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

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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. STEM CELLS TRANSLATIONAL MEDICINE *2015;4:1–5*

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

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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 immunoisolation 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 peritransplant 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 immunoisolating 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 1β (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 passaging 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 10^7 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 $\sim 2.5 \times 10^5$ cells per cm^2. A single thawed vial from a master or working cell bank of 10^7 cells can be expanded rapidly to $>10^{10}$

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

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

[a]Data from [12].
[b]Data from [13].
[c]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.

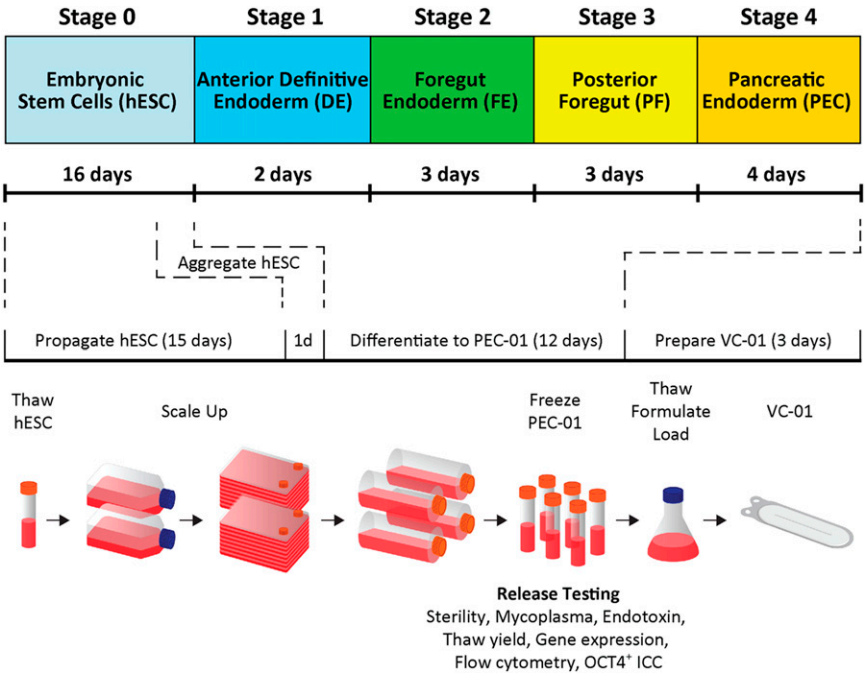

Figure 1. Manufacturing process for pancreatic endoderm cells. CyT49 cells are thawed and expanded for 15 days in adherent culture, followed by a 1-day aggregation step in suspension (stage 0). Aggregation and differentiation are performed using arrays of roller bottles. Differentiation is performed 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.

euploid CyT49 cells in a 4-passage, 15-day, process using a series of T-flasks and 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

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density, harvest density, culture media composition, volumes, and handling processes.

3. Suspension aggregation at scale. PEC-01 is differentiated from aggregates of pluripotent CyT49 cells in dynamic suspension culture. Aggregates were generated initially in 6-well plates under rotational culture, and lots of up to $\sim 3.3 \times 10^9$ differentiated PEC-01 have been produced in this format [7]. Under conditions that promote cell-cell contact and adhesion appropriately, aggregation of dissociated single cells proceeds to form uniform spheroids of undifferentiated cells 100–200 μm in diameter over the course of 1 day of culture. The horizontal rotation format did not lend itself easily to scaling, however, and presented a potential future bottleneck, particularly when considering the requirements for eventual commercial manufacturing. An alternative approach using roller bottles was therefore devised that supported aggregation at scale, including lots of $>10^{10}$ cells (Fig. 1). Amenable to both scale-up via bottle size and scale-out with roller bottle arrays, the approach could conceivably be used for the aggregation of $>10^{11}$ cells or >100 L volumes. The roller bottle format has been qualified and is being used for clinical manufacture of PEC-01.
4. Suspension differentiation to PEC-01 at scale. The directed differentiation of CyT49 aggregates to PEC-01 is performed in suspension, through four stages that provide developmental cues for specification. Although developed in horizontal rotational culture, PEC-01 differentiation has also been adapted to a scalable format in roller bottles (Fig. 1). The roller bottle system reduces the handling steps of large process runs considerably and provides a platform for differentiation that has the potential to accommodate 10^{10}–10^{11} cells. Multiple PEC-01 manufacturing runs have been prepared in roller bottles with cGMP and qualified for clinical trials.
5. Cryopreservation and release testing of PEC-01. Incorporation of a process hold point is a crucial component in manufacturing of candidate biologic therapeutic agents. ViaCyte has established controlled rate freezing that supports efficient thaw yields of PEC-01. End of process cryopreservation permits lot-release testing to be conducted on thawed test material, characterizing and qualifying the product safety and identity requirements. Clinical PEC-01 lots are tested with release assays for sterility, mycoplasma, endotoxin, thaw yield, composition by flow cytometry, gene expression using the nCounter Analysis System (NanoString Technologies, Seattle, WA, http://www.nanostring.com), and undifferentiated cells by OCT4 immunocytochemistry.

PEC-01 manufacturing is therefore an integrated and controlled process. Standardized procedures, batch records, cGMP, and lot release testing are all implemented in the production of a functional pancreatic population on a scale enabling clinical testing.

The VC-01 combination product is being evaluated as an allotransplant that might not require chronic immunosuppression. After lot-release, PEC-01 is thawed and cultured for several days of recovery, formulated, loaded into Encaptra devices, and implanted subcutaneously in a minimally invasive procedure (Fig. 1). The Encaptra system is both biocompatible and biostable, with the device membranes excluding host cells and preventing biodistribution of the engrafted cells. The device is designed to protect grafts from host alloimmunity and β-cell autoimmunity, facilitate vascularization, with free exchange of oxygen, proteins, nutrients, and, in particular, glucose in and insulin out. Similar devices have previously demonstrated protection of allografts in autoimmune or immunized rodent hosts [15, 16] and in nonhuman primates without immunosuppression [17]. A key safety advantage of durable macroencapsulation in the subcutaneous space is that the graft can be monitored via ultrasonography and removed should the need arise. ViaCyte's phase I/II trial was initiated in September 2014 and will implant VC-01 in an open-label, dose-escalating study of safety, tolerability, and efficacy. ViaCyte's paradigm for producing PEC-01 is therefore the first complete manufacturing process for a pluripotent cell-based therapeutic for T1D.

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. Although several of these might be customized from existing technologies, new players in the bioprocessing space are also being drawn toward the opportunities in cellular therapeutic manufacturing. It is, therefore, possible to envision eventual commercial manufacturing performed at scales in line with traditional bioproduction.

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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.

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References

1 Scientific Summary of the Collaborative Islet Transplant Registry (CITR) 2010 (Seventh) Annual Report. Available at https://web.emmes.com/study/isl/reports/01062012_7thAnnualReport.pdf. Accessed January 30, 2015.

2 Gaba RC, Garcia-Roca R, Oberholzer J. Pancreatic islet cell transplantation: An update for interventional radiologists. J Vasc Interv Radiol 2012;23:583–594.

3 Organ Procurement and Transplantation Network/Scientific Registry Transplant Recipients (OPTN/SRTR) 2012 Annual Data Report: Deceased Organ Donation. Available at http://srtr.transplant.hrsa.gov/annual_reports/2012/pdf/07_dod_13.pdf. Accessed January 30, 2015.

4 Plesner A, Verchere CB. Advances and challenges in islet transplantation: Islet procurement rates and lessons learned from suboptimal islet transplantation. J Transplant 2011;2011:979527.

5 Srinivasan P, Huang GC, Amiel SA et al. Islet cell transplantation. Postgrad Med J 2007;83:224–229.

6 Kroon E, Martinson LA, Kadoya K et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443–452.

7 Schulz TC, Young HY, Agulnick AD et al. A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS One 2012;7:e37004.

8 Kelly OG, Chan MY, Martinson LA et al. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. Nat Biotechnol 2011;29:750–756.

9 Blum B, Hrvatin SS, Schuetz C et al. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. Nat Biotechnol 2012;30:261–264.

10 Rezania A, Bruin JE, Riedel MJ et al. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012;61:2016–2029.

11 Rezania A, Bruin JE, Xu J et al. Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. STEM CELLS 2013;31:2432–2442.

12 Rezania A, Bruin JE, Arora P et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol 2014;32:1121–1133.

13 Pagliuca FW, Millman JR, Gürtler M et al. Generation of functional human pancreatic β cells in vitro. Cell 2014;159:428–439.

14 Kirk K, Hao E, Lahmy R et al. Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. Stem Cell Res (Amst) 2014;12:807–814.

15 Sweet IR, Yanay O, Waldron L et al. Treatment of diabetic rats with encapsulated islets. J Cell Mol Med 2008;12(suppl 6B):2644–2650.

16 Kumagai-Braesch M, Jacobson S, Mori H et al. The TheraCyte^{TM} device protects against islet allograft rejection in immunized hosts. Cell Transplant 2013;22:1137–1146.

17 Sasikala M, Rao GV, Vijayalakshmi V et al. Long-term functions of encapsulated islets grafted in nonhuman primates without immunosuppression. Transplantation 2013;96:624–632.