Co-transplantation of encapsulated human mesenchymal stem cells improves the viability of human islet xenografts in a mouse model of type I diabetes

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Target audience: Medical professionals and researchers who are interested in islet transplantation.

Purpose: The Edmonton protocol of allogeneic islet transplantation needs continuous treatment with immunosuppressant drugs[1]. As a possible solution to this issue, transplantation of microencapsulated islets has been investigated extensively[2] and patient studies have been initiated[3]. In this study, we aimed to improve xenotransplanted human islet cell survival by co-transplantation with encapsulated human mesenchymal stem cells (hMSCs) in an immunologically and physiologically demanding subcutaneous (s.c.) environment, and monitoring the fate of encapsulated islets using ^{19}F MRI and bioluminescent imaging (BLI).

Methods: Perfluoro-15-crown-5-ether (PFPE) was incorporated into the alginate/protamine sulfate/alginate (APA) microcapsules by a double emulsion method in order to obtain 6% v/v PFPE per ml of primary alginate[4]. Twelve to fifteen weeks old female NOD/ShiLtj mice were used as a model of type I diabetes mellitus. Blood glucose levels were monitored on weekly basis and only those mice with at least 3 consecutive readings of above 300 mg/dL were considered diabetic and used in the study. Prior to encapsulation, human islets were transduced to express luciferase; and then encapsulated in the PFPE-modified APA capsules to get one islet per capsule. hMSCs were encapsulated separately in non-modified APA capsules to get 5 hMSCs per capsule. 5000 encapsulated human islets with and without 50,000 encapsulated hMSCs were transplanted s.c. into diabetic NOD/ShiLtj mice (n=3). To assess the *in vivo* viability of transplanted human islets, BLI was performed on day 1, 7 and 14 after s.c. transplantation. MRI was performed with a Bruker 11.7T scanner using a dual-tuned ^{19}F /^{1}H surface coil. The parameters for ^{1}H/^{19}F MRI were: RARE, TR=4/3s, E=58/58ms, SI=1/1mm, matrix=256x160/256x160, NA=2/8, FOV=3.2x2/3.2x2cm. For statistical significance, a Student's t-test with a significance level of $p<0.05$ was used.

Results: As compared to encapsulated human islets alone, there was a significant increase in the BLI signal of transplanted encapsulated human islets after co-transplantation of encapsulated hMSCs (**Fig. 1**). This beneficial effect was most profound on day 1 post-transplantation with over five times higher mean signal intensity ($p<0.05$, student t test). Although over time the differences in islet cell survival diminished, the BLI was consistently and significantly higher in case of islets co-transplanted with hMSCs. On ^{19}F MRI, the transplanted fluorocapsules were clearly visible (**Fig. 2A, B**). The fluorine signal in the hMSC co-tranplantation experiments had a speckled appearance (**Fig. 2B**), indicating that the hMSC capsules (that were not fluorine-labeled) were located in between the fluoroencapsulated human islets.

Discussion: Since there is virtually no background signal of fluorine *in vivo*, ^{19}F MRI of the PFPE tracer allowed us to monitor the implanted fluorocapsules with a very high level of specificity. Overall, islet survival *in vivo* is affected largely by immunorejection and a lack of sufficient vascular perfusion of the implant. While encapsulation can protect islets from immediate immunorejection, a foreign body reaction of invading macrophages can "choke" the capsule wall. By co-transplantation of hMSCs, we can improve the implant survival through a dual mechanism of immunomodulation, proangiogenesis. The close proximity of the two different capsules as observed in our MRI results may be critical to obtain optimal effects of hMSCs through secreted soluble factors. While the beneficial effect of MSC co-transplantation has been shown previously either in syngenic[5] or allograft scenarios[6], notably this study has shown beneficial effects using a subcutaneous transplantation site and human-to-mouse xenogeneic environment.

Conclusion: ^{19}F MRI can be successfully used to image transplanted fluoroencapsulated islets *in vivo*. Co-transplantation of encapsulated hMSCs improves the viability of islets in a stringent xenograft scenario. Studies are ongoing to further improve this approach for long-term implant survival.

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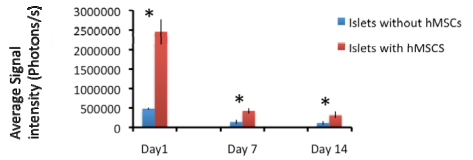

Figure 1: BLI signal from human islets transplanted with or without hMSCs. The presence of hMSCs in close vicinity of transplanted human islets increased viability (*$p<0.05$, n=3).

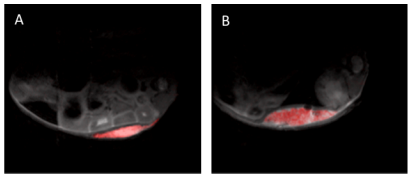

Figure 2: Representative 11.7T T2-w MR images overlaid with ^{19}F images (day 7 post-transplantation). (A) Microencapsulated human islets transplanted subcutaneously without MSCs. (B) Microencapsulated human islets co-transplanted subcutaneously with encapsulated hMSCs.