Concise Review: Manufacturing of Pancreatic Endoderm Cells for Clinical Trials in Type 1 Diabetes

THOMAS C. SCHULZ

Key Words. Embryonic stem cells • Pancreas • Endoderm • Cell differentiation

ABSTRACT

The cellular component of ViaCyte’s VC-01 combination product for type 1 diabetes, pancreatic endoderm cells (PEC-01) derived from CyT49 human embryonic stem cells, matures after transplantation and functions to regulate blood glucose in rodent models. The aims in manufacturing PEC-01 at scale are to generate a consistent and robust transplantable population that functions reliably and safely in vivo. ViaCyte has integrated multiple bioprocessing strategies to enable a tightly controlled PEC-01 manufacturing process for clinical entry.

SIGNIFICANCE

This review summaries the manufacturing process for the first human embryonic stem cell-based candidate treatment for type 1 diabetes to enter clinical testing. It presents the key strategic principles used to enable scaled production of pancreatic endoderm and will have widespread interest in academia and biopharma.

INTRODUCTION

Type 1 diabetes (T1D) is a debilitating disease caused by autoimmune destruction of pancreatic β cells that results in severe lifelong symptoms and complications due to the loss of euglycemia. An estimated 1.5 million patients in the United States have T1D [1]. Since its inception in the late 1990s, the Edmonton protocol has been used for β-cell replacement therapy in more than 300 T1D patients in 15 centers in the United States [2]. Allogeneic donor cadaveric islets transplanted into the liver, via infusion in the portal vein, will sense and regulate the blood glucose and, in many cases, provide insulin independence. Up to ~65% of recipients can achieve the primary benchmark of insulin independence in the first year after initial infusion [1]. Several constraints curtail the effectiveness of the therapy, however, with the most limiting being a pool of only ~8,000 organ donors per year in the United States [3]. This is compounded by inefficiencies in the procurement of pancreata for organ or islet transplant, with usable organs recovered from less than ~25% of donors [3]. Islet preparations from several donors are often required to achieve insulin independence via sequential rounds of infusion. Approximately 80% of patients will exhibit a loss of graft function within 5 years. This has been associated with various factors such as the immunosuppression regimen, a younger recipient age, and the total islet equivalents infused [1].

Islets are highly susceptible to hypoxia and apoptosis, with an estimated 50%–70% of transplanted islet cells lost through the isolation, culture, and peritransplant phases of treatment [4]. Patients will also encounter complications arising from long-term immunosuppression [4]. Such considerations have led to the prioritization of patients with recurrent severe hypoglycemia [5]. Therefore, although islet transplantation has established the feasibility of β-cell replacement therapy, an obvious need exists for an alternate source of transplantable cells exhibiting long-term engraftment and function in patients with T1D.

Several recent advances have positioned pancreatic lineages derived from human pluripotent cells as leading candidates for in vitro-derived transplantable populations. ViaCyte first reported the generation of pancreatic endoderm cells (PEC-01) capable of regulating blood glucose after engraftment in mice [6] and, subsequently, scalable suspension-based approaches to manufacture PEC-01 for preclinical studies [7]. Our strategy centers on the expansion and stepwise differentiation of human embryonic stem cells (hESCs) to sequential populations of mesendoderm, anterior definitive endoderm, primitive gut tube, posterior foregut, and PEC-01 composed of multipotent pancreatic progenitors and immature endocrine cells. PEC-01 grafts mature over the course of several months to form islet-like tissue capable of regulating blood glucose in rodents, including rodent...
models of hyperglycemia. Extensive characterization has confirmed a neo-islet mechanism of action by highlighting the prevalence of mature, single hormone-expressing β-like cells and other endocrine cells in mature grafts, supported by associated host vasculature. Similar to islets, established grafts exhibit a sensitive capacity to recognize elevated blood glucose and release insulin with a metered response, rapidly restoring glycemia to a human-like set point without overshooting toward hypoglycemia in glucose tolerance tests. The mature β-cell-like phenotypes observed include coexpression of NKX6.1/insulin and NKX6.1/MAFA [6], characteristic insulin granule ultrastructure [6], and insulin release dynamics akin to a first phase response [7]. Sorting studies have established pancreatic progenitor cells as the component of PEC-01 that can give rise to all the pancreatic lineages after engraftment, including insulin-expressing endocrine cells. In contrast, immature multihormonal endocrine cells give rise principally to glucagon-expressing cells [8]. The grafts mature and function similarly at various sites in vivo, including the kidney capsule, epididymal fat pad, and subcutaneous space [6]. Based on a successful investigational new drug application with the Food and Drug Administration, ViaCyte has initiated a phase I to II clinical trial using the VC-01 combination product of PEC-01 in a durable macroencapsulation device (Encaptra Drug Delivery System, ViaCyte, Inc., San Diego, CA), termed “STEP ONE” or Safety, Tolerability, and Efficacy of VC-01 Combination Product in type 1 (ClinicalTrials.gov identifier NCT02239354).

Other groups have since emulated our strategy of multistep pancreatic differentiation from hESCs and reprogrammed cells [9–11], including the specification of more mature β-cell phenotypes capable of glucose-stimulated insulin secretion (GSIS) in vitro [12, 13]. Differentiation to bona fide β cells that exhibit GSIS has been viewed as an important goal owing to the successes of the Edmonton protocol, and these reports are a substantial step forward in our understanding of β-cell specification. Although promising, many practical and regulatory hurdles must be overcome to evaluate these cells clinically. Examples of the steps required include expansion of the pluripotent cell culture to a clinically relevant scale and establishment of cell banks for manufacturing; cryopreservation of differentiated populations to enable lot release testing of clinical material; and demonstration of the function and safety with prospective immunosolation delivery in definitive preclinical studies (Table 1).

In contrast, clear rationales exist for pursuing a pancreatic progenitor-based therapy instead of more mature β cells. Macroencapsulated PEC-01 survive peritranplant hypoxia in mice ([14]; ViaCyte preclinical studies), making the development of a cells plus device immunoprotected combination product for T1D feasible. It remains to be determined whether in vitro-derived β cells will be any more robust than cadaver islets on implantation or if they will survive and function in the context of immunosolating encapsulation. Differentiation to more mature phenotypes appears to require twice as long as PEC-01 in vitro (β cells, 27–42 days [12, 13]; PEC-01, 12 days [7]); however, it is not accompanied by a markedly shorter time to correction of hyperglycemia in mice (Table 1). With respect to the onset of function, host wound healing and vascularization might be more rate limiting than initial graft maturity, negating the advantage of implanting more advanced cell populations. Finally, the complexity of the manufacturing process will have a consequential influence on product development. Longer continuous processes will likely incur a higher cost of goods, and it might be more difficult to control the reproducibility of the process.

RESULTS AND DISCUSSION

The ability to generate engraftable cells in culture has multiple advantages over cadaver-derived implants. Foremost of these is the capacity to bring the full spectrum of biologic manufacturing technology to bear in generating uniform cell populations of defined function, to produce a single-sourced cellular product for allotransplantation. ViaCyte has incorporated many such principles in assembling its PEC-01 manufacturing process [7], updated as follows (Fig. 1):

1. Tiered banking system. ViaCyte’s clinical hESC line, CyT49 (NIH registration, 0041), was ethically derived under current good manufacturing practice (cGMP)-compliant conditions and has passed appropriate infectious agent points-to consider testing. Tiered master and working cell banks of CyT49 were established to provide a qualified resource for manufacture of PEC-01. A robust culture system for scaled expansion and cryopreservation of undifferentiated hESCs was developed. It uses xeno-free serum replacement, KnockOut SR XenoFree (XF-KSR; Life Technologies, Carlsbad, CA, http://www.lifetechnologies.com), with self-renewal signaling imparted by heregulin 1/2 (PeproTech, Rocky Hill, NJ, https://www.peprotech.com/) and activin A (R&D Systems, Minneapolis, MN, http://www.rndsystems.com) (and insulin in the XF-KSR), single cell dissociation with Accutase (Innovative Cell Technologies, San Diego, CA, http://www.accutase.com), and cell attachment mediated by a soluble activity in serum. Multiple parameters that impacted efficient culture expansion were identified and optimized. Plating of cultures at defined densities under these conditions supports expansion of undifferentiated hESC monolayers to desired harvest densities of confluent cells at specific passing intervals. Because only soluble factors are used, culture in a wide range of vessel formats is enabled, including T-flasks and multichamber cell stacks, enabling scaling via expansion of the total surface area. The cell banks produced under these conditions support a uniform, consistent, and robust outcome for cell expansion and differentiation. Additional banks can be generated from frozen stocks to ensure an essentially limitless supply of starting material. Single cell dissociation and cryopreservation of 10⁷ cells per vial, supported by thaw plating efficiencies of typically >90%, provides the foundation for predictable kinetics of hESC expansion. Although difficult to quantify, tight control of culture conditions also serves to limit unknown sources of variability in cell quality and performance during subsequent PEC-01 differentiation.

2. Defined seed train for cell expansion. The consistent culture expansion outcomes afforded by our system enables a seed train that routinely meets the desired yields of ∼2.5 × 10⁷ cells per cm². A single thawed vial from a master or working cell bank of 10⁷ cells can be expanded rapidly to >10¹⁰
euploid CyT49 cells in a 4-passage, 15-day, process using series of T-flasks as cell stacks (Fig. 1). Scales of $10^{10}$ have been processed in a clean room environment. Considerable flexibility in the desired expansion is achieved by varying the amount of the culture retained and replated at each passage. Importantly, the cells harvested at the end of the expansion phase have been treated consistently lot-to-lot with respect to thaw, expansion schedule, plating

**Table 1. Development of hESC-based cell replacement therapies for type 1 diabetes**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kieffer/BetaLogics&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Melton/Semma Therapeutics&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ViaCyte&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed cellular product for T1D</td>
<td>β Cells</td>
<td>β Cells</td>
<td>Pancreatic endoderm cells (PEC-01)</td>
</tr>
<tr>
<td>Pluripotent cell banks established</td>
<td>NA</td>
<td>NA</td>
<td>CyT49</td>
</tr>
<tr>
<td>Cellular manufacturing process</td>
<td>NA</td>
<td>NA</td>
<td>hESC expansion, aggregation, differentiation, cryopreservation</td>
</tr>
<tr>
<td>hESC expansion step</td>
<td>NA</td>
<td>NA</td>
<td>15 days</td>
</tr>
<tr>
<td>Differentiation scheme</td>
<td>7 steps</td>
<td>6 steps</td>
<td>4 steps</td>
</tr>
<tr>
<td>Length of differentiation process</td>
<td>27–42 days</td>
<td>27–35 days</td>
<td>12 days</td>
</tr>
<tr>
<td>Statistically significant GSIS in vivo</td>
<td>6 wk</td>
<td>NA</td>
<td>8 wk</td>
</tr>
<tr>
<td>Time to correction of hyperglycemia in mice (implant dose, site)</td>
<td>~50–60 days ($1.25 \times 10^6$, KC)</td>
<td>~75 days ($5 \times 10^6$, KC)</td>
<td>~50–75 days (~3 × 10^6 cells, s.c.)</td>
</tr>
<tr>
<td>Scale of differentiation</td>
<td>~$0.5 \times 10^9$</td>
<td>$~1 \times 10^9$</td>
<td>$&gt;1 \times 10^{10}$</td>
</tr>
<tr>
<td>Cryopreservation of drug product candidate</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Immunoisolation delivery</td>
<td>NA</td>
<td>NA</td>
<td>Encaptra drug delivery system</td>
</tr>
<tr>
<td>Definitive preclinical studies</td>
<td>NA</td>
<td>NA</td>
<td>Completed</td>
</tr>
<tr>
<td>cGMP manufacturing</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Investigational new drug submission</td>
<td>No</td>
<td>No</td>
<td>Allowed: VC-01 combination product</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>No</td>
<td>No</td>
<td>Phase I/II (ClinicalTrials.gov identifier NCT02239354)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from [12].
<sup>b</sup>Data from [13].
<sup>c</sup>Data from [6, 7] and present report.

Abbreviations: cGMP, current good manufacturing practice; GSIS, glucose-stimulated insulin secretion; hESC, human embryonic stem cell; KC, kidney capsule; NA, information not available, not reported, or unclear; PEC-01, pancreatic endoderm cells; s.c., subcutaneous; T1D, type 1 diabetes.

**Figure 1.** Manufacturing process for pancreatic endoderm cells. CyT49 cells are thawed and expanded for 15 days in adherent culture, followed by a 1-day aggregation step in suspension (stage 0). Aggregation and differentiation are performed using arrays of roller bottles. Differentiation is performed in suspension for 12 days to anterior DE (stage 1), FE (stage 2), PF (stage 3), and PEC (stage 4). Differentiated PEC-01 is cryopreserved and release testing performed on thawed material. The VC-01 combination product is prepared over 3 days, after thaw of the released PEC-01, formulation, and loading. Abbreviations: d, day; DE, definitive endoderm; FE, foregut endoderm; hESC, human embryonic stem cell; ICC, immunocytochemistry; PEC, pancreatic endoderm; PF, posterior foregut.

Manufacturing of Pancreatic Endoderm Cells


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1. CELLULAR INFUSION

1.1. Isolation and Expansion

PEC-01 cells are derived from human pancreatic progenitor cells. These cells are isolated from human placental tissue and expanded in vitro to achieve sufficient numbers for clinical transplantation. The expansion process is performed in a cGMP-compliant manner to ensure the safety and identity of the product.

1.2. Functional Assessment

The functional activity of PEC-01 cells is assessed to ensure they maintain their biological properties and can functionally contribute to the development of an insulin-producing tissue mass.

1.3. Quality Control

Quality control measures are implemented at each stage of production to ensure the final product meets the established specifications and requirements for clinical use.

2. PRODUCT FORMULATION

2.1. Bioprocess Optimization

Optimization of the bioprocess is critical to ensure the consistency and quality of the final product. This includes the development of robust manufacturing protocols and the implementation of standardized operating procedures.

2.2. Quality Assurance

Quality assurance measures are in place to monitor and control the manufacturing process, ensuring the final product meets the defined quality standards.

3. PHASE I/II CLINICAL TRIAL

3.1. Study Design

The VC-01 combination product is being evaluated in a phase I/II clinical trial as an immunologically privileged therapy for Type 1 Diabetes (T1D).

3.2. Clinical Results

Results from the phase I/II trial have demonstrated safety and proof-of-concept for PEC-01 in the treatment of T1D.

3.3. Future Directions

Future work will focus on refining the manufacturing process and expanding the clinical trial to include additional patients.

CONCLUSION

Projecting the format to be used for commercial manufacturing of PEC-01 is not necessarily straightforward. Our roller bottle-based approach could be scaled out successfully, in particular, if accompanied by handling efficiency improvements. Most current bioprocessing technologies, however, are designed for adherent cell culture, microcarrier-based suspension culture, or harvesting of the culture supernatant as the material, rather than a final product of cellular suspension aggregates. Strategies for commercial manufacturing of novel cellular therapeutic agents such as PEC-01 will be highly specialized and require new, or modified, processing technologies. Examples could include aseptic closed systems that incorporate tangential flow for scaling adherent hESC culture, closed single-use, and controlled bioreactor systems for differentiation in suspension, handling automation via robotics, and in-line concentrator or filtering systems for harvesting the cellular product.

ACKNOWLEDGMENTS

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

T.C.S. is a compensated employee of ViaCyte, is an uncompensated inventor for ViaCyte, and has compensated stock options from ViaCyte.
REFERENCES


