# Safety and Viability of Microencapsulated Human Islets Transplanted Into Diabetic Humans

Bernard E. Tuch, fracp, phd[1,2]
Gregory W. Keogh, fracs[3]
Lindy J. Williams, bsc[2]
Wei Wu, phd[2]
Jayne L. Foster, phd[2]
Vijayganapathy Vaithilingam, msc[2]
Robert Philips, franzcr[4]

**OBJECTIVE** — Transplantation of insulin-producing cells placed inside microcapsules is being trialled to overcome the need for immunosuppressive therapy.

**RESEARCH DESIGN AND METHODS** — Four type 1 diabetic patients with no detectable C-peptide received an intraperitoneal infusion of islets inside microcapsules of barium alginate (mean 178,200 islet equivalents on each of eight occasions).

**RESULTS** — C-peptide was detected on day 1 post-transplantation, and blood glucose levels and insulin requirements decreased. C-peptide was undetectable by 1–4 weeks. In a multi-islet recipient, C-peptide was detected at 6 weeks after the third infusion and remains detectable at 2.5 years. Neither insulin requirements nor glycemic control was affected. Capsules recovered at 16 months were surrounded by fibrous tissue and contained necrotic islets. No major side effects or infection occurred.

**CONCLUSIONS** — While allografting of encapsulated human islets is safe, efficacy of the cells needs to improve for the therapy to make an impact on the clinical scene.

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Transplantation of insulin-producing cells placed inside capsules is a strategy that is being trialled to overcome the need for immunosuppressive therapy in insulin-dependent diabetic people (1). We have made microcapsules of barium alginate and shown that insulin-producing porcine cells can function as efficiently inside such capsules as when nonencapsulated (2). Moreover, human islets placed in these capsules normalize blood glucose levels when engrafted in diabetic mice (3,4). In the current study, we transplanted four type 1 diabetic humans with encapsulated human islets.

## RESEARCH DESIGN AND METHODS

— Cadaver pancreata were obtained with consent, and islets were isolated by digestion with collagenase NB1 premium grade and neutral protease NB (Serva, Germany). Islets were placed in barium alginate microcapsules (2), and their median average diameter from eight islet preparations was 340 μm (range 255–750).

Median viability of the encapsulated islets, assessed with the fluorescent dyes carboxyfluorescein diacetate and propidium iodide (2), was 73% (range 60–80%). Purity was 68% (range 50–88%), and insulin content (2) was 1.1 mU/islet equivalents (IEQs) (range 0.1–35). The median stimulation index of the islets exposed to 20 mmol/l glucose, compared with 2.8 mmol/l glucose, for 1 h was 1.22 (range 1.0–2.4). The median number of IEQs transplanted on each occasion was 178,200 (range 98,200–227,900). Conditioned culture medium was free of microbial contamination.

Of the 14 diabetic people screened for phase 1 of the clinical trial, 7 were selected and 4 were transplanted over a period of 19 months. The seven with long-standing type 1 diabetes had no endogenous insulin production (no C-peptide in serum during an arginine tolerance test and none in 24-h urine), BMI $<25$ kg/m$^2$, and weight $<70$ kg. Those transplanted had antibodies to neither GAD nor islet cell surface antigens (ICA512). One person received four islet infusions over 7 months with three in the 1st month; a second received two infusions 10 months apart; and the other two recipients received one infusion each (see Tables A1 and A2 in the online appendix available at http://care.diabetesjournals.org/cgi/content/full/dc09-0744/DC1). Infusions were carried out on an outpatient basis in the Department of Medical Imaging, Prince of Wales Hospital, Sydney, Australia.

No immunosuppression was used, but recipients did take both a mild anti-inflammatory agent (Atorvastatin 20 mg) and antioxidants (vitamin A 50,000 units, vitamin B6 100 mg, and vitamin E 750 units) after each transplant. For the last two islet infusions, exenatide 5 μg b.i.d. was administered in an attempt to enhance β-cell survival and function.

Approval for all the procedures performed was obtained from the Institutional Human Research Ethics Committee.

## RESULTS

— C-peptide was detected in urine on the 1st day after the islet infusion (median 0.59 nmol/l [range 0.11–1.79]; 0.15 nmol/mmol creatinine [range 0.06–0.25]) with levels declining thereafter and becoming undetectable at 1–4 weeks (median 10 days) later. Both blood glucose levels and insulin requirements were also lower on the 1st day after transplantation by an average (means ± SEM)

From the [1]Australian Foundation for Diabetes Research, Sydney, Australia; the [2]Diabetes Transplant Unit, Prince of Wales Hospital, Sydney, Australia; the [3]Department of Surgery, Prince of Wales Hospital, Sydney Australia; and the [4]Department of Medical Imaging, Prince of Wales Hospital, Sydney, Australia.
Corresponding author: Bernard Tuch, btuch@alumni.sydney.edu.au.
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of 36 ± 8% and 22 ± 3%, respectively, but not thereafter. An arginine tolerance test was carried out on the 7th day, and plasma C-peptide was undetectable.

In the recipient of the four islet infusions, urinary C-peptide was detected at 6 weeks after the third infusion and continues to remain detectable at 2.5 years. C-peptide levels are 0.06–0.34 nmol/l or 0.02–0.06 nmol/mmol creatinine. The small amount of insulin being produced did not alter insulin requirements or glycemic control.

To better understand what was occurring in the transplanted capsules, a laparoscopy was performed in the recipient of the four islet infusions at 16 months after the first infusion. Large numbers of capsules were found scattered throughout the peritoneal cavity in clusters attached to the parietal peritoneum (Fig. 1*A*), spleen, omentum, and kidney. A biopsy showed the capsules to be intact and surrounded by fibrous tissue containing thin-walled capillaries with a mild histiocytic response. Islets were necrotic (Fig. 1*B*).

Antibodies were detected to GAD but not ICA512 in three recipients. The titer became elevated 4 weeks after the first infusion in two of the recipients and it was 14 weeks after the fourth infusion in the third recipient. In all these recipients, antibodies continue to remain detectable 1.1−2.5 years after the initial infusion.

Cytotoxic antibodies were detected at 4 and 8 weeks after the first infusion in two patients, one who received four infusions and the other a single infusion. The titer, 56 and 32%, respectively, declined with time but was still detectable when last measured at 1.9 and 0.6 years, respectively (titers of 19% for both).

There were no serious adverse events. Nausea did occur in the two recipients of exenatide, and the medication was stopped earlier than anticipated because of this symptom in one person. There was a trend for blood glucose levels to be lower when exenatide was administered, probably because of delayed gastric emptying since urinary C-peptide was sometimes undetectable. Wound infections did not occur, and all recipients were discharged within an hour of the completion of the infusion.

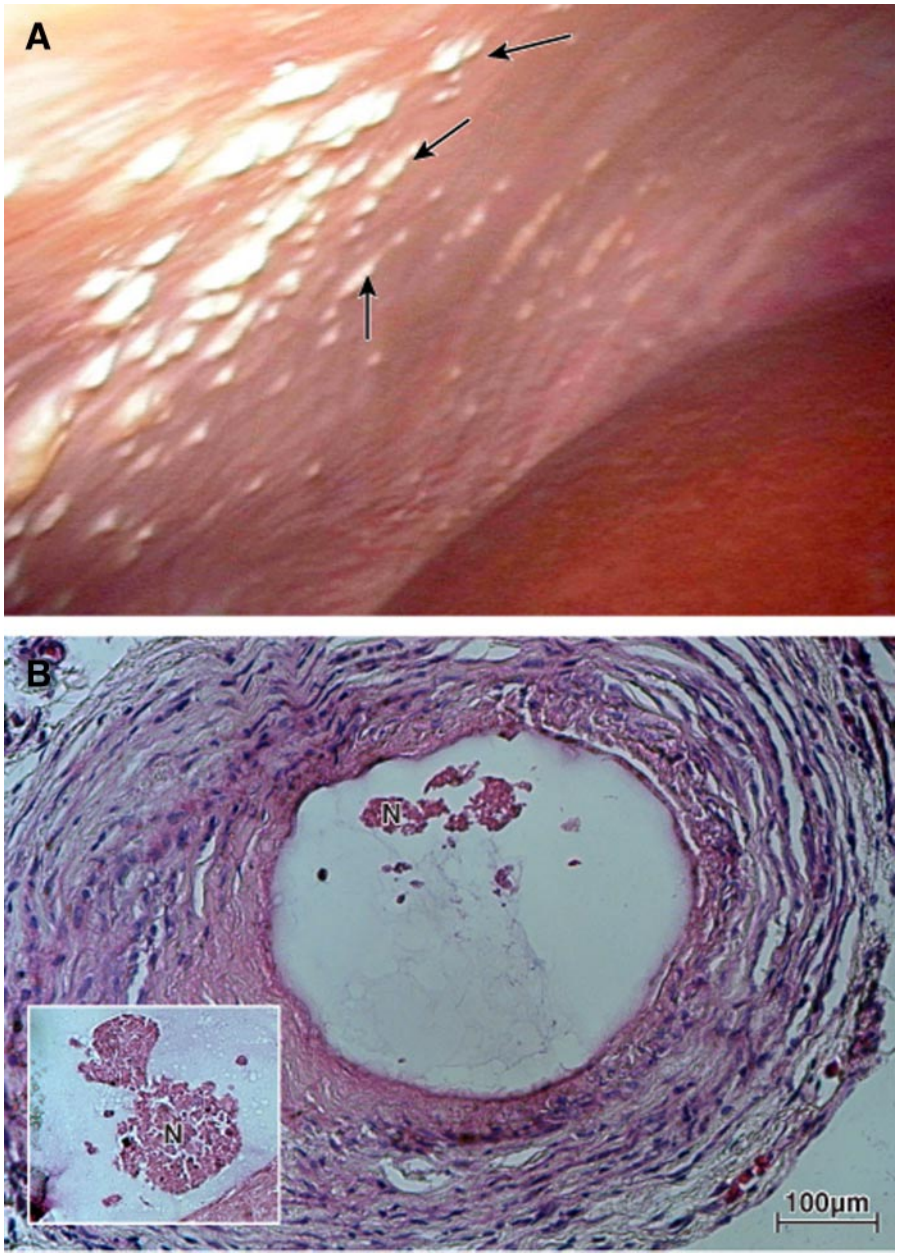


**Figure 1—***Biopsy of the encapsulated human islets taken from the recipient of four islet infusions (total of 779,000 IEQs) 16 months after the first infusion.* A*: Clusters of capsules on parietal peritoneum* (black arrows *indicate some of the many clusters).* B*: Higher magnification showing necrotic islets (N) inside capsules (artifactually distorted because of fixing) surrounded by fibrous tissue containing few blood vessels. (A high-quality digital representation of this figure is available in the online issue.)*

**CONCLUSIONS** — Phase 1 of the trial shows that the infusion of encapsulated human islets is safe, although cytotoxic antibodies can develop. All recipients had normal renal function after several decades of type 1 diabetes, which makes them extremely unlikely to ever develop renal dysfunction and potentially require a renal transplant. In such a situation, the occurrence of cytotoxic antibodies is mostly of passing interest.

The laparoscopy performed on a recipient of encapsulated human islets was novel with the capsules intact and the islets necrotic. This outcome is different from that of the one other report (5) of a laparoscopy performed in a human receiving encapsulated insulin-producing cells. In that case, viable endocrine cells were observed in the encapsulated neonatal porcine cells 9 years after transplantation (5).

Loss of graft function within days of transplantation was likely due to either ischemic necrosis or an inflammatory process, possibly initiated by fibrinogen adhering to the capsule surface (6). Cytokines could enter the capsule through its pores (250 kDa) and destroy the β-cells.

That there was some late graft function lasting for years in the recipient of the four infusions might be explained by a small number of ductal cells in the graft differentiating into β-cells (7).

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