been proposed that, the nature of the stressful conditions caused by acute tissue damage, such as pancreatectomy or PDL may activate cell plasticity and signaling pathways in specific cell lineages that may differ from the ones activated after β-cell ablation. Although, in our model there was no extensive damage to the native pancreas, it seems that the type of injury provided by the platform in close contact with the organ triggered the signaling pathways involved in activation of proliferative mechanisms in the various cell types of the native pancreas. It has been proposed that repair of pancreas mass reduction occurs through regeneration and that repair of exocrine pancreas after duct ligation occurs through remodeling [30]. It is unlikely that these processes have occurred in our model, since no pancreas removal/β-cell ablation or duct ligation took place. Thus, it is likely that the cells from the native pancreas proliferated, migrated, and organized themselves in a pancreas-like organ inside the platform.

The histologic analysis of the biohybrid platform showed the growth of a well-structured pancreatic parenchyma in which acinar areas, pancreatic ducts, and islet-like structures (immunostained for insulin) were identified. Interestingly, no alteration in glucose metabolism, as determined by glucose and c-peptide serum levels, was observed in the animals bearing the platform. It was intriguing to

observe the high proliferative capacity of the duct cells. The number of ducts was higher intra-implant comparing to native pancreas.

It is likely that some features (size of the pore, architecture) of our synthetic matrix provided a suitable microenvironment that allowed the colonization of the platform the parenchymal and non-parenchymal pancreas cells. The average pore diameter of the polyether-polyurethane platform was 474 μm as previously reported [22], essential characteristic to vascularization process in platform [31]. Moreover, the potential for cells to migrate into platform is influenced by the biophysical nature, the porosity and it interconnectivity [32,33].

The newly tissue that infiltrated the platform was initially (day 15) composed of an extracellular matrix, dense inflammatory infiltrate, spindle-shaped fibroblasts, clusters of pancreatic cells (islets, acinis, ducts) and blood vessels of various sizes. The pancreatic structures remained organized until day 45 post-implantation. This is consistent with our finding that apoptotic cells were identified in fibrovascular tissue but not in the newly pancreatic tissue. Interestingly, some ductal cells were also positive for insulin immunostaining. This finding is in agreement with a number of studies demonstrating that extra-islet cells (acinar and ductal) under stressful conditions are reprogrammed into insulin producers [34–37].

In previous studies, we have demonstrated the ability of the polyether-polyurethane matrix to induce angiogenesis as determined by a number of vascular markers [38]. As expected, newly formed blood vessels were seen in the fibrovascular tissue and in the platform-induced pancreatic tissue. It has been proposed that induction of a functional capillary network inside any platform, is a

requirement and advantage of any construct involving biomaterials [39]. Thus, it is likely that platform-induced angiogenesis has contributed not only to the development of the pancreatic tissue, but also to the maintenance of the newly formed structures. It has been demonstrated that endothelial cells of islets can synthetize growth factors as connective tissue growth factor (CTGF) and hepatocyte growth factor (HGF), which participate in β cells proliferation and differentiation, besides of regulation of glucose response (reviewed by Cao & Wang 2014; Narayanan et al. 2017).

The inflammatory component of the host response to the implant was assessed by measuring TNF-α and CCL2 levels and by estimating numbers of leukocyte number indirectly, using MPO and NAG activities. There was a consistent pattern in the cytokine production and in the inflammatory enzyme activities. The peak achieved at day 15 post-implantation was followed by a decrease at days 30 and 45. In the last two intervals examined, the newly formed pancreatic tissue remained well organized. The insulin-producing cells formed islet-like structures and the ducts and acini were well delimited. This is consistent with the work by Criscimanna et al (2014) that induced ablation of infiltrating pancreatic macrophages by use saporin-conjugated antibody against CD11b/Mac1(Mac1-SAP), leading to failure of pancreatic regeneration [36]. It seems that the resolution inflammatory phase (days 30 and 45), in which macrophages are actively involved, provided the suitable microenvironment for the maintenance of the pancreatic tissue.

The main findings that have emerged from this work were that the polyether-polyurethane polymeric platform placed adjacent to the animal's pancreas was able to induce migration, proliferation, and organization of a pancreas-like organ inside the platform. Positive cells to insulin inside the platform were organized forming islet-like structures and some ductal cells were also positive for insulin immunostaining. This experimental model of pancreas proliferation holds potential to be exploited to restore and/or expand exocrine (acini, ducts) and endocrine β-cell mass in pancreatic injuries and in high metabolic demand.

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Statement of author contributions

Conception and design, or analysis and interpretation of data, LXP, PPC, SPA and ACV Conception and design, analysis and interpretation of data, PPC,SPA drafting the article and revising it critically for important intellectual content. LAAO, SAA, MGTL, LCC and CTRV carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Conflict of interest

The authors declare that they have no conflict of interest.

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Legends and Figures

Figure 1: Pancreatic tissue growth into the synthetic matrix. Microscopic appearance of the native pancreas (A) and progressive infiltration of the pancreatic tissue in the platform (B-15 days; C-30 days and D-45 days). (lupa magnification – Bar 0,5 cm – Hematoxylin-Eosin). E) Percentage of pancreatic parenchyma area intra platform. F) Platform wet weight values after implantation. Data are expressed as mean ± SEM.

Figure 2: Endocrine and exocrine components of the newly formed pancreatic tissue. A) Number of islets/field. **B)** Number of pancreatic acini/field. **C)** Number of pancreatic ducts/field. **D)** Representative histological sections (H&E) of native pancreas showing islets (i), acini, ducts (arrows). **E-F)** Representative histological sections (H&E) of platform - induced pancreatic tissue showing islets (i), acini, ducts (arrows) *synthetic matrix. Data are expressed as mean ± SEM. *Significant difference between the groups (ANOVA) * $P < 0.05$.

Figure 3: Kinetics of insulin-producing cells growth inside the synthetic matrix. The number of insulin-producing cells remained unaltered during the experimental period (A). Data are expressed as mean ± SEM. B) Representative micrograph of immunohistochemistry staining for insulin showing islets-like structure. *synthetic matrix. C) Representative micrograph showing ducts-like structure staining for insulin (insert, duct stained in detail). Scale bar represent 50μm.

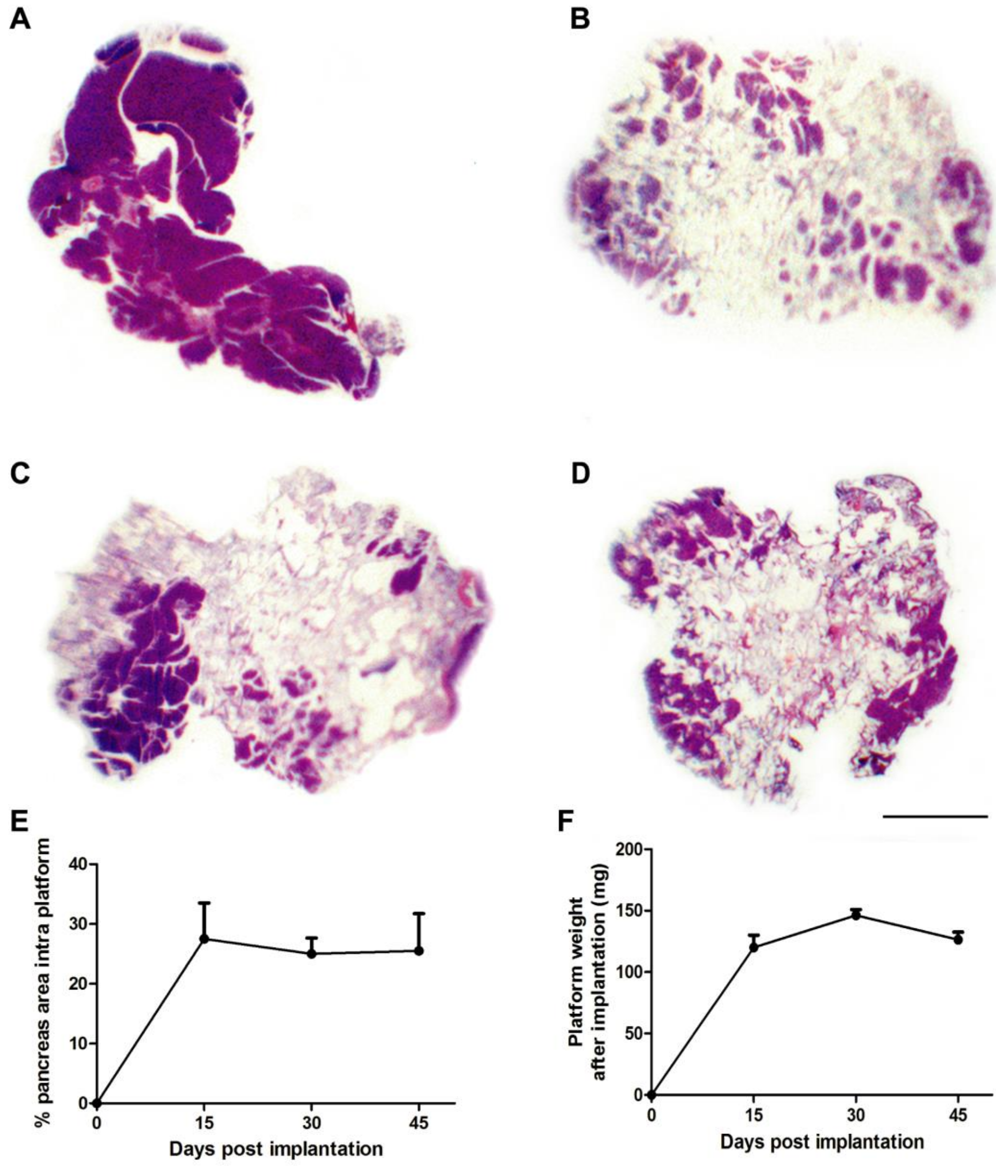
Figure 4: Vascular analysis of the platform-induced pancreas. A) Morphometric analysis of the blood vessels number in the native pancreas and inside of platform. Representative histological sections (H&E) of blood vessels in native pancreas (B) and in the platform-induced pancreatic tissue (C). Arrows = blood vessels. Data are expressed as mean ± SEM. *Significant difference between the groups (ANOVA) * $P < 0.05$. Bar 50μm.

Figure 5: Apoptosis analysis in the platform-induced pancreatic tissue. Representative histological sections of apoptotic cells staining to TUNEL in the pancreatic tissue induced by the synthetic matrix (A and B). Note that positive staining was found in the non-parenchymal fibrovascular tissue (B), but not in the newly formed pancreatic tissue (A). Scale bar, 25μm. C) Morphometric analysis of apoptotic cells staining to TUNEL in the fibrovascular tissue. Data are expressed as mean ± SEM.

Figure 6: Kinetics of inflammatory markers in the newly formed pancreatic tissue. Increase in TNF-α (A) and CCL2 (B) levels was observed 15 days after implantation, followed by a decrease at 30 and 45 days. Similarly, increase in MPO activity (C) and NAG activity (D) was observed 15 days after implantation, followed by a decrease at 30 and 45 days. Data are expressed as mean ± SEM. *Significant difference between the groups (ANOVA) * $P < 0.05$.

Figure 7: Glucose metabolism in animals bearing newly formed pancreatic tissue. A) Fast blood glucose level measured before and 15, 30 and 45 days after implantation (**B)** Serum c-peptide levels (ng/mL) measured before and 15, 30 and 45 days after implantation. Data are expressed as mean ± SEM.

Figure 1



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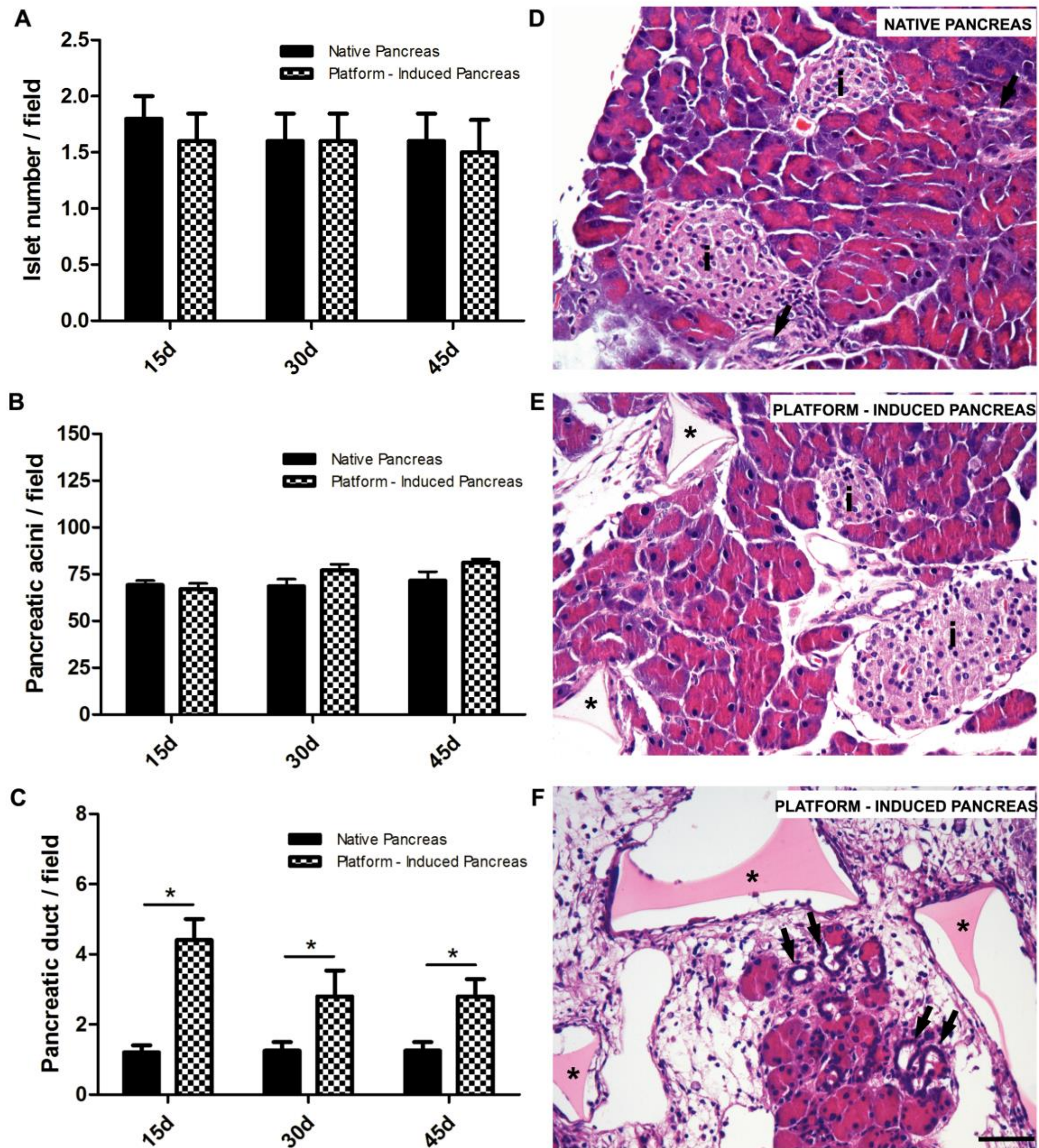

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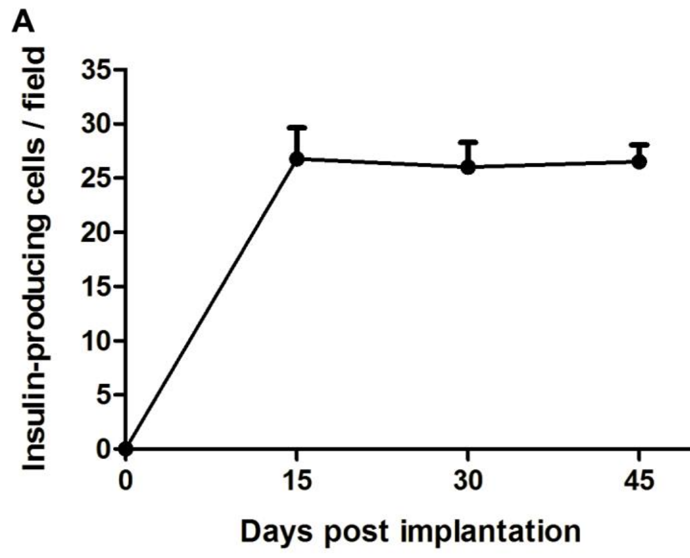
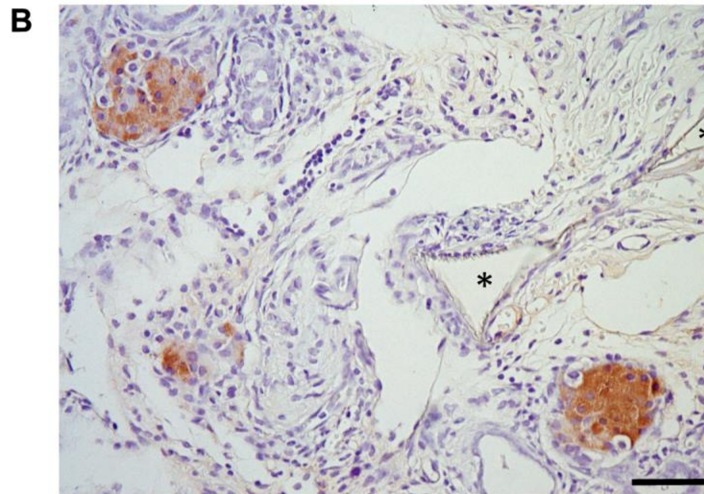




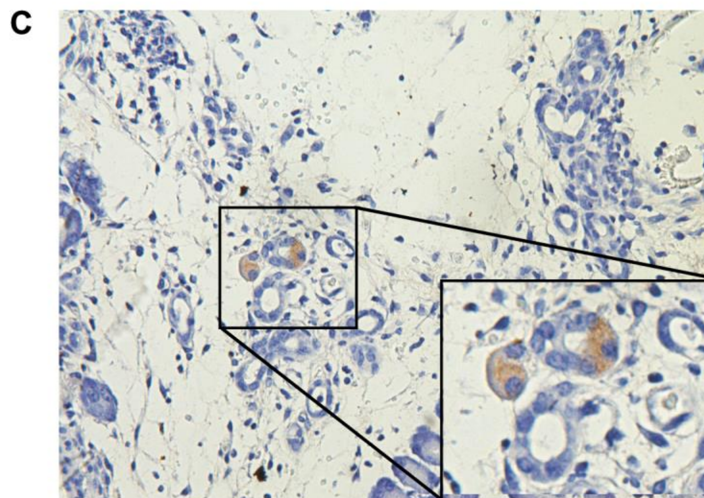
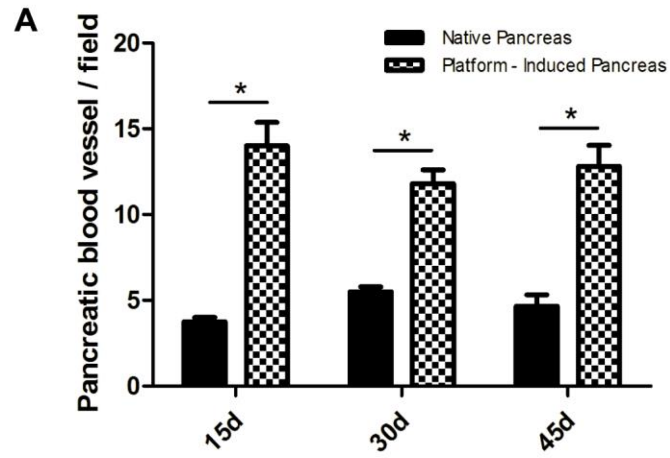
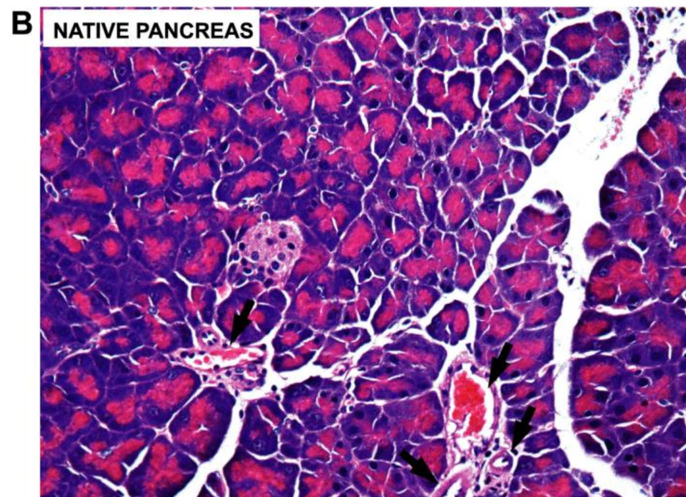


Figure 4





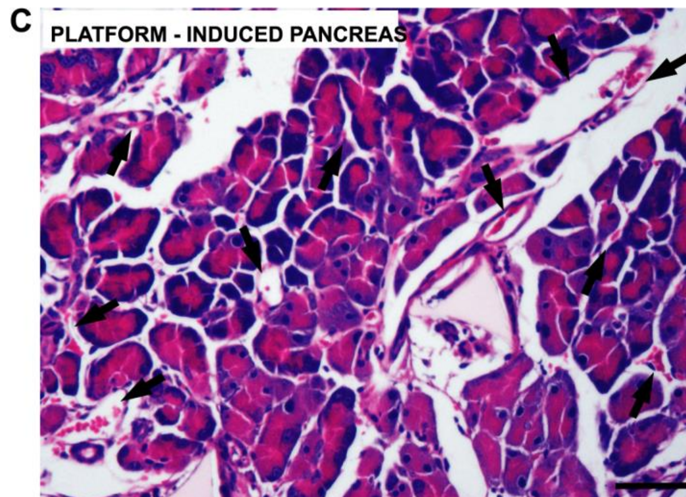


Figure 5

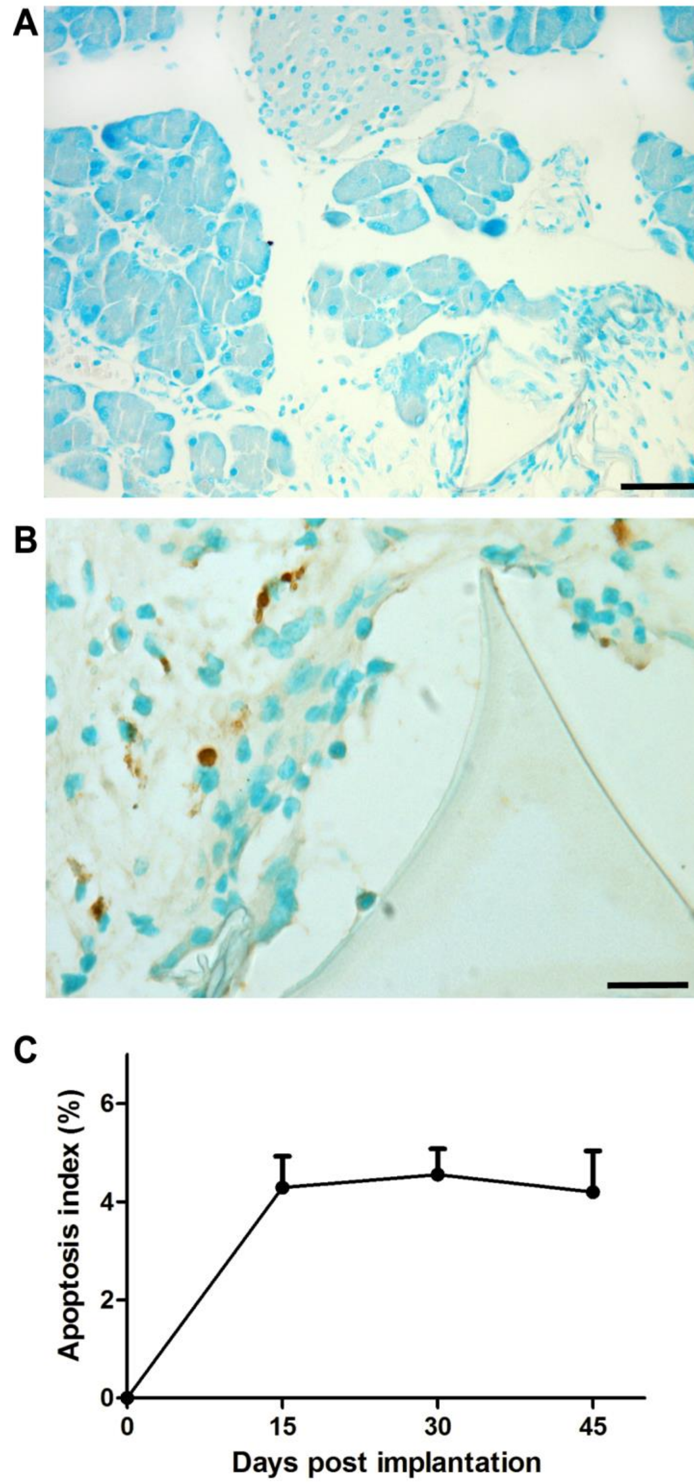


Figure 6

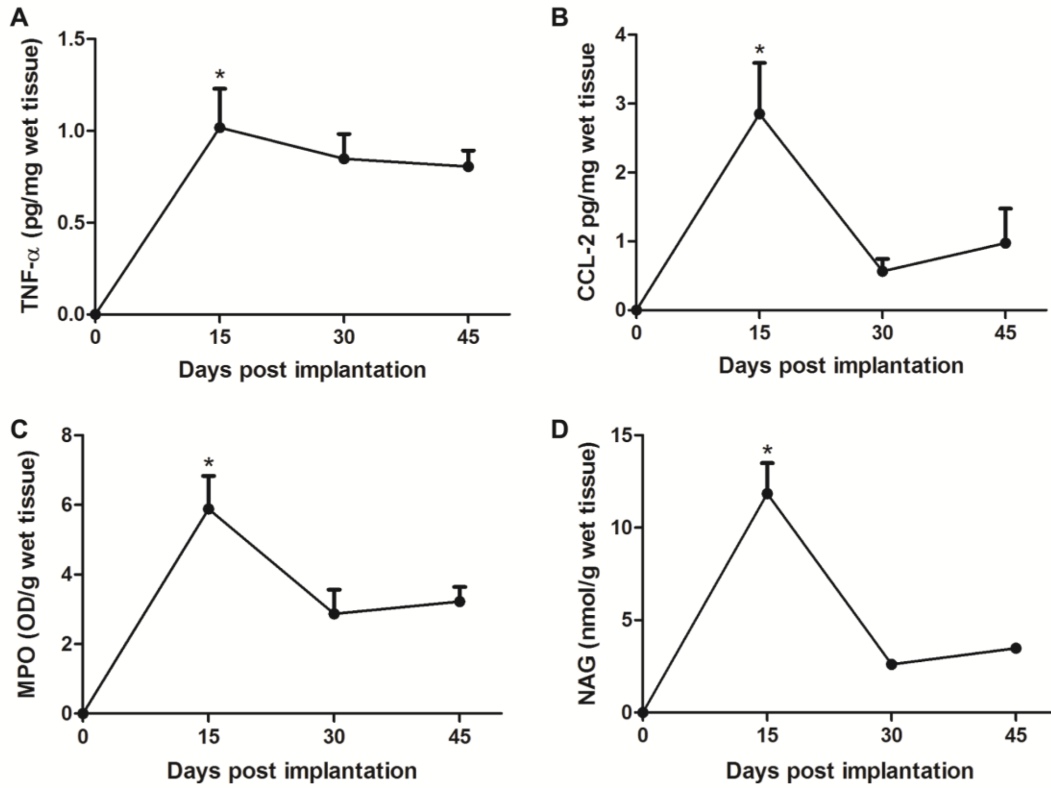
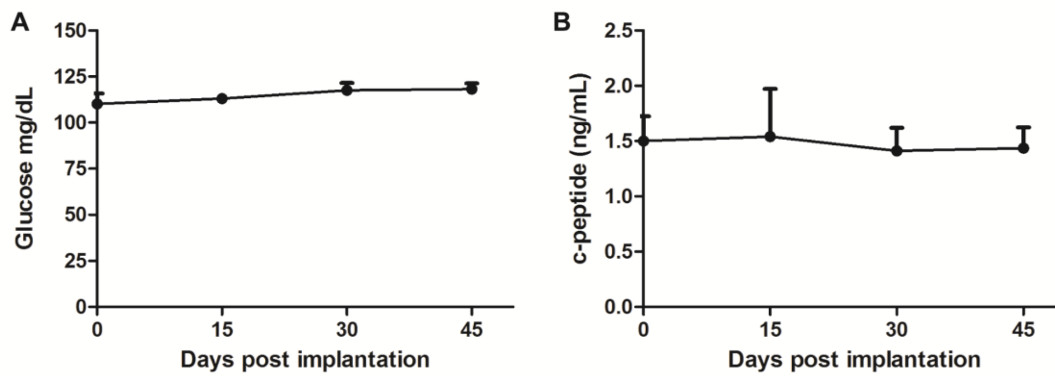


Figure 7



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Synthetic matrix of polyether-polyurethane as a biological platform for pancreatic regeneration

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abstract

Aims: Several alternative cellular approaches using biomaterials to host insulin-producing cells derived from stem cells have been developed to overcome the limitations of type 1 diabetes treatment (exogenous insulin injection). However, none seem to fulfill all requirements needed to induce pancreatic cells successful colonization of the scaffolds. Here, we report a polymeric platform adherent to the native mice pancreas filled with human adipose stem cells (hASCs) that was able to induce growth of pancreatic parenchyma.
Main methods: Synthetic polyether-polyurethane discs were placed adjacent to pancreas of normoglycemic and streptozotocin-induced diabetic mice. At day 4 post implantation, 1×10^6 hASCs were injected intra-implant in groups of normoglycemic and diabetic mice. Immunohistochemistry analysis of the implants was performed to identify insulin positive cells in the newly formed tissue. In addition, metabolic, inflammatory and angiogenic parameters were carried out in those mice.
Key findings: This study provides evidence of the ability of a biohybrid device to induce the growth of differentiated pancreas parenchyma in both normoglycemic and streptozotocin-induced diabetic mice as detected by histological analysis. Glucose metabolism and body weight of hyperglycemic mice bearing hASCs implants improved.
Significance: The synthetic porous scaffold bearing hASC cells placed adjacent to the native animal pancreas exhibits the potential to be exploited in future cell-based type 1 diabetes therapies.
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1. Introduction

Type 1 diabetes mellitus (T1D) is an autoimmune disease that causes the death of β cells, resulting in insulin deficiency and consequently hyperglycemic state. Control of blood glucose levels by exogenous insulin use, physical activity and healthy diet are essential to avoid long-term complications such as circulatory disorders, retinopathy and kidney failure [1,2]. Despite the longer survival of patients by improvement in the management of T1D, epidemiological data shows that the incidence of type 1 diabetes is increasing by 3% per year worldwide. Currently there are 542,000 cases in the age group under 15 years old in the world [3,4]. Furthermore, glucose control through exogenous insulin administration often renders patients susceptible to severe episodes of hypoglycemia by not mimicking the physiologic secretion of the hormone [5–8]. Another approach to control glucose metabolism involves transplantation of pancreatic islet cells to restore endogenous insulin production under physiologic regulation [9]. Limitations of this procedure include the need of immunosuppressant therapy, limited supply of organ donation, and short survival of the grafts [10].

Tissue engineering associated with cell therapy has enabled the development of new approaches to overcome the limitations of current treatments for T1D. Researchers have been combining islets with biomaterials to provide mechanical support and a suitable extracellular environment for islets cells. To maintain their survival and function in vitro and in vivo; otherwise, without the biomaterials use, the probability of these cells to fail engraftment increases [11]. However, for this to work, a pre-vascularized microenvironment suitable for islet cell growth and viability able to nourish and oxygenate the new tissue is essential [12,13]. Tissue engineering approach for the development of a bioartificial pancreas could ensure optimal implantation site to favor both engraftment and long-term function of islet cells [14,15].

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Tissue engineering is composed by three pillars: cells, signaling molecules, and natural/synthetic constructs that work as scaffolds [16–20]. Scaffolds play a fundamental role by providing support for the interaction between cells, molecules and the extracellular matrix [17,18]. The application of stem cells into scaffolds already brought some benefits for the treatment of various diseases, including diabetes mellitus (DM) [21,22]. In fact, transplantation of insulin-producing cells derived from stem cells into diabetic mice has shown varying abilities to reverse STZ induced hyperglycemia in rodent models [23,24]. However, none of the available scaffold system seem to fulfill all the requirements for sustained insulin-producing cell survival [11].

Our group has used the implant of synthetic matrix of polyether polyurethane (sponge disc) to induce subcutaneous and peritoneal fibrovascular tissue proliferation, in which inflammation, angiogenesis, fibrogenesis, and cytokine production could be easily assessed. The placement of this synthetic matrix intraperitoneally firmly adheres to visceral organs (liver, intestines or both) containing parenchymal and non-parenchymal cells from these organs [25,26]. These observations suggested that synthetic matrix of polyether polyurethane integrates well with the surrounding environment [26]. Thus, our hypothesis was that by placing this scaffold at the pancreas site in mice, we would induce pancreatic tissue proliferation. Moreover, the scaffold could be used for hosting mesenchymal stem cells from adipose human tissue (hASCs), which have been used in regenerative medicine for the treatment of Type 1 diabetes mellitus [27].

2. Material and methods

2.1. Porosity analysis of a synthetic polyether-polyurethane matrix

Samples of a synthetic polyether-polyurethane matrix were scanned using a compact desktop micro-CT scanner (SkyScan 1174, Bruker micro-CT, Belgium), with 40 kV of source voltage, 800 μA source current, and 10.03 μm pixel size. (Supplementary material – SM1).

2.2. Animals

The Ethics Committee of Animal Use (CEUA) of the Federal University of Minas Gerais, (protocol number 43/2015) approved all animal procedures. We used 8-week-old male C57BL/6 mice ($n = 10$/group) provided by Centro de Bioterismo (CEBIO) of the Federal University of Minas Gerais (UFMG). All mice were provided with standard chow pellets (NUVILAB CR-1 Brazil) and water ad libitum in our pathogen-free facility, and maintained under a 12-h light/dark cycle. The chemical composition of NUVILAB CR-1 is dry matter (89,88%), crude protein (22,23%), crude fiber (5,73%), calcium (0,92%) and phosphorus (0,87%).

2.3. Isolation and culture of human adipose tissue-derived stem cells

The human adipose stem cells (hASCs) were isolated by Laboratory of Cellular and Molecular Immunology/Department of Biochemistry and Immunology of the Federal University Minas Gerais, as standardized by that research group [28]. The hASCs were maintained in a fresh basal medium and once the cells reached 70–80% confluence, they were replaced in new cell culture flasks. The cells were expanded until passage five and then used in the assays.

2.4. Experimental design

Synthetic polyether-polyurethane matrices in disc format, 5 mm thick × 8 mm in diameter (Vitafoam Ltd., Manchester, U.K.), were soaked overnight in 70% ethanol and sterilized by boiling in distilled water for 30 min prior to implantation [29]. The implant model was described in Supplementary material – SM2 and demonstrated in Supplementary Fig. 2 – SF2.

At day 4 post implantation, the animals were divided in two groups of which one received PBS intra-implant and the other group was transplanted with 1×10^6 hASCs into the implant. After 15 days, the animals were anesthetized with ketamine/xylazine and euthanized. The implant and pancreas were carefully dissected from the adherent tissue, removed, and weighted. They were then processed as described below for various assays. Fig. 1 shows the design protocol for this experiment.

2.5. Induction of diabetes mellitus

Diabetes Mellitus was induced by Streptozotocin (STZ) (Sigma–Aldrich, St. Louis, MO, USA) dissolved in 10 mM citrate buffer (pH 4.5). STZ doses were determined according to the animals' body weight and administered intraperitoneally in a single 150 mg/kg injection [30]. Only mice with fasting blood glucose levels of >200 mg/dL were used as recipients [31]. The blood glucose levels were measured before injection and after one week with On Call® Plus Blood Glucose Meter (ACON Laboratories, Inc. San Diego CA, USA). The animals' blood glucose levels were measured weekly throughout the experiment. The animals were weighted weekly. Oral glucose tolerance test (OGTT) was carried out in the end of the experiment (day 21). Glucose (2 mg/g body weight) was administered intraperitoneally and measured at 15, 30, 60, and 90 min post-injection. The animals were housed in polypropylene cages, provided with chow pellets and water ad libitum, and maintained under a 12-h light/dark cycle. At day 4 post implantation, two diabetics groups were established, in which one received 1×10^6 transplanted hASCs by injection into the implant, while the other received vehicle only. The following day, the animals were anesthetized with ketamine/xylazine and killed by cervical dislocation. The implant and pancreas were carefully dissected from the adherent tissue, removed, and weighted. They were then processed as described below for the various assays. The blood was collected, centrifuged at 4 °C for 10 min at 3000 rpm to obtain serum to measure c-peptide level. C-peptide levels were measured using the C-peptide ELISA Kit (DRG Instruments GmbH, Germany) according to manufacturer's protocol.

2.6. Histological and immunohistochemical (IHC) analysis

The implants were removed along with the integrated pancreas; collected and fixed in 10% formol for 24 h. After paraffin inclusion, sections with 5-mm thickness were stained with hematoxylin and eosin (H&E), while other sections were processed for immunohistochemical analysis. A total of 30 fields/slide were captured with a digital camera JVC TK-1270/JCB connected to a microscope with a magnification of ×400 to analyze the number of blood vessels/field and the number of insulin producing-cells/field. The morphometric parameters were analyzed by Image Pro Plus 7.0 software.

To verify if the hASCs remained into the implant at the end of the experiment, sections of frozen samples were processed for immunofluorescent staining to human nuclei (HuNu) and co-stained with anti-insulin antibody to verify if the insulin producing-cells intra-implant were derived from the human stem cells.

All protocols for immunohistochemistry are described in the Supplementary material – SM3.

2.7. Measurement of cytokines levels and determination of myeloperoxidase (MPO)

The cytokines VEGF and TNF-α in the implants were determined by Immunoassay Kits (R and D Systems, USA) according to manufacturer's protocol (details in Supplementary material – SM4).

The assessments of neutrophils in the samples were made by indirect measurements. Myeloperoxidase (MPO) enzyme activity was

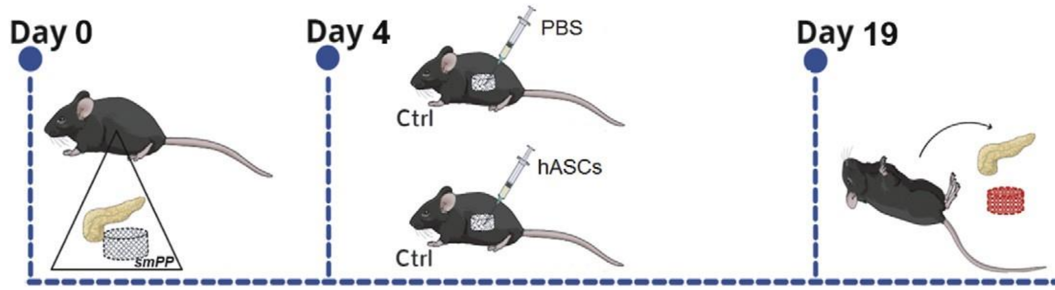


Fig. 1. Diagram representing the time line of the experimental protocol. At day 0, the polyether/polyurethane synthetic matrix (smPP) was implanted adjacent to the pancreas of the animals. At day 4, the control animals (normoglycemic) were divided in two groups: one received PBS and the other received 1×10^6 hASCs in 100 μL intra-implant. At day 19, the animals were anesthetized and killed for removal of implant and pancreas for morphometric and biochemical analyses. hASCs (human adipose stem cells).

measured according to protocol described in Supplementary material – SM5.

2.8. Statistical analysis

Quantitative data was presented as mean ± SEM. The assumptions of normality and homoscedasticity were determined for subsequent statistical analysis. Statistical analysis were performed using the GraphPad Prism 5.0 software, by Student's t-test for unpaired groups. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Synthetic matrix porosity

The synthetic matrix has been structurally characterized by computed microtomography (mCT). Supplementary Fig. 1A and B show a 3D volumetric view of the matrix and of the pores interconnectivity. In this biomaterial, most pores present a diameter between 400 and 600 μm (Supplementary Fig. 1C). The total percentage of porosity and mean pore diameter were calculated by micro-CT scanner analysis software, showing that 96,05% of the synthetic matrix used in this study is formed by pores and all these pores are clear, because showed the same value to open porosity parameter (Table 1 - Supplementary material).

3.2. Insulin-producing cells grew inside the implant

The implant of polyether-polyurethane disc (scaffold) at the interface with pancreas induced growth of pancreatic parenchyma inside the mesh of the synthetic matrix. To quantify the infiltration of pancreatic parenchyma into the implant, we measured the number of insulin-producing cells. Interestingly, implants from control animals that received hASCs showed higher number of positive cells to insulin than implants of control animals that received only vehicle (Fig.2).

Through immunohistochemical analysis, we also evaluated cell proliferation inside the implant and observed that all three cell lineages (acinar, ductal, and islets cells) were positive for CDC47 (cell proliferation marker) (Supplementary Fig. 3). Furthermore, we did the assessment of the islets morphology and observed that the islets inside the matrix were similar to the ones in the pancreas in their ability to express insulin (Supplementary Fig. 4).

Table 1
Analysis porosity.

Total porosity	Open porosity	Structure separation (pores medium diameter)
96.05%	96.05%	474.28 μm

3.3. Adipose stem cells increased matrix vascularization and inflammation in diabetic mice

Based on the development of pancreatic tissue within the implant of normoglycemic animals, we decided to evaluate if the same process occurs in diabetic animals. Therefore, we induced type 1 diabetes in the animals and followed the same experimental design (Fig. 3A). First, we assessed the fibrovascular tissue development inside the synthetic matrix, quantifying the number of blood vessels/field and VEGF level inside the implant from diabetic animals. We noticed a significant increase in these parameters in the implants with adipose stem cells from the diabetic animals when compared with the ones without stem cells (Fig. 3B–D). We also evaluated inflammatory parameters, measuring the levels of pro-inflammatory cytokine TNF-α and MPO activity (indirect measure of neutrophils presence) in the implants from diabetic animals. Both parameters were reduced in the implants containing hASCs from diabetic animals (Fig. 3E–F).

3.4. The biological platform associated to hASCs improved metabolic parameters, survival and insulin producing-cell proliferation in diabetic mice

We evaluated the fasting blood glucose levels of diabetic animals weekly. The diabetic mice that received hASCs intra-implant showed significantly lower fasting blood glucose levels compared to the diabetic group without hASCs (Fig. 4A).

We also carried out an oral glucose tolerance test (OGTT) and observed that the diabetic mice that received hASC presented better glucose disposal by the OGTT comparing with diabetic mice without hASCs at the end of the experiment (Fig. 4B).

The weekly analysis of the animal's body weight supported the efficacy of our biological platform to improve glucose metabolism in diabetic animals. Diabetic animals that received hASC intra-implant gained weight, in contrast to diabetic animals without hASCs in the implants (Fig. 4C). The survival curves showed that diabetic animals that were not transplanted with stem cells died during the experimental period. In contrast, in the group of diabetic animals that received hASCs we did not observe deaths (Fig. 4D).

In addition, to evaluate insulin levels in diabetic animals, we measured the serum c-peptide levels. As shown in Fig. 4E, diabetic mice with hASCs intra-implant showed higher c-peptide levels compared to diabetic mice without hASCs (Fig. 4E).

Moreover, pancreatic parenchyma grew to inside of the mesh of the synthetic matrix implanted in diabetic animals and this growth was greater in implants that received hASCs. We quantified the infiltration of the pancreatic parenchyma by measuring the number of insulin-producing cells. Our results show that the infiltration of insulin-producing cells was higher in implants from diabetic mice that received the adipose stem cells than in implants from diabetic animals that received vehicle only (Fig. 5A and B).

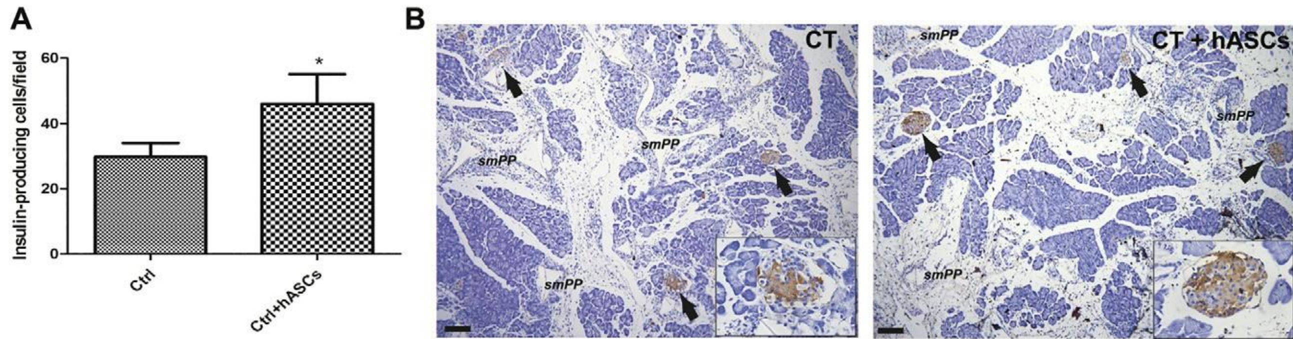


Fig. 2. Growth of pancreatic parenchyma inside synthetic matrix. A) Insulin-producing cells number/field counted intra-implant. Data are expressed as mean ± SEM. (*) represent significant difference between groups (p b 0.05). B) Representative micrographs of immunohistochemistry staining for insulin. Black arrow indicates islets and in detail, islets in higher magnifications. smPP (synthetic matrix of polyether-polyurethane); CT (control non-diabetic); CT+ hASCs (control non-diabetic inoculated with hASCs); scale bar represent 100 μm.

3.5. hASCs are not present in the implant at the end of the experiment

By immunohistochemical analysis we did not observe positive cells to HuNu intra-implant after two weeks of the cells inoculation, indicating that the hASCs were no longer present in the implant. The co-staining of insulin and HuNu intra-implant, showed the presence of islet with insulin producing-cells, but not of human cells, suggesting that insulin producing-cells originate from the animal and not from transplanted stem cells (Fig. 6).

4. Discussion

Currently, pancreatic and islet transplantation have provided therapeutic options for type 1 diabetes mellitus, since the standard treatment – insulin administration – cannot guarantee physiological regulation of glucose metabolism [5,32]. However, several adversities, such as complications associated with the surgical transplantation, the need for continued use of immunosuppressant medication, the shortage of transplant material, and organ donation, pose restrictions to this therapeutic modality. Stem cell therapies based on mesenchymal stem cells have emerged as therapeutic alternatives for diabetes control. However, this approach also has limitations, including the availability of adequate scaffolds for hosting the cells [33].

In this study, we proposed to use the synthetic matrix of polyether-polyurethane as a polymeric biological platform to induce the proliferation of pancreatic parenchyma that would also host stem cells. We show that the synthetic matrix, induced the formation of a fibrovascular tissue containing the main components of the exocrine and endocrine pancreas (acinar cells, ductal cells and insulin-producing cells) in both diabetic and non-diabetic mice. More importantly, inoculation of hASCs intra-implant was efficient to improve the survival of diabetic animals, glucose metabolism and weight gain.

It is possible that the biochemical (polyether-polyurethane) and biophysical (porous diameter 474 μm and implant size 8 mm diameter 5 mm thickness) features of the synthetic matrix have contributed to the development of the pancreatic parenchyma. It has been proposed that the pore size of a scaffold determines the success of the vascularization process post implantation, since diameter smaller than 400 μm limits the growth and diameter of blood vessels [34]. Besides, 3D microenvironment is important to development of extracellular matrix and the cell-matrix contact is essential for the function of the new tissue [13,35]. The pancreatic proliferation that occurred in our model could be also related to the inflammatory response induced by the implantation itself, similar to what occurs in lesion models, as in partial-pancreatectomy [36] and partial duct ligation [37,38]. We observed in this study by immunohistochemistry staining for a cell proliferation marker, that pancreatic cells are proliferating inside the implant, corroborating with Dor and colleagues who demonstrated a self-duplication capacity of pancreatic cells post injury [39].

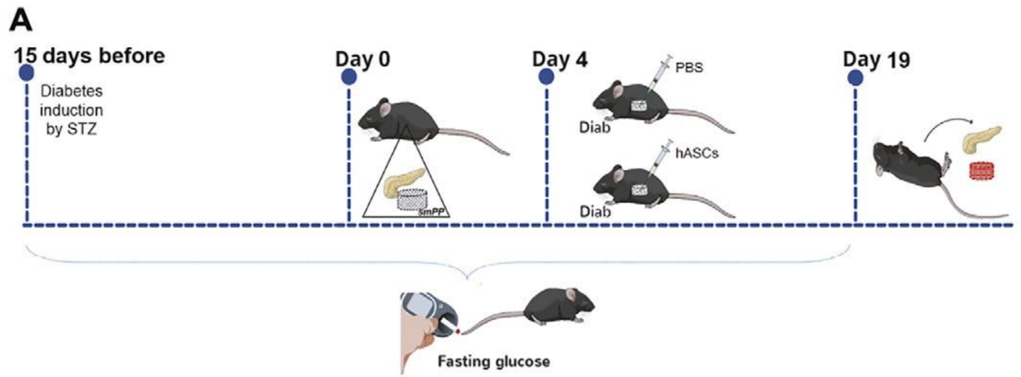
Neovascularization is part of the local inflammatory response induced by polyether-polyurethane sponge discs implanted intraperitoneally in mice [40,41]. Thus, we suggest that it may have played an important role in pancreatic proliferation [12,13,42]. Definitely, poor vascularization in healing processes of diabetic animals is well known [43], and Oviedo-Socarrás et al. demonstrated decreased angiogenesis in intraperitoneal implants of diabetic rats [44]. In our study, we observed that the hASCs enhanced angiogenesis in the implants of diabetic animals as shown by the increase in the number of vessels and high VEGF content. These findings are consistent with the pro-angiogenic effect of adipose stem cells in vivo and in vitro [45]. Moreover, other studies have demonstrated the direct and paracrine action of hASCs as pro-angiogenic cells by releasing VEGF and HIF-1α and inducing expression of cytokines by fibroblasts, improving revascularization in the ischemic skin flap [46].

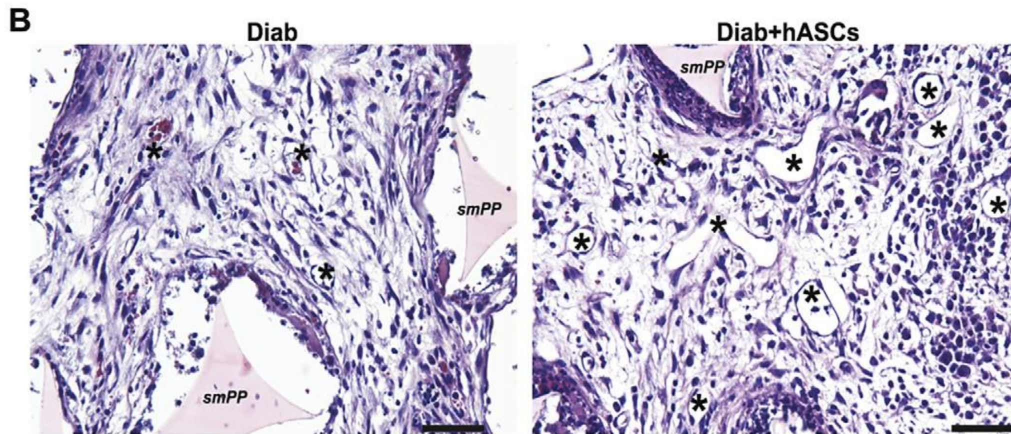
The resolution of the inflammatory process observed in implants bearing hASCs in diabetic animals was ameliorated, as evidenced by decreased levels of inflammatory cytokine TNF-α and high MPO activity, when compared to implants without hASCs. This results corroborate the findings of Stucky et al. who observed that encapsulated mesenchymal stem cells in alginate microspheres attenuated local inflammation, decreasing TNF- α, among other cytokines [47]. Indeed, studies have shown immunosuppressive properties of these cells, by inhibition of the differentiation/proliferation of immune cells or by the capacity to alter their cytokine secretion [10,48].

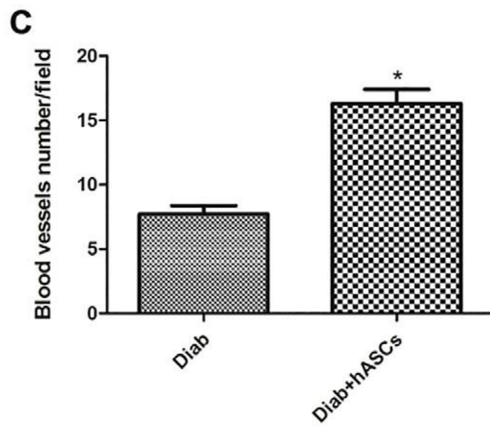
In hASC-bearing implants, there were increased number of insulin producing-cells compared to implants without stem cells. Many studies have associated mesenchymal stem cells with pancreas regeneration, mainly islet cells, by direct differentiation or indirectly by influencing the microenvironment where they are present [38,49,50]. We propose

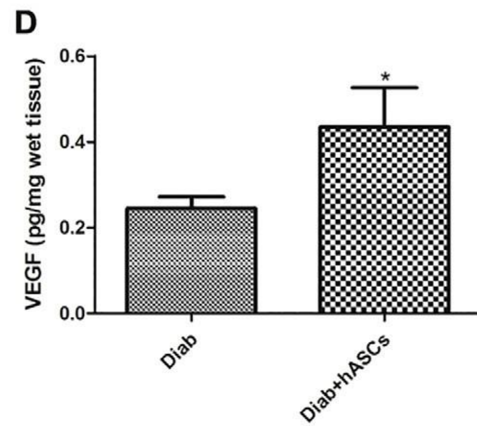
Fig. 3. hASCs improved angiogenic and inflammatory parameters in the biological platform of diabetic mice. A) Diagram representing the time line of diabetes induction, sponge implantation, hASCs injection intra-implant. Single intraperitoneal injection of STZ (150-mg/kg) was given to a group of animals 15 days before implantation. At this time point, smPPs were implanted on the pancreas surface of streptozotocin-induced diabetic mice. At day 4 after implantation, the diabetic mice were divided in two groups: one received PBS and the other received hASCs injection intra-implant. The animals' fasting glucose levels were evaluated weekly. At day 15 after implantation, the animals were anesthetized and killed to collect the implant and pancreas for morphometric and biochemical analyses. hASCs (human adipose stem cells), smPP (synthetic polyether/polyurethane matrix). B) Representative H&E staining histological sections of fibrovascular tissue intra-implant showing blood vessels in each group; eosinophilic triangular shapes correspond to porous of the smPP (synthetic matrix of polyether-polyurethane), Diab (diabetic); Diab + hASCs (diabetic inoculated with hASCs). (*) blood vessels. Scale bars represent 50 μm. C) Blood vessels number/field. D) VEGF levels. E) TNF- α levels. F) MPO activity – indirect measure of neutrophils. Data are expressed as mean ± SEM. (*) represent a significant difference between groups (p b 0.05). MPO (myeloperoxidase).

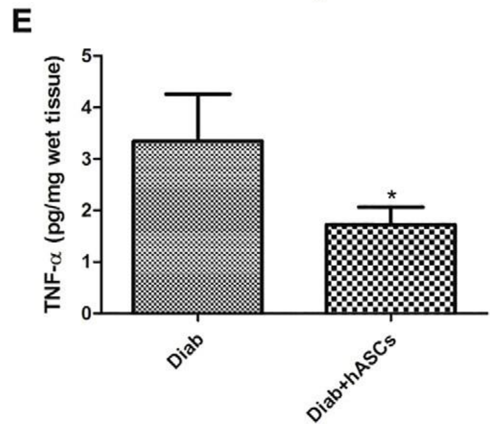
that the results observed here are related to the paracrine effects of hASCs in terms of inducing inflammation resolution (reducing inflammatory cytokines) and by increasing angiogenesis. Mesenchymal stem cells are known to be qualified in promoting regeneration of diverse tissues by secreting trophic factors such as growth factors, pro-angiogenic cytokines, anti-apoptotic cytokines, and others [51]. Moreover, studies

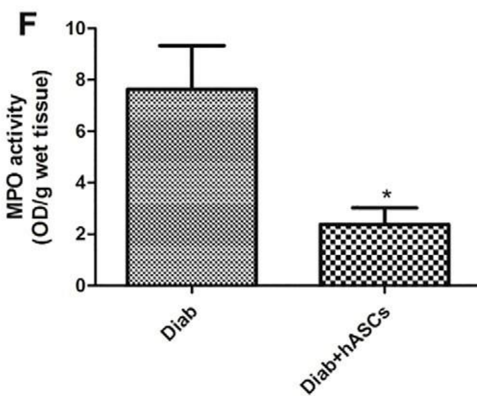












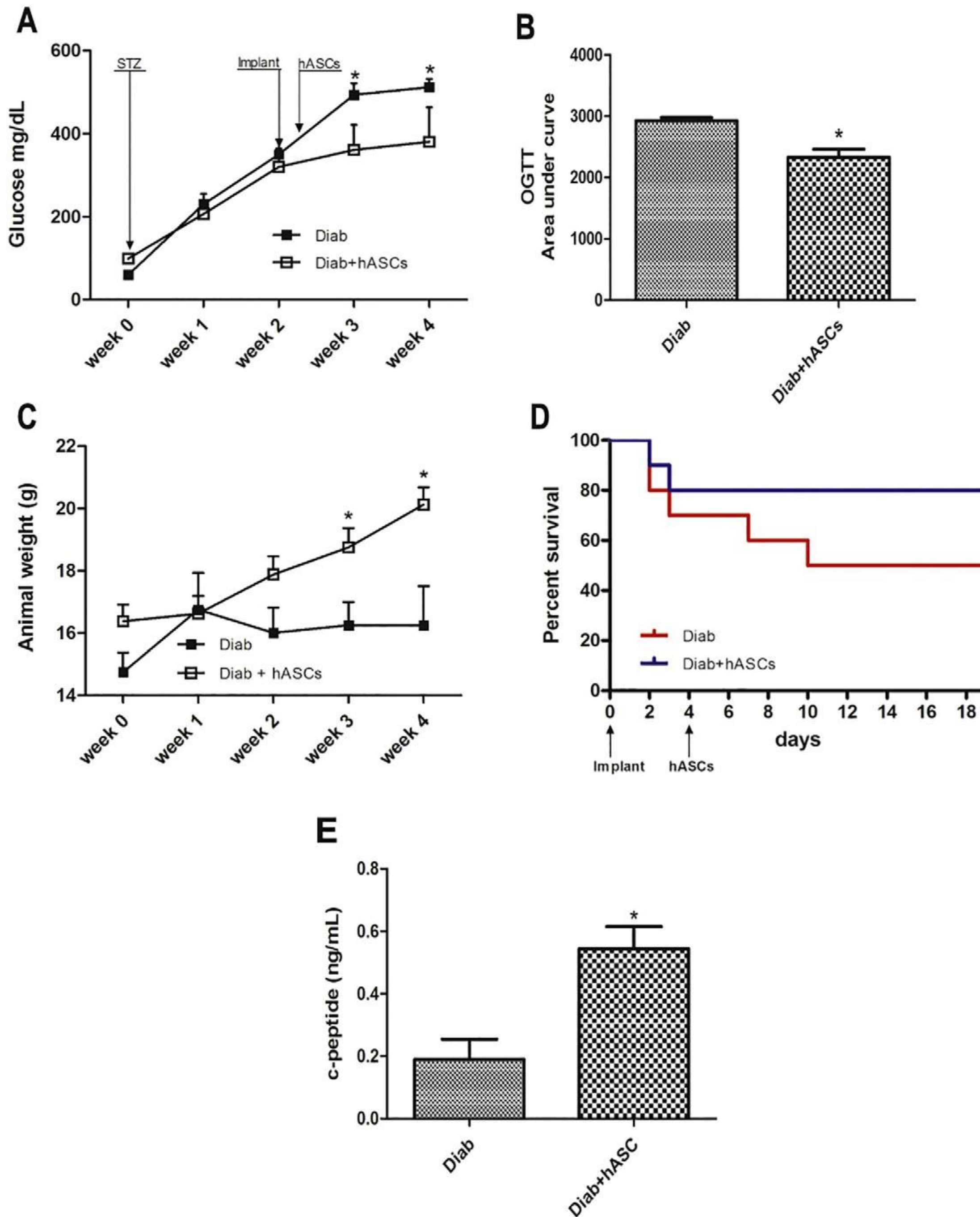


Fig. 4. Improvement of metabolic parameters and survival of diabetic mice. A) Fast blood glucose level measured weekly. B) Oral glucose tolerance test (OGTT), result was measured as the area under the curve (AUC) for 90 min. C) Body weight of diabetic animals evaluated weekly. D) Percent survival of animals in each group during experimental period. E) Serum c-peptide levels (ng/mL). Data are expressed as mean ± SEM. (*) represent significant difference between groups (p b 0.05). Diab (diabetic); Diab + hASCs (diabetic inoculated with hASCs).

have demonstrated the paracrine action of mesenchymal stem cells more than their self-differentiation [50,52]. In the present study, the animals that received stem cells did not presented any tumor formations, or cellular alterations (data not shown).

The absence of long-term engraftment detected in the implants of synthetic matrix of polyether–polyurethane could be possibly due to an immunological reaction to the human cells. Many in vitro studies indicated that mesenchymal stem cells are non-immunogenic, however, in vivo models suggest that these cells could be rejected, by alteration of MHC expression after cell differentiation and consequently action of T lymphocytes [53]. Furthermore, it is possible that even cells with low MHC undergo action of natural killing cells (NK cells) [53]. In fact, recent study showed that NK cells act rejecting MSCs in the liver [54]. Nevertheless, our analysis showed that implants that received hASCs had higher number of insulin producing-cells; and the fact that these cells originate from the animal itself, reinforce our hypothesis that hASCs indirectly improve cell proliferation, possibly by paracrine factors, right after transplantation.

Despite the advances in research, tissue engineering has still been looking for biomaterials that provide an optimal environment for cell-matrix interaction [18,20]. Here, we demonstrated that the synthetic porous scaffold (polyether-polyurethane) bearing hASC cells placed adjacent to the native animal pancreas exhibits the potential to be exploited in cell-based type 1 diabetes therapy.

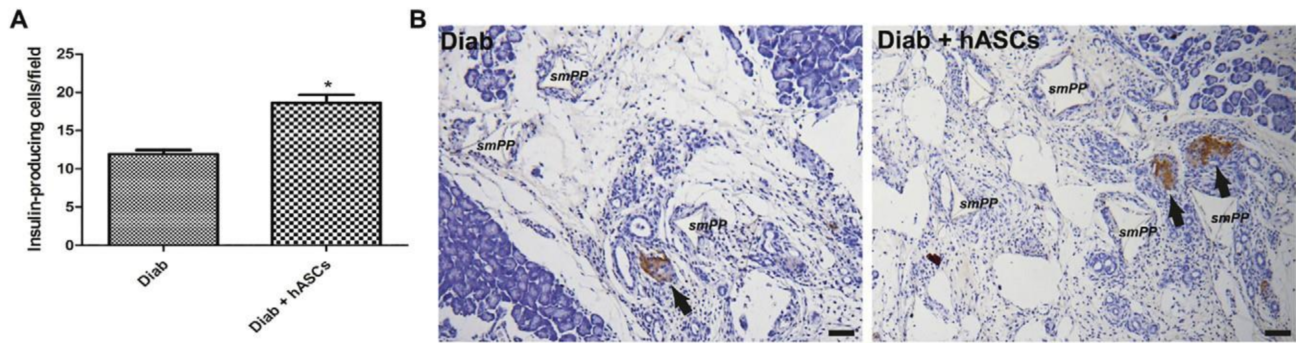


Fig. 5. Insulin-producing cell. A) Insulin-producing cell number/field counted intra-implant. B) Representative micrographs of immunohistochemistry staining for insulin. Black arrow indicates islets. Data are expressed as mean ± SEM. (*) represent significant difference between groups (p b 0.05). Diab (diabetic); Diab + hASCs (diabetic inoculated with hASCs); smPP (synthetic matrix of polyether-polyurethane). Scale bar represent 100 μm.

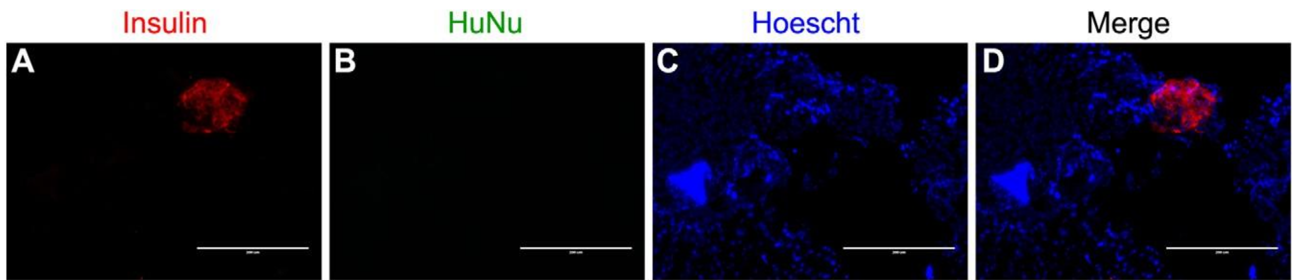


Fig. 6. Co-staining to insulin and hASCs intra-implant. Representative images of A) Insulin producing-cells positive staining in red; B) negative staining in green to HuNu, indicating absence of hASCs; C) nuclear staining in blue; D) Representative images of co-staining of insulin and HuNu intra-implant (merge).

5. Conclusion

This study provides evidence of the ability of a biohybrid device to induce the growth of differentiated pancreas parenchyma in both normoglycemic and streptozotocin-induced diabetic mice as detected by histological analysis. Glucose metabolism and body weight of hyperglycemic mice bearing hASCs implants improved. Recognizing the need for a scaffold design in diabetes research, intraperitoneal implantation of polyether-polyurethane sponge discs in mice has been shown to induce proliferation of fibrovascular tissue that is capable of functioning as a scaffold and to permit the induction of growth of functional pancreatic parenchyma.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2017.03.015.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary Material

Material and Methods

SM1. Porosity analysis of the synthetic matrix of polyether-polyurethane by micro-CT scanner: The samples were attached to a stage that rotated 180° with images acquired every 0.7°. The acquired shadow projections (16-bit TIFF format) were further reconstructed into 2D slices using the NRecon software interface (v.1.6.9.18, Skyscan, Bruker micro-CT, Belgium). The three dimensional models were assembled using CTAn software (v.1.15.4.0, Bruker micro-CT, Belgium), and CTVol software (v.2.3.1.0, Skyscan, Bruker micro-CT, Belgium) was used for 3D volumetric visualization.

SM2. Implant model: All animals were anesthetized with a mixture of ketamine and xylazine (60 mg/kg and 10 mg/kg, respectively). The abdominal hair was shaved and the skin wiped with 70% ethanol. An incision (< 1 cm long) was made in the upper left quadrant of the animals' abdomen. The implant discs were placed directly on the pancreas to create an interface between the synthetic matrix and the biological tissue. The incisions were closed with silk braided absorbable suture. The animals were kept isolated (one animal/cage) and monitored post-surgery for any signs of infection or discomfort.

SM3. Histological and immunohistochemical (IHC) analysis. The sections were deparaffinized, hydrated, and submitted to subsequent blocking steps: endogenous peroxidase block with 30% H2O2: methanol (1:1) for 15 minutes and 5% BSA for 1 hour at ambient temperature. The slides were then incubated overnight at room temperature with the primary antibody: guinea pig antibody anti-insulin (1:500 - Dako); mouse monoclonal antibody anti CDC-47 (1:300 - Neomarkers), followed by incubation with Dako kit LINK and HRP–streptavidin (1 hour each). DAB chromagen was used to visualize the peroxidase activity. Sections were then counterstained with hematoxylin. The negative control was performed by omitting the primary antibody and carried out simultaneously. To immunofluorescence analysis, sections were deparaffinized and followed to block processing with 3% BSA for 30 minutes at room temperature. Staining for insulin producing cell was performed with insulin anti - guinea pig polyconal antibody (1:100 Dako), followed by incubation with Alexia 555 anti-guinea pig (abcam) and counterstaining with Hoescht. The negative control was performed by omitting the primary antibody and carried out simultaneously. The specimens were observed under an immunofluorescence microscope.

Others implant samples were harvested at 2 weeks after received the cell transplant, fixed with paraformaldehyde 4% overnight, washed in PBS 1x five times (30 minutes each), inserted in sucrose solution 20% and 40% (2h in each) and embedded in an optimal cutting temperature compound (O.C.T compound TISSUE-TEK® - USA) and frozen. The frozen sample was sliced in section of 5μm at -25°C and slides of beginning, middle and end part of sample were analyzed.

The sections were staining for immunofluorescence to HuNu (primary antibody anti-human nuclei 1:20 and secondary antibody anti-mouse IgG Alexa 488). Other sections were co-staining for immunofluorescence to insulin (primary antibody anti-insulin 1:100 and secondary antibody Alexa Fluor 555 anti-mouse) and HuNu (primary antibody anti-human nuclei 1:20 and secondary antibody anti-mouse IgG Alexa 488). The nuclei was staining with Hoescht and so the sections were analyzed in a fluorescent microscope (Evos FL).

SM4. **Measurement of cytokines levels:** The implants were homogenized in PBS pH 7.4 containing 0.05% Tween and centrifuged at 10,000 ×g for 30 min. Following that procedure, 100 µl of the supernatant were used to measure the cytokines. Dilutions of cell-free supernatants were added to ELISA plates coated with a specific murine monoclonal antibody against the cytokine (VEGF and TNF-α), followed by the addition of a second horseradish peroxidase-conjugated polyclonal antibody, also against the cytokine. After washing to remove any unbound antibody-enzyme reagent, a substrate solution (50 µL of a 1:1 solution of hydrogen peroxide and tetramethylbenzidine 10 mg/mL in DMSO) was added to the wells. Color development was stopped after 20 min incubation with 2 M sulfuric acid (50 µL) and color intensity was measured at 540 nm on a spectrophotometer (Thermoplate). Standards were 0.5-log10 dilutions of recombinant murine cytokines from 7.5 pg/mL to 1000 pg/mL (100 µL). The results were expressed as a picogram of cytokine/mg of wet tissue.

SM5. Determination of myeloperoxidase (MPO): The tissues were homogenized in sodium phosphate buffer ($NaPO_4$ 0,05M pH 5,4), and centrifuged at 12000g for 10 min. MPO activity was assayed by measuring the change in absorbance (optical density; OD) at 450 nm using tetramethylbenzidine (1.6 mM) and H_2O_2 (0.3 mM). The reaction was terminated by adding 50 µl of H_2SO_4 (4M). Results were expressed as a change in OD per g of wet tissue.

Supplementary Figures Legends

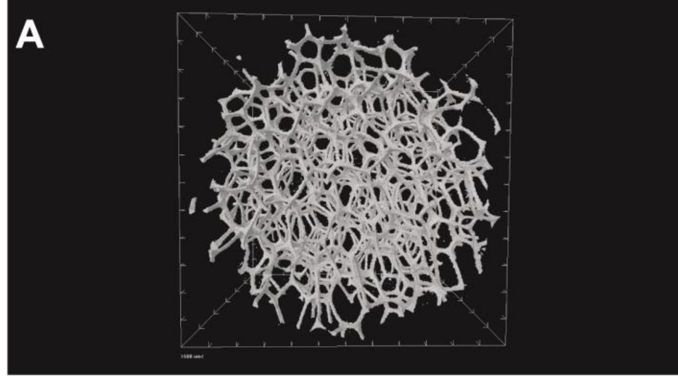
SF1. Characterization of the synthetic matrix of polyether/polyurethane (smPP) by computed microtomography (mCT). A) mCT image showed an overhead 3D view of smPP. B) mCT image showed a side 3D view of smPP. C) Frequency distribution plots of total pore counts based on pore size, analyzed across all of the biomaterial structure.

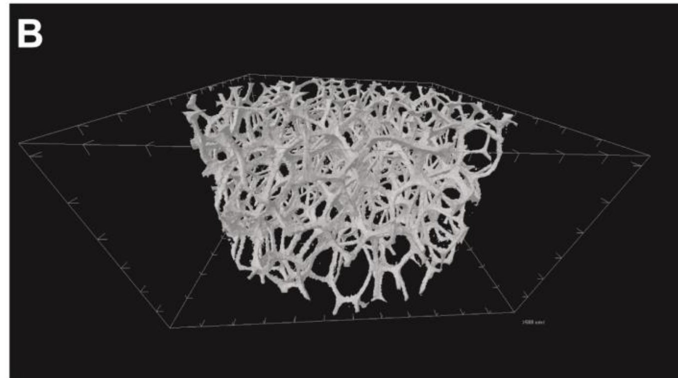
SF2. Implantation of polyether polyurethane sponges adjacent to the pancreas in C57BL/6 mice. The animals were anesthetized and submitted to sequence of procedures for implantation. A) incision, B) pancreas localization, C) suture of the smPP directly to pancreas, D and E) introducing the smPP into abdominal space, F) incision closure. smPP (synthetic matrix of polyether polyurethane).

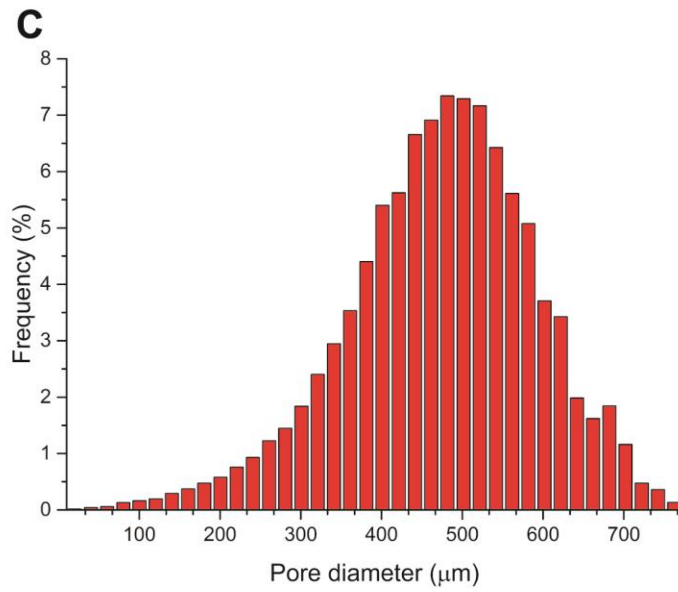
SF3: Cell proliferation marker. Acinar, ductal and islets cells were positive to cell proliferation marker CDC47 by immunohistochemistry staining. A) Overview into implant. Arrowhead indicates positive cells to CDC47. Scale bars represent 100 µm. B) Detailed view of islet cells (arrows) and acinar cells (arrowheads) positive to CDC47. C) Detailed view of ducts cells positive to CDC47 (arrowheads). smPP (synthetic matrix of polyether-polyurethane). Scale bars represent 50 µm.

SF4. Comparing the islets in the pancreas and the islets into the synthetic matrix (A-C) synthetic matrix; (D-F) pancreas. Representative micrographs of immunohistochemistry for insulin. (A,B,D,E) black arrow indicates islets and (C and F) immunofluorescence staining for insulin (red) with DAPI counterstaining. smPP (synthetic matrix of polyether-polyurethane). (A,C,D,E) Scale bar represent 200 µm. (B and E) Scale bar represent 50 µm.

SF1







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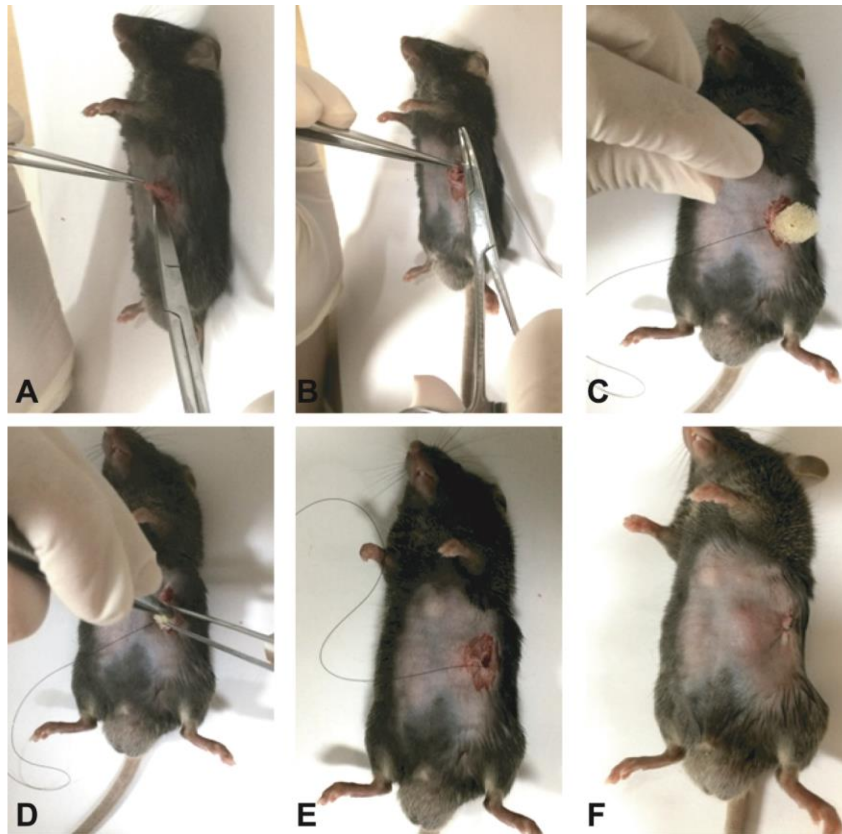

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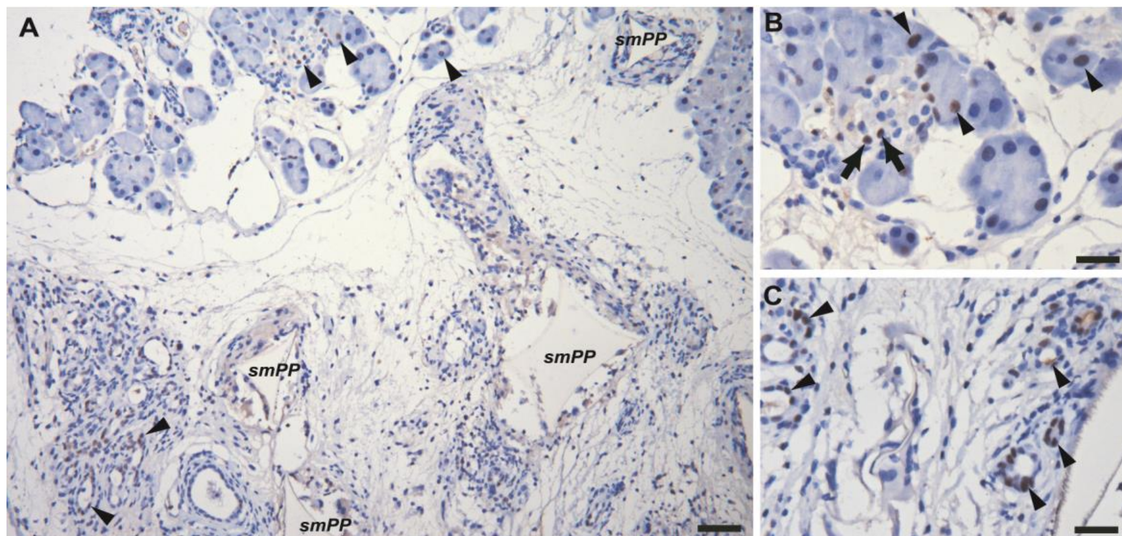

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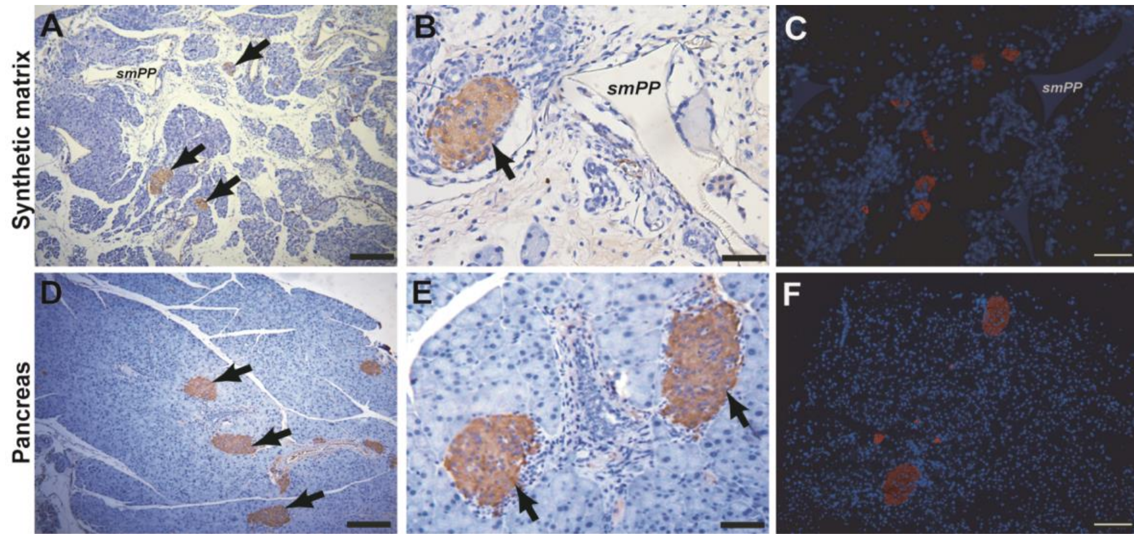

10. Considerações Finais

O pâncreas é um órgão glandular de extrema importância para fisiologia do organismo por secretar enzimas digestivas e hormônios. Lesões em seus compartimentos exócrino e endócrino desencadeiam complicações clínicas que podem afetar a saúde do indivíduo de forma permanente.

Estudos tem mostrado que o pâncreas é um órgão que apresenta certa plasticidade, ou seja, suas células diferenciadas são capazes de perderem sua característica funcional de estado maduro e adotar características de outros tipos celulares. Essa capacidade de desdiferenciação está relacionada ao controle de expressão de determinados genes, que definem o fenótipo final de cada célula.

Modelos animais de lesão ao parênquima do pâncreas tem auxiliado no entendimento de como as células pancreáticas reagem nos contextos de estímulos de stress/lesão, como nas pancreatites e diabetes. Estes estudos são importantes para se conhecer os mecanismos que as células são capazes de desenvolver durante um estímulo de regeneração do órgão.

Neste presente trabalho nós propusemos o uso de uma matriz sintética de poliéter-poliuretano como uma plataforma biológica para proliferação pancreática. Nós mostramos que apenas o estímulo do implante, sem adição de fatores externos, é capaz de desencadear uma resposta proliferativa de células exócrinas e endócrinas do pâncreas. As células foram capazes de proliferar, migrar e formar uma estrutura parenquimatosa como do pâncreas nativo no interior da plataforma. De forma interessante, este novo parênquima

em organização não afetou o metabolismo glicêmico dos animais, sugerindo que o organismo foi capaz de reconhecer e apresentar um controle fisiológico.

Acreditamos que os estímulos inflamatórios e angiogênicos do processo do implante da matriz sintética sejam capazes de estimular as células pancreáticas a uma resposta proliferativa. Além disso, o implante da matriz primeiramente gera todo um ambiente propício de suporte de tecido, por induzir o desenvolvimento de um tecido fibrovascular, com matriz extracelular e uma rede vascular, capaz de sustentar e nutrir as células do pâncreas que estão proliferando e migrando para a plataforma.

Conseguimos neste trabalho associar células tronco mesenquimais ao implante e verificamos que de fato tais células são capazes de atuarem de forma parácrina melhorando os sintomas do diabetes experimental e foi capaz de melhorar a reposta do implante em animais diabéticos quanto ao desenvolvimento de um parênquima intra-implante.

Diante dos resultados obtidos, está claro a capacidade do implante como um modelo de proliferação pancreática, abrindo novas perspectivas de estudos, com o objetivo de entender os mecanismos que ocorrem nesse novo modelo de proliferação proposto.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO
Certificamos que o Protocolo nº. 43 / 2015, relativo ao projeto intitulado "Avaliação da resposta tissular pós-transplante de células tronco em biomaterial implantado junto ao pâncreas de animais diabéticos", que tem como responsável Paula Peixoto Campos Lopes, está de acordo com os Princípios Éticos da Experimentação Animal, adotados pela Comissão de Ética no Uso de Animais (CEUA/UFMG), tendo sido aprovado na reunião de 22/05/2015. Este certificado espira-se em 22/05/2020.

CERTIFICATE
We hereby certify that the Protocol nº. 43 / 2015, related to the Project entilted "Evaluation of tissue response after stem cell transplantation in biomaterial implanted in the pancreas of diabetic animals", under the supervision of Paula Peixoto Campos Lopes, is in agreement with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation (CEUA/UFMG), and was approved in 22/05/2015. This certificate expires in 22/05/2020.

Gleide Fernandes de Avelar
Coordenador(a) da CEUA/UFMG
Belo Horizonte, 22/05/2015.

Atenciosamente.

Sistema CEUA-UFMG
https://www.ufmg.br/bioetica/cetea/ceua/

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