Commentary – A hard lesson about transplanting islets into prevascularized devices

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The iconic quote "You cannot learn anything from success, you only learn from failure" applies to all research endeavors. Unfortunately, a common reaction amongst investigators is to feel unrewarded by publishing a failed endeavor, especially if the work is sponsored by a commercial entity. The authors of the accompanying paper[1] are therefore to be applauded for publishing the details of their study because only in that way can their valuable findings be disseminated so as to benefit the field of islet transplantation.

So, what can we learn? Why was there nearly complete lack of engraftment? Could the failure have been expected by the prior published work in small animals? How should devices and test methods be modified in the future to increase the probability of success?

WHAT WAS THE DESIGN OF THE CELL POUCH?

From the limited information publicly available, we understand the Cell Pouch™ used in this study to be a retrievable device for islet implantation in subcutaneous tissue. It consisted of a porous mesh fabricated from fibers in a regular pattern. The interstices of the mesh were large enough for ingrowth of vascularized tissue, and the mesh had no immunobarrier properties. The human trial employed the standard immunosuppression that is required for islet transplants. Solid cylindrical plugs were placed in the mesh with regular spacing. After an extended period following implantation, the plugs were removed so as to create empty cylindrical channels in which isolated islet preparations were placed, after which the channels were sealed. Oxygen was provided to the islets by diffusion from the prevascularized tissue around the periphery of the cylindrical spaces. Oxygen would also be provided by any additional neovascularization that occurred within the islet tissue in the cylindrical channels. From various sources[1-3], we estimate that each channel had a diameter of about 3 mm, a length of about 4 cm, and a contained volume of about 0.3 cm^3. Using the islet loading data in the paper and the volume of an islet equivalent (IE), 1.77×10^{-6} cm^3/IE, the volume fraction of islet tissue was about 14 and 32% in Cases 1 and 2, respectively. The islet volume fraction was potentially higher in case 3, but there is a discrepancy in the number of channels used to calculate islet loading per channel between Table 1 and the description in the text. In addition to islets, the preparations contained varying amounts of exocrine tissue, which added to the total tissue loading and increased the total volume fraction of tissue to about 16 and 43% in cases 1 and 2, respectively The low purity of islet preparations in Case 3 means that the total volume fraction tissue was even higher.

WHAT WERE THE CLINICAL OBSERVATIONS?

Although the concept behind the Cell Pouch™ had merit, it did not work. An increase of C-peptide was found in the blood of subjects immediately after implantation, which likely represents the release of stored insulin in dying beta cells. Subsequently, virtually no C-peptide was found in the circulation, nor was there any notable fall in insulin requirements. This was the case despite large numbers of cells being placed into the pouches. Case number 3 received nearly 1.3 million islet equivalents (IEQ), which is substantially more than what is usually required to reverse type 1 diabetes when islets are transplanted into the liver[4]. Histological evaluation of the pouches showed that

some patches of beta cells did survive in limited sections, but they probably represent only a small fraction of what was transplanted. One must conclude that the vast majority of the large number of islets transplanted did not survive.

WHAT CAUSED FAILURE OF ENGRAFTMENT?

It is possible that all four islet preparations were of such poor quality that they died immediately after implantation. We view this as extremely unlikely. Seroma formation, which frequently occurs following implant of mesh materials[5], was observed in Cases 1 and 3 and ultimately resolved itself. It is conceivable that seroma interfered with vascular tissue ingrowth or with the neovascularization process. We view this scenario as also unlikely.

The authors concluded that massive islet death resulted from hypoxia. This is reasonable from various viewpoints. Islet tissue under severe hypoxia likely died well within 1 day, similar to substantial islet loss observed 1 day after implantation in the kidney capsule[6]. Such loss might be prevented in the Cell Pouch™ if substantial vascularization occurred within the implanted islet tissue in a very short period of time, e.g., 1 day after implantation. Vessels arising from the prevascularized bed around the cylinder periphery would have to migrate and proliferate throughout the cylindrical space in this time period, and that did not happen. In freely transplanted islets, the first signs of angiogenesis do not appear until 2 days after transplantation, and 1-2 weeks is needed for the entire vascularization process to be completed[7]. In the case of neovascularization induced by the microarchitecture of porous materials without exogenous angiogenic factors, the rate at which new vessels migrate is markedly smaller[8]. While waiting for sufficient neovascularization, might the islet tissue be kept alive by a prevascularized bed at the periphery? The paper gives no significant evidence that this occurred. Limited histology in Figures 4 and 6 does not provide clear boundaries or dimensions (Figures 4A and 6A are identical but different magnifications are indicated). More importantly, even if a prevascularized network formed and was sufficient to provide a uniform pO_2 of 40 mmHg (typical for the microvasculature) around the cylinder, a simple engineering calculation using published parameter estimates[9] suggests most of the tissue would die. The maximum cylinder radius over which oxygen could diffuse until it would be totally consumed[10] is about 430 and 220 μm for the conditions of cases 1 and 2, respectively. These estimates are only a small fraction of the 1,500 μm cylinder radius used in the Cell Pouch™, which means most of the tissue would be expected to die. Furthermore, previous studies of islet encapsulation in hollow fiber and tubular membranes[11,12] demonstrated viability after implantation only when the volume fraction of tissue was extremely small, about 1% or less, because of hypoxia-induced cell death at higher loadings, thereby necessitating lengths of membrane tubing that were very large and impractical for clinical use[13-16]. One should, therefore, anticipate that the loading levels and cylinder diameter used in this study would lead to islet tissue death. The significant numbers of acinar and duct cells present, especially in Case 3, further magnify the problem because the death of acinar cells could lead to the release of proteolytic enzymes that could also contribute to beta cell death. Thus, failure of the Cell Pouch™ is not surprising from every viewpoint examined.

WAS FAILURE PREDICTABLE FROM THE PUBLISHED STUDY WITH SMALL ANIMALS?

The previous study in a mouse model[2] with a scaled down Cell Pouch™ demonstrated long-term insulin independence with implantation of 200 islets. However, achievement of euglycemia occurred more slowly, and the response to an IVGTT was slightly poorer, than with implants in the kidney capsule, though both IVGTT results were substantially worse than in naïve animals. There was no quantitative information about survival of implanted islets in explanted devices, and the evidence for neovascularization anywhere was similarly not quantitative and showed little more than a sparse distribution of vessels. Most critically, 200 islets would occupy only about 0.5% of the reported 75 μl volume of the single channel. The aforementioned studies with implanted hollow fibers and tubular diffusion chambers showed that most islets remain viable at such low volume fractions. In this critical aspect, the previous study[2] did not test the Achilles heel of this approach, i.e., the need for high islet volume fraction in the cylindrical spaces of clinical devices. Combined with the marginal glycemia results, the prior study did not provide any encouragement that the larger Cell Pouch™ and much higher islet density required in

humans would succeed. A variant of the oft-quoted message applies: "Learn from history or you're doomed to repeat it."

How should devices and test methods be modified in the future?

Given the advances in understanding immunosuppression and immunoisolation, and the expanding efforts with differentiated pluripotent cell-derived insulin-secreting cells, the critical remaining problem is the supply of sufficient oxygen to keep the cells alive and functioning. The latter aspect is even more difficult to achieve because deepening hypoxia can reduce the insulin secretory capacity of beta cells while still maintaining islets viable[17].

Current efforts to deal with oxygen supply limitations include exogenous supply of oxygen[18] and neovascularization around devices[19] and/or within tissue, as attempted with the Cell Pouch™. In the latter approach, sufficient oxygen supply requires robust neovascularization with an extensive vascular network[20]. It is not sufficient to observe some degree of neovascularization and declare success. In the native pancreas, each beta cell is adjacent to a blood vessel[21]. While such intimate vascularization may not be required for an implantable device, we currently do not know what is needed. A quantitative understanding of the relationship between characteristics of a neovascularized bed and maintenance of cell viability and function needs to be established. We also need validated methods for quantifying the extent of neovascularization. Only then will we be able to examine the efficacy of any particular method. The Cell Pouch™ relies on whatever vascular structure the neovascularization process provides. It may be necessary instead to develop preformed vascular structures within the tissue that are designed in advance to provide sufficient oxygen according to known engineering concepts. Without such microscale considerations, neovascularization will not succeed. Because of the low pO_2 of the blood in the microvasculature, neovascularization around a device limits the avascular thickness of tissue that can be supported. Use of exogenous oxygen is likely necessary to provide support for maintenance of much higher tissue densities and correspondingly smaller implanted devices for clinical use.

This work also speaks to insufficiencies in the way in which translational research in animals in this field is carried out. We need to do a better job before devices get to human testing. We need to use quantitatively reliable methods, such as measurement of morphometric parameters[22,23] or oxygen consumption rate[24] to characterize how much viable tissue is implanted and how much is left when the implant is excised. If vascularization is important, we need to quantitatively assess the state of the microvasculature at the device-host tissue interface. The biggest lesson to learn from this failed study is that we have to up our game in the quality of translational research if we are to avoid repetition of these kinds of results.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

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