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Pancreatic islet transplantation into the bone marrow

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ABSTRACT

Bone marrow is currently being considered as an alternative site for pancreatic islet transplantation. The goal of the present review is to report preclinical and clinical studies taking advantage of this new implantation site. Preclinical studies in mice demonstrated that syngeneic islets could survive in bone marrow indefinitely with a higher success in providing euglycemia compared to islets transplanted into the traditionally used implantation site, namely, the liver. In concordant and discordant xenogeneic models, the immune response was more stringent when islets were transplanted to the bone marrow as compared to the traditional implantation site in rodents, the kidney capsule. As demonstrated by histology, cellular and humoral rejection was prevented by islet protected by micro-encapsulation in calcium-alginate beads, and a similar degree of fibrotic reaction was induced at both site, although functional studies in diabetic animals are still needed. In clinical settings, a pilot study of four patients who received islet auto-transplantation into the bone marrow after a total pancreatectomy had been showed to be safe and feasible. Three out of four patients had a functioning graft, as measured by serum C-peptide, at an average follow-up of 545 ± 369 days.

In conclusion, islet transplantation into the bone marrow may be a viable alternative to the

liver as an implantation site, particularly with the perspective of transplanting encapsulated xenogeneic islets. Meanwhile, the efficacy of immunosuppressive drugs would still require to be evaluated in allogeneic and xenogeneic preclinical models of islet cell transplantation into the bone marrow.

INTRODUCTION

Beta cell replacement therapy, namely islets or solid organ pancreas transplantation, is currently the sole cure for type I diabetes mellitus. Since 2000 the Edmonton protocol provided clear evidence that islet transplantation could consistently restore euglycemia¹. The rate of insulin independence has been constantly increasing over the years; from 27% at 3-year in 1996 to 44% in the 2000's and currently up to 50%, approaching the results obtained in solid organ pancreas transplantation². The number of islet transplantations have been expanding worldwide; however, this is still being undermined by the shortage of available donor organs^{3,4}. Transplantation of encapsulated xenogeneic islets represents a potentially viable option that has been studied for decades. Meanwhile, the site of transplantation has long been debated because encapsulated islets require more space and likely to be more oxygen-dependent. Although the peritoneal cavity can accommodate such large volume of encapsulated islets, it suffers from low oxygen pressure due to its limited vascularization^{5,6}. Conversely, owing to its rich vascularization and easy accessibility, the bone marrow has thus been proposed as an alternative site for autologous and allogeneic islet transplantation^{7,8}.

PRECLINICAL STUDIES

The first group to investigate and report transplantation of pancreatic islets into the bone marrow were Salazar-Bañuelos et al from Mexico^{9,10}. The authors reported that syngeneic and allogeneic rat islets survived in rat bone marrow up to 21 days post-transplantation. Xenografts from Tilapia were however acutely rejected. Cantarelli et al¹¹ further demonstrated that syngeneic islets could survive in the bone marrow for over one year, and presumably, indefinitely. In a murine model, marginal mass islet transplantation into the bone marrow (125-250 IEQ) had a higher probability in reaching euglycemia compared to islets transplanted into the liver. Fasting glycemia, insulin and glucagon serum levels, beta-cell function, insulin resistance, oral and intravenous glucose tolerance tests were normal up to 9 months post-transplantation. Importantly the transplanted islets in the bone marrow did not compromise the hematopoietic activity. The same group also reported that allogeneic islet transplanted in the bone marrow of mice were rejected simultaneously as islets transplanted into the liver¹² and that syngeneic islet engraftment was delayed when transplanted in high number into the bone marrow¹³. Recent results by the same group indicate that anti-CD3 are more efficient to prevent rejection in the liver compared to the bone marrow in the allogeneic setting¹⁴. A potential shortcoming of these studies is that in murine models the liver might not be an ideal representation of the clinical situation, since the islets cause thrombus, hepatic ischemic injuries, and β -cell loss¹⁵; this is due to the fact that the size of islets is conserved between species, making rodent islets too big for liver vasculature. A Chinese group transplanted allogeneic islet in the bone marrow of five diabetic (streptozotocin-induced) and immunosuppressed rhesus monkeys (ATG, cortisone, tacrolimus and sirolimus)¹⁶. The authors reported that such procedure was technically easier compared to the liver and allowed a correction of diabetes in all five animals. The authors reported a median of 102 days of normoglycemia compared to 39 and 58 days in a control group of two animals with the islet transplanted into the liver. Our group had similar success in glucose normalization and function transplanting syngeneic islet into the bone marrow of diabetic mice¹⁷. We further sought to analyze the survival of xenogeneic rat and human islets in this setting. Such rat and human islets transplanted without im-

munosuppression into femurs of mice were able to reverse diabetes, however, rejection seemed to be accelerated (7 to 9 days) as compared to the usual transplantation site, namely, under the kidney capsule (10 to 14 days). Xenograft rejection was associated with infiltration of macrophages, CD4⁺ and CD8⁺ T cells. Taking advantage of our previously developed islet encapsulation protocols¹⁸⁻²¹, we showed that the encapsulation of rat islet in calcium-alginate beads protected the islet from immune response up to one-month post-transplantation. We observed a similar degree of fibrotic reaction around the capsules into the bone marrow compared to the kidney capsule. We then tested the feasibility of transplanting encapsulated islets in a rat model (Figure 1, unpublished data). To that aim, we transplanted encapsulated in calcium-alginate beads human islet (isolated using the Ricordi protocol with local adaptations²²⁻²⁶) and transplanted into the bone marrow or kidney capsule of normoglycemic adult Sprague-Dawley rat as previously described^{17,27,28}. Briefly, transplantation into the bone marrow was performed via a medial incision at the anterior surface of the knee joint. The femur was reamed using 29, 22 and 18-gauge needles successively. Encapsulated islet were then injected using an 18-gauge needle. Skin closure was performed using non-absorbable 5.0 sutures. Histological examination showed no major difference in fibrosis development in the bone marrow as compared to the kidney capsule 30 days post-transplantation (Figure 1). Of note, the encapsulation and transplantation process did not damage the islet. The “shrunked” aspect of the capsule is likely to be due to the dehydration and decalcification processes used in the procedure for histological sections. The physiological discrepancy between the size of rat femurs and the volume of capsules did not allow us to transplant sufficient numbers of capsules to study graft function. Scaling-up these experiments to a large animal model would allow sufficient numbers of encapsulated islet cells to be transplanted and, thus, pave the way toward clinical application.

CLINICAL TRIALS

A first pilot study of four patients performed by Piemonti et al⁷ evaluated the safety and feasibility of islet autotransplantation into the bone marrow after total pancreatectomy. Three of the four patients had

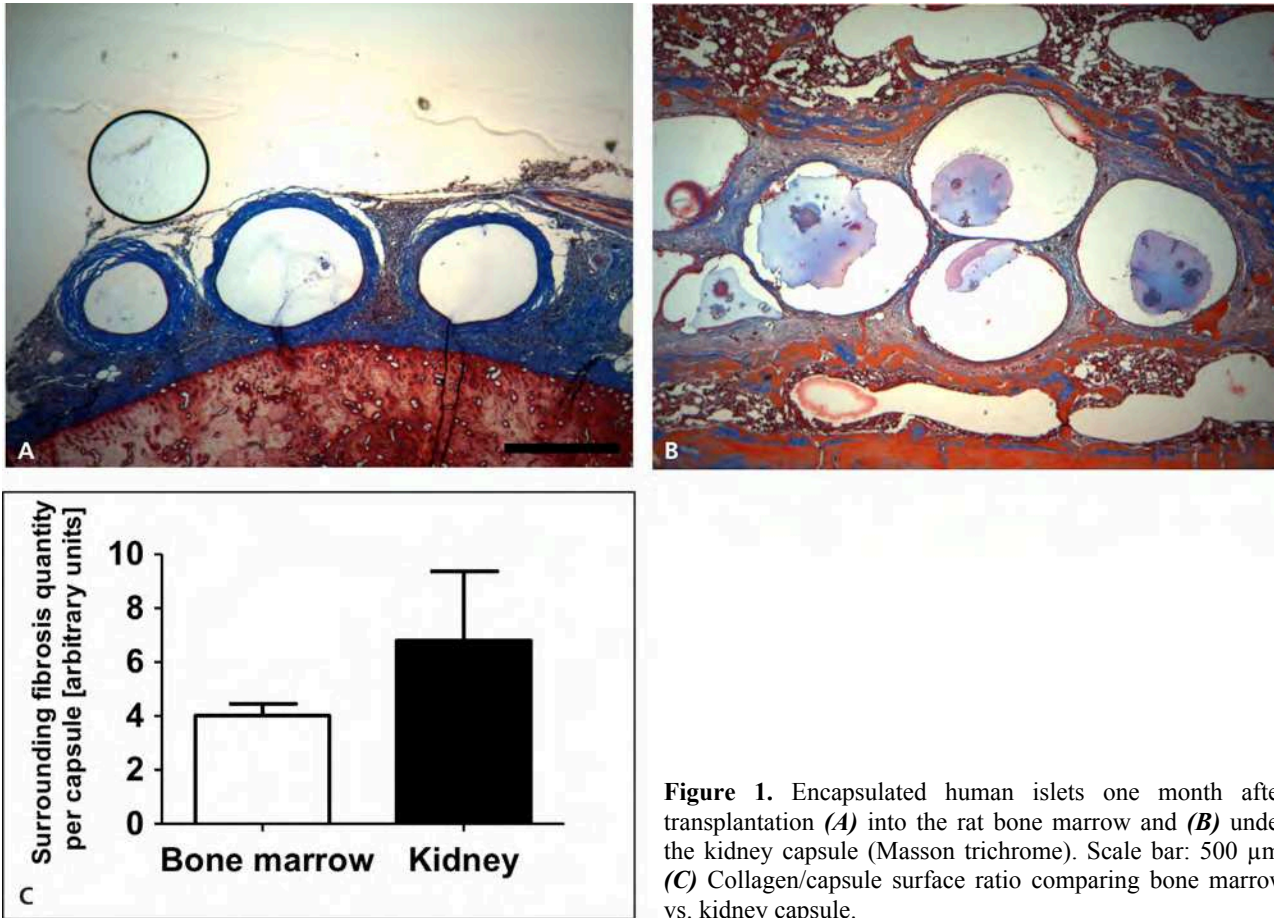


Figure 1. Encapsulated human islets one month after transplantation (**A**) into the rat bone marrow and (**B**) under the kidney capsule (Masson trichrome). Scale bar: 500 μ m. (**C**) Collagen/capsule surface ratio comparing bone marrow vs. kidney capsule.

a high risk of anastomotic leakage in case of the Whipple procedure and were scheduled for a total pancreatectomy, and one patient suffered from hemorrhage after Whipple procedure which required totalization at day 34. The indications for surgery were chronic pancreatitis, ductal adenocarcinoma ($n=2$) and neuroendocrine carcinoma. Three out of the four patients had a functioning graft as measured by C-peptide at an average follow-up of 545 ± 369 days. Islet function remained present up to 944 days post-transplantation. One patient died of bleeding on day 4 after islet autotransplantation due to the rupture of the gastroduodenal artery. Although the risk of tumor cell embolization seems to be low, particularly with purified islet preparations, the risk-benefit balance should be carefully weighted in each patient. The said research group is currently undertaking a prospective randomized safety-efficacy study comparing bone marrow to the liver as an implantation site for allogeneic islet grafts in patients for type 1 diabetes (NCT01722682).

DISCUSSION

Bone marrow is a highly vascularized and easily accessible site, offering space and mechanical protection, thus allowing the perspective of repeated islet infusions and transplantations¹⁷. Preclinical studies have shown the feasibility of the method and demonstrated results comparable to the standard implantation site, namely, the liver, in the syngeneic setting. However, the bone marrow site is associated with potent rejection in the allo- or xenogeneic settings. This issue can potentially be addressed by immunoisolation of the islets, for example by micro-encapsulation. The density of the vascular network in the bone addresses the critical issue of oxygen supply in the setting of encapsulated islet transplantation¹¹. The liver is still currently the preferred site for islet transplantation albeit several disadvantages are noted; these include the immediate blood-mediated inflammatory reaction (IBMIR) that destroys up to 75% of the islets immediately after infusion²⁹; the risk of bleeding following portal vein catheterization by a percuta-

neous approach³⁰; and the risk of increased portal pressure following the procedure due to the 500 μm diameter of the capsules³¹. The perspective of transplanting encapsulated porcine xenogeneic islets into patients certainly warrants the exploration of alternative transplantation sites³². We demonstrated the feasibility of transplanting xenogeneic islets into the bone marrow of rodent. Successful placement of encapsulated islet into rodent femurs is however technically demanding. Such procedure caused moderate fibrosis around the capsules at one month however a trend toward less fibrosis was observed in the bone marrow compared to the kidney capsule. Further studies are needed in large animal models to determine the pros and cons of the bone marrow as a potential site for encapsulated xenogeneic islets.

In the clinical setting, promising results have been reported; the bone marrow was an acceptable alternative site for autotransplantation in a pilot study, meanwhile both the bone marrow and liver are currently being compared in a prospective randomized trial of allogeneic islet transplantation. The perspective of setting up a clinical trial using encapsulated xenoislets is thus required to consider the bone marrow as a potential transplantation site.

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CONFLICT OF INTERESTS:

The Authors have no financial disclosure.

REFERENCES

- Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343(4): 230-238.
- Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, Oberholzer J, Odorico JS, Garfinkel MR, Levy M, Pattou F, Berney T, Secchi A, Messinger S, Senior PA, Maffi P, Posselt A, Stock PG, Kaufman DB, Luo X, Kandeel F, Cagliero E, Turgeon NA, Witkowski P, Naji A, O'Connell PJ, Greenbaum C, Kudva YC, Brayman KL, Aull MJ, Larsen C, Kay TW, Fernandez LA, Vantyghem MC, Bellin M, Shapiro AM. Improvement in outcomes of clinical islet transplantation: 1999-2010. *Diabetes Care* 2012; 35(7): 1436-1445.
- Meier R, Montanari E, Morel P, Bühler L. Thérapies cellulaires: l'avenir de la transplantation d'organes? *Swiss medical forum – Forum médical suisse* 2015; 15(52-53): 1203-1205.
- Niclauss N, Meier R, Bedat B, Berishvili E, Berney T. Beta-Cell Replacement: Pancreas and Islet Cell Transplantation. *Endocr Dev* 2016; 31: 146-162.
- Siebers U, Horcher A, Bretzel RG, Klock G, Zimmermann U, Federlin K, Zekorn T. Transplantation of free and microencapsulated islets in rats: evidence for the requirement of an increased islet mass for transplantation into the peritoneal site. *Int J Artif Organs* 1993; 16(2): 96-99.
- Giraldo JA, Weaver JD, Stabler CL. Tissue engineering approaches to enhancing clinical islet transplantation through tissue engineering strategies. *J Diabetes Sci Technol* 2010; 4(5): 1238-1247.
- Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, Mercalli A, Scavini M, Esposito A, Peccatori J, Cantarelli E, Messina C, Bernardi M, Del Maschio A, Staudacher C, Doglioni C, Ciceri F, Secchi A, Piemonti L. Autologous pancreatic islet transplantation in human bone marrow. *Diabetes* 2013; 62(10): 3523-3531.
- Ciceri F, Piemonti L. Bone marrow and pancreatic islets: an old story with new perspectives. *Cell Transplant* 2010; 19(12): 1511-1522.
- Salazar-Banuelos A, Wright JR, Jr., Sigalet D, Benitez-Bribiesca L. Pancreatic islet transplantation into the bone marrow of the rat. *Am J Surg* 2008; 195(5): 674-678; discussion 8.
- Salazar-Banuelos A, Wright J, Sigalet D, Benitez-Bribiesca L. The bone marrow as a potential receptor site for pancreatic islet grafts. *Arch Med Res* 2008; 39(1): 139-141.
- Cantarelli E, Melzi R, Mercalli A, Sordi V, Ferrari G, Lederer CW, Mrak E, Rubinacci A, Ponzoni M, Sitia G, Guidotti LG, Bonifacio E, Piemonti L. Bone marrow as an alternative site for islet transplantation. *Blood* 2009; 114(20): 4566-4574.
- Cantarelli E, Citro A, Dugnani E, Aiolfi R, Dilucia P, Sitia G, et al. The relevance of site on immune responses after islet transplantation: bone marrow (bm) vs liver. Congress abstract, Cell Transplant Society 2013.
- Cantarelli E, Citro A, Pellegrini S, Dugnani E, Mercalli A, Piemonti L. The islet transplant site influences the anti-cd3 efficacy: bone marrow (bm) vs liver. Congress abstract, 5th European pancreas and islet transplant association Winter Symposium 2015.
- Cantarelli E, Citro A, Pellegrini S, Piemonti L. Transplant site influences the immune response against allogeneic islets in the presence of anti-cd3: bone marrow (BM) vs liver. Congress abstract, 6th European pancreas and islet transplant association Winter Symposium 2016.
- Hara M, Yin D, Dizon RF, Shen J, Chong AS, Bindokas VP. A mouse model for studying intrahepatic islet transplantation. *Transplantation* 2004; 78(4): 615-618.
- Wang C, Du X, He S, Chen Y, Liu J, Shi M, et al. The bone marrow cavity is suitable for islet transplantation in diabetic rhesus monkey. Congress abstract, International Pancreas and Islet Transplant Association 2013.

17. Meier RP, Seebach JD, Morel P, Mahou R, Borot S, Giovannoni L, Parnaud G, Montanari E, Bosco D, Wandrey C, Berney T, Bühler LH, Muller YD. Survival of free and encapsulated human and rat islet xenografts transplanted into the mouse bone marrow. *PLoS One* 2014; 9(3): e91268.
18. Lavallard V, Armanet M, Parnaud G, Meyer J, Barbieux C, Montanari E, Meier R, Morel P, Berney T, Bosco D. Cell rearrangement in transplanted human islets. *Faseb J* 2016; 30(2): 748-760.
19. Meier RP, Navarro-Alvarez N, Morel P, Schuurman HJ, Strom S, Buhler LH. Current status of hepatocyte xenotransplantation. *Int J Surg* 2015; 23(Pt B): 273-279.
20. Meier RP, Mahou R, Morel P, Meyer J, Montanari E, Muller YD, Christofilopoulos P, Wandrey C, Gonelle-Gispert C, Bühler LH. Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. *J Hepatol* 2015; 62(3): 634-641.
21. Meier RP, Muller YD, Gutzwiller EM, Spahr L, Negro F, Krause KH, Schaller K, Wandrey C, Sgroi A, Morel P, Bühler LH. [Cell transplantation: current treatments and future prospects]. *Rev Med Suisse* 2014; 10(435): 1350-1355.
22. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* 1988; 37(4): 413-420.
23. Bucher P, Mathe Z, Morel P, Bosco D, Andres A, Kurfuest M, Friedrich O, Raemsch-Guenther N, Buhler LH, Berney T. Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. *Transplantation* 2005; 79(1): 91-97.
24. Niclauss N, Bosco D, Morel P, Demuylder-Mischler S, Brault C, Milliat-Guittard L, Colin C, Parnaud G, Muller YD, Giovannoni L, Meier R, Toso C, Badet L, Benhamou PY, Berney T. Influence of donor age on islet isolation and transplantation outcome. *Transplantation* 2011; 91(3): 360-366.
25. Meier RP, Sert I, Morel P, Muller YD, Borot S, Badet L, Toso C, Bosco D, Berney T. Islet of Langerhans isolation from pediatric and juvenile donor pancreases. *Transpl Int* 2014; 27(9): 949-955.
26. Niclauss N, Sgroi A, Morel P, Baertschiger R, Armanet M, Wojtuszczyz A, Parnaud G, Muller Y, Berney T, Bosco D. Computer-assisted digital image analysis to quantify the mass and purity of isolated human islets before transplantation. *Transplantation* 2008; 86(11): 1603-1609.
27. Mahou R, Kolláriková G, Gonelle-Gispert C, Meier R, Schmitt F, Tran N, et al.: Combined Electrostatic and Covalent Polymer Networks for Cell Microencapsulation Macromolecular Symposia 2013; accepted (ahead of print).
28. Mahou R, Bühler L, Wandrey C. Alginate-Poly(ethylene glycol) Hybrid Microspheres for Primary Cell Microencapsulation *Materials* 2014;7(1):275-86.
29. Nilsson B, Ekdahl KN, Korsgren O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant* 2011; 16(6): 620-626.
30. Bucher P, Mathe Z, Bosco D, Becker C, Kessler L, Greget M, Benhamou PY, Andres A, Oberholzer J, Buhler L, Morel P, Berney T. Morbidity associated with intraportal islet transplantation. *Transplant Proc* 2004; 36(4): 1119-1120.
31. Toso C, Oberholzer J, Ceausoglu I, Ris F, Rochat B, Rehor A, Bucher P, Wandrey C, Schuldt U, Belenger J, Bosco D, Morel P, Hunkeler D. Intra-portal injection of 400-microm microcapsules in a large-animal model. *Transpl Int* 2003; 16(6): 405-410.
32. Merani S, Toso C, Emamaullee J, Shapiro AM. Optimal implantation site for pancreatic islet transplantation. *Br J Surg* 2008; 95(12): 1449-1461.