A New Double Capsule for Human and Porcine Islet Transplantation

Safley S.A, Gordon K, Barber G, Holdcraft R, Turgeon N, Larsen C.P, Gazda L., Weber C.J*
Emory Univ School of Medicine, Department of Surgery, Atlanta, GA USA (ssafley@emory.edu)

INTRODUCTION AND OBJECTIVE

The current status of human islet transplantation as a therapy for Type 1 diabetes mellitus (T1DM) requires new approaches to enhance donor islet viability and long-term function. One such approach is islet encapsulation. The availability of highly purified reagents (such as endotoxin-free alginates) and recently improved protocols for microcapsule generation (such as our new 'double capsule' technique) are promising.

The application of human islet allografts for patients with T1DM is a long-term goal (Bellin, 2012). However, alternative approaches will be required for large scale islet replacement. One approach is the use of xenogeneic porcine islets. We have found that encapsulation of xenogeneic adult porcine islets (APIs) in single barium-gelled alginate capsules protected them from rejection for about 3 weeks (23 ± 22 days) in NOD mice (Cui, 2009). However, if recipients were treated with selective immuno-modulatory agents, these encapsulated xenografts functioned for 300 ± 96 days in NODs (Cui, 2009).

We have found that in immunosuppressed diabetic non-human primates (NHPs), APIs in single barium-gelled alginate capsules functioned for about 3 weeks, with limited host cellular responses. However, host IgG was found within the microcapsules; and *in vitro* studies showed rapid destruction of APIs in the presence of NHP serum and complement.

We have designed a new double microcapsule that excludes IgG. The objective of this study was to determine whether double-encapsulation is more effective than single encapsulation for promoting the function of human islets and APIs in diabetic NOD mice, and whether double encapsulation allows reduction in immunosuppression.

MATERIALS AND METHODS

Human islets were provided by the Emory Transplant Center Human Islet Isolation Laboratory. APIs were isolated the laboratory of Dr. Weber at Emory from adult pig pancreases provided by Drs. Lawrence Gazda and Robert Holdcraft, Rogosin Institute, Xenia Ohio, and prepared as described (Brandhorst, 1999). Human islets and API were encapsulated in single barium-gelled alginate capsules (Cui, 2009) or in our new double capsule and transplanted i.p. in diabetic NOD mice (9,000 IEQ/mouse) given no immunosup-pression or costimulatory blockade (CoB) (CTLA4-Ig plus anti-CD154 mAb). Our double capsule is composed of an inner compartment of Ca^{++}-gelled 3.2% low viscosity, high mannnuronic acid (LVM) alginate coated with one layer of 0.05% poly-L-lysine (PLL), and an outer capsule of Sr^{++}-gelled 2% LVM (Figure 1). Graft function was monitored by measurement of random blood glucose (BG), serum porcine C peptide, *in vivo* glucose tolerance tests (GTT), and histologic analyses of graft biopsies. Host immune responses were characterized by phenotyping peritoneal cellular infiltrates.

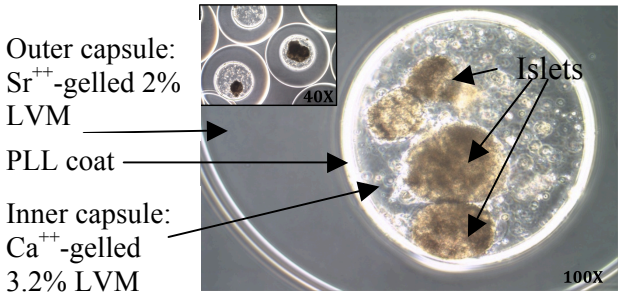

Figure 1. Double Capsule: Inner islet-containing compart-ment of Ca-gelled 3.2% LVM alginate covered with 0.05% PLL; outer Sr-gelled 2% LVM alginate coating, 100X Mag, Inset = double encapsulated islets at 40X magnification.

RESULTS AND DISCUSSION

Double encapsulation augments islet graft function in the absence of immunosuppression. APIs in double capsules functioned significantly longer in diabetic NOD mice than APIs in single Ba-gelled alginate capsules (80 ± 26 days [n=8] *vs* 23 ± 22 days[n=50], p=0.0003, Table 1). There was a trend toward longer function for human islets in double capsules (40, 33, and 12 days), compared to single Ba-gelled alginate capsules (13 ± 4days [n=20], p=NS, Table 1). Both double capsules and Ba-gelled single capsules were intact when recovered from mice at rejection, showing that microcapsule breakage was not a factor in the failure of their API grafts. However, the majority of single Ba-gelled capsules were covered with adherent host cells, and the islets within the capsules appeared to be undergoing destruction (1). The results for double-encapsulated APIs were variable, with 90-100% capsules completely covered with host cells in 2 of 8 untreated mice (days 51 and 98 post-transplant), 50% of capsules completely covered in 2 of 8 mice (days 24 and 129 post-transplant), and 90-100% of capsules clean with minimal, if any, adherent host cells in 4 of 8 mice (days 29, 54, 85, and 107 post-transplant) (Figure 2).

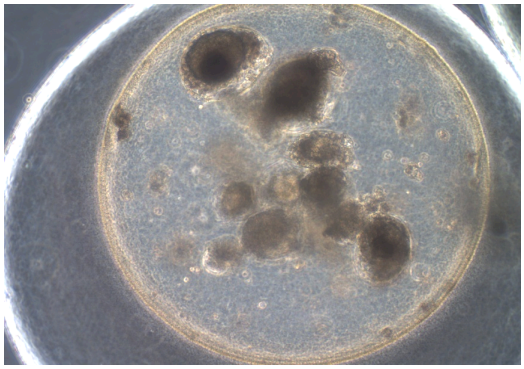

Figure 2. Double-encapsulated APIs functioning in a NOD mouse given no immunosuppression, Day 107 post-transplant.

Double encapsulation synergizes with targeted immuosuppression to promote long-term function of APIs in vivo. APIs in double capsules functioned long-term in streptozotocin (SZN)-diabetic NOD-Scid mice (197 ± 80 days, n=8) (Table 1). Similarly, human islets functioned for 178 and 248 days in two SZN-diabetic NOD-Scid mice (Table 1), demonstrating that islets in double capsules exhibit robust function for well over 150 days in the peritoneal cavity of mice. In diabetic NOD mice given costimulatory blockade (CoB) with CTLA4-Ig and MR1 (500 μg and 250μg, respectively, QOD for 10 days and then weekly), double-encapsulated APIs functioned for 198 ± 52 days (n=10), and human islets functioned for 205 ± 97 days (n=8) (Table 1). These results were comparable to our findings with APIs and human islets in single Ba-gelled capsules in NOD mice given CoB (300 ± 96 [n=39] and 387 ± 96 [n=17], respectively) (Table 1). These results show that in the presence of immunsuppression, both double capsules and single capsules promote long-term function of islet xenografts.

Our new capsule may allow reduced immunosuppression. With double encapsulation, a single agent (non-depleting anti-CD4 mAb, YTS177) allowed APIs to function for 208 ± 41 days (n=6), which is similar to graft survival in mice treated with CoB (198 ± 52 days [n=10], Table 1). In contrast, with the single Ba-gelled alginate capsule, islet xenografts survived a significantly shorter time in NODs given monotherapy compared to dual therapy (167 ± 49 days vs 300 ± 96 days, p=0.0001) (Table 1).

CONCLUSIONS

We conclude that double-encapsulation is more effective than single encapsulation (Ba-gelled alginate) for promoting the function of human islets and APIs in diabetic NOD mice in the absence of imunosuppression. Like single encapsulation, double encapsulation synergizes with immunosuppression to prolong the function of human islets and APIs in diabetic NODs. Also, double encapsulation, but not single encapsulation, promotes long-term islet xenograft function with a single immunomodulatory agent (a non-depleting anti-CD4 monoclonal antibody). Our findings suggest that this technique, combined with targeted host immunosuppression, may be applicable for encapsulated human islet and porcine islet xenografts in patients with Type 1 diabetes in the future.

Table 1. Survival of APIs and Human Islets in Double vs. Single Capsules in Diabetic Mice given Various Immunotherapies

Host	Graft	Rx	Double Capsule		Single Ba-gelled Capsule	
			Average Days Function	n	Average Days Function	n
NOD	APIs	CoB	198 ± 52	10	300 ± 96*	39
NOD	APIs	YTS177.9	208 ± 41	6	167± 49	8
NOD	APIs	None	80 ± 26*	8	23 ± 22	50
Scid	APIs	None	197 ± 80	8	146 ± 85	43
NOD	Human	CoB	205 ± 97	8	387± 96*	17
NOD	Human	YTS177.9	Not done	-	167, 219, 219	3
NOD	Human	None	40, 33, 12	3	13 ± 4	20
Scid	Human	None	178, 248	2	109 ± 48	16

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ACKNOWLEDGMENTS

We gratefully acknowledge Michael Dornish and Jan Egil Melvik (Novamatrix/FMC Biopolymer) for ultrapure alginates, and Malcomb and Musette Powell, as well as the Juvenile Diabetes Foundation for their funding which supported this work in the Elizabeth Brooke Gottlich Diabetes Research and Islet Transplant Laboratory, Emory University.