



Novocell, Inc.

**Alan J Lewis[†],
Melissa Carpenter,
Allan Robins &
Emmanuel Baetge**

*†Author for correspondence
Novocell, Inc., 3550 General
Atomics Court, Building 2,
Room 503, San Diego, CA
92121, USA
Tel.: +1 858 455 3708
Fax: +1 858 455 3962
E-mail: alewis@novocell.com*

Novocell, Inc. is a stem cell engineering company creating, delivering and commercializing cell and drug therapies for diabetes and other chronic diseases. The use of human embryonic stem cells provides a scalable source of any differentiated lineage that has potential for cell replacement therapy, as well as tools for drug discovery to create regenerative medicines.

Throughout the 1960s and 1970s, the pharmaceutical industry focused almost exclusively on small-molecule drugs to treat diseases. The 1980s and 1990s witnessed the era of genetic engineering, resulting in the development of innovative biologics such as monoclonal antibodies and recombinant proteins, as well as the development of new tools to identify small molecule drugs. These transformational technologies helped start the biotechnology industry. As we enter the 'Century of the Cell', the advent of stem cell engineering offers the potential to produce cellular replacement disease-modifying treatments and potential cures for many debilitating, degenerative disorders, including diabetes and cancer. To embrace this revolution Novocell has three primary technology platforms, discussed below.

Stem cell-directed lineage

Novocell is the first company to efficiently engineer human embryonic stem cells (hESCs) into definitive endoderm (DE), the gatekeeper cells that differentiate into pancreas, liver, lung and many other cells, tissues and organs. This provides a platform to create cell therapies, develop drug discovery opportunities in regenerative medicines, and assays for ADME/toxicity, as well as find therapies that target key cancer stem cells.

Cell encapsulation

Novocell utilizes a biocompatible polyethylene glycol (PEG) conformal coating that enables transplanted cells to survive and function by protecting them from immune rejection. It is designed to eliminate the need for continuous immunosuppressant drugs that are necessary for allograft transplants. The technology is being used in the current proof-of-principle primary islet transplant Phase I/II clinical trial in Type 1 diabetics.

Drug discovery

Novocell is developing defined media conditions for the growth and expansion of cancer stem cells (CSCs). These CSCs are being used to identify specific druggable targets for cancer treatment. Novocell is also developing drug discovery assays for regenerative medicine and for drug ADME/toxicity prediction using liver and intestinal cells.

Novocell: a history

Novocell was formed in August 1999 by acquiring all of the assets and liabilities of Neocrin Company, including key licenses to cell encapsulation technology invented by Jeffrey Hubbell and assigned to the University of Texas, USA. In August 2004, Novocell merged with CyThera, Inc., San Diego, CA, USA to combine the delivery technology with a potential for renewable islet cell sources derived from stem cells. CyThera, focused on developing human stem cells for the treatment of human degenerative disease, merged in July 2004 with Bresagen, Inc., Athens, GA, USA. Bresagen is one of the few suppliers of 'presidentially approved' hESC lines and has access to NIH-funds to distribute and characterize these lines. Novocell's primary therapeutic target is diabetes and our proprietary technology platform has the potential to provide a renewable supply of functional human insulin-producing cells for the treatment of diabetes, thereby overcoming the major obstacle of islet supply.

Novocell's unique accomplishments

- Demonstrated, in small and large animal studies, that encapsulated cells are protected from immune rejection after implantation, enabling the development of a new therapy for major diseases such as diabetes.
- Demonstrated preliminary evidence of safety and function in a Phase I/II proof-of-principle clinical trial in patients with Type 1 diabetes using encapsulated primary islets.

- Established six human ESC lines, including a clinical-grade line.
- Developed DE cells from hESCs.
- Developed insulin-producing cells from hESC-derived DE.

Differentiation of hESCs to endoderm

A unique property of hESCs obtained from the inner cell mass of blastocysts is their ability to differentiate into all the cell types that comprise the human body.

The generation of fully differentiated cells throughout the body occurs by a series of developmental stages. The first stage, known as gastrulation, results in the generation of the three somatic lineages (Figure 1): the ectoderm, mesoderm and endoderm. The endoderm subsequently gives rise to a number of therapeutically important structures including the lungs, liver, thyroids, thymus, intestine, stomach, bladder and pancreas.

The development of cell therapies involving these organs has been hampered by the inability to control and direct differentiation of hESCs to DE. The Novocell team accomplished this ground-breaking feat by developing a reproducible and efficient protocol [1-4] that has been replicated in numerous laboratories around the world. This work provided a solution that now opens the door to generation of the endoderm-derived mature cell types, such as insulin-producing cells and hepatocytes.

Diabetes program

Novocell's encapsulation and stem cell technologies have the potential to treat many human cellular degenerative diseases and disorders. The first application of these technologies uses proprietary cell encapsulation of insulin-producing cells to treat diabetes. The product will be biocompatible and allows subcutaneous implantation without requirement for long-term immunosuppression.

Diabetes is the fifth leading cause of death by disease in the USA alone. According to the American Diabetes Association (ADA) the total annual economic cost of diabetes in 2002 for the USA was estimated to be US\$132 billion. According to the International Diabetes Federation (IDF), it is estimated that currently some 194 million people worldwide, or 5.1% in the adult population, have diabetes and that this will increase to 333 million, or 6.3%, by 2025.

The efforts of James Shapiro and colleagues, creators of the Edmonton Protocol for islet transplantation, demonstrate that hepatic

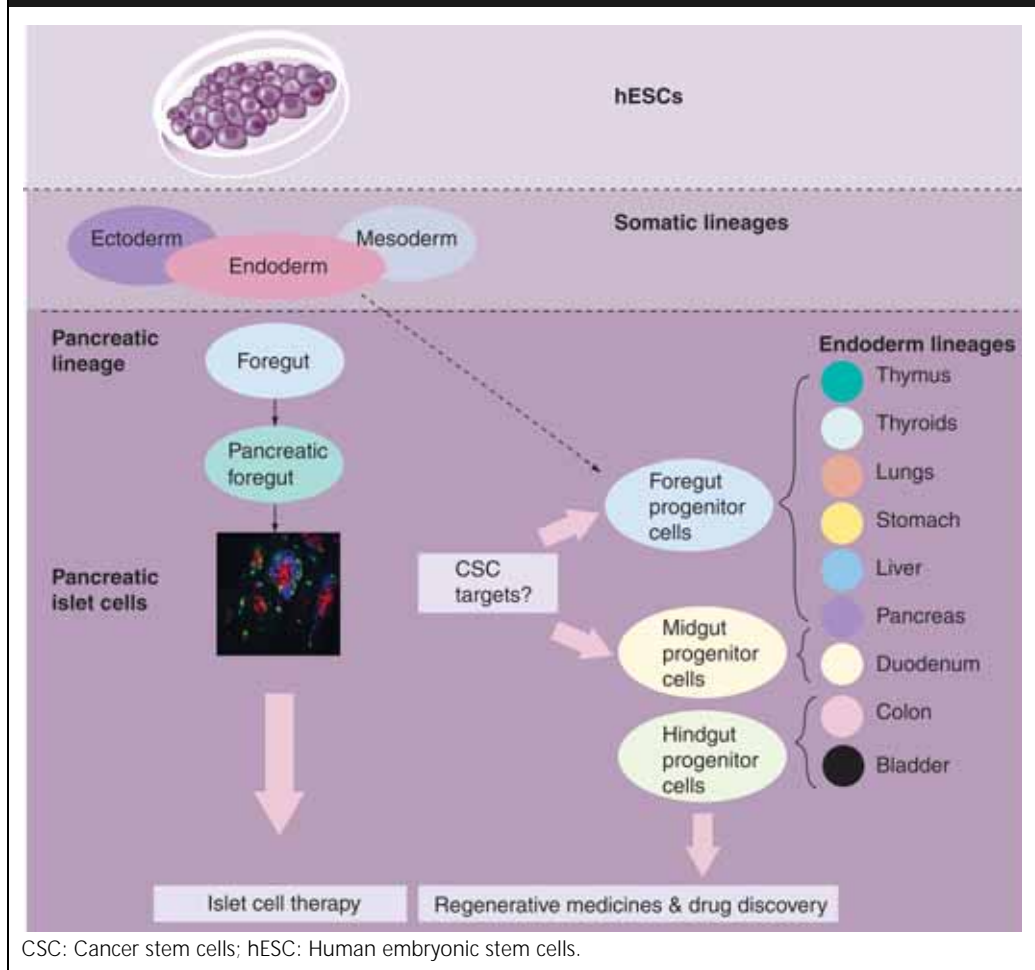
engraftment of human primary islets can provide relief from daily insulin therapy and lead to normalization of blood glucose with predicted long-term amelioration of diabetes-related complications. All Type I and insulin-requiring Type II diabetics (up to 40% of Type II patients) are candidates for islet cell transplantation. Nevertheless, there remains a severe shortage of functional primary human islet cells for these proven transplantation treatments. One possible means for alleviating this paucity of transplantable insulin-producing cells would be to produce such cells from hESCs, which are functionally immortal and are considered to have the potential to generate every cell type found in the human body. hESCs are unique amongst stem cells with regard to these properties.

Novocell's milestones for its stem cell engineering programs include:

- Establishment of hESC lines according to clinical standards for application to cell-replacement therapies such as diabetes;
- Development of ways to efficiently differentiate hESCs into functioning insulin-producing cells that can ultimately be implanted into humans;
- Application of the company's encapsulation technology to allow delivery of cells without immunosuppression in diabetic patients;
- Development of methods to scale-up production of cells to allow efficient commercialization of the technology;
- Identification of targets for the discovery of novel anticancer agents to treat those cancers linked to endoderm organs such as pancreas, colon and lung.

Novocell's strategy for the generation of functional insulin-producing cells is to guide the hESCs through a step-by-step process that mimics early embryological development of the pancreas. The differentiation of hESCs to insulin-producing cells occurs through a series of specifying and patterning events whereby hESCs transition through mesendoderm, endoderm and foregut endoderm to form pancreatic endoderm and endocrine precursor cells. We observe the temporal expression of the principal markers specifying each transition including HNF1B, HNF4a, HNF6, PDX1, PTF1A, HLXB9, NKX6.1, NKX2.2, PAX4, PAX6, NEUROD1, ISL1 and IAAP. As a result of this directed differentiation strategy Novocell has generated cell populations in which the principal islet hormones, including insulin, glucagon, ghrelin, pancreatic polypeptide

Figure 1. hESC differentiate into endoderm lineages that have enormous potential for cell therapy and drug discovery.



and somatostatin, are expressed at the mRNA and protein levels. The cells generated have many of the properties of fetal pancreatic islets. A manuscript detailing these results has been recently published [5]. Novocell is currently working on scale-up technologies to generate sufficient quantities of insulin-producing cells for clinical entry.

Cell encapsulation

While there have been many different approaches to encapsulating cells, including insulin-producing islets, none of these have been successful in humans to date.

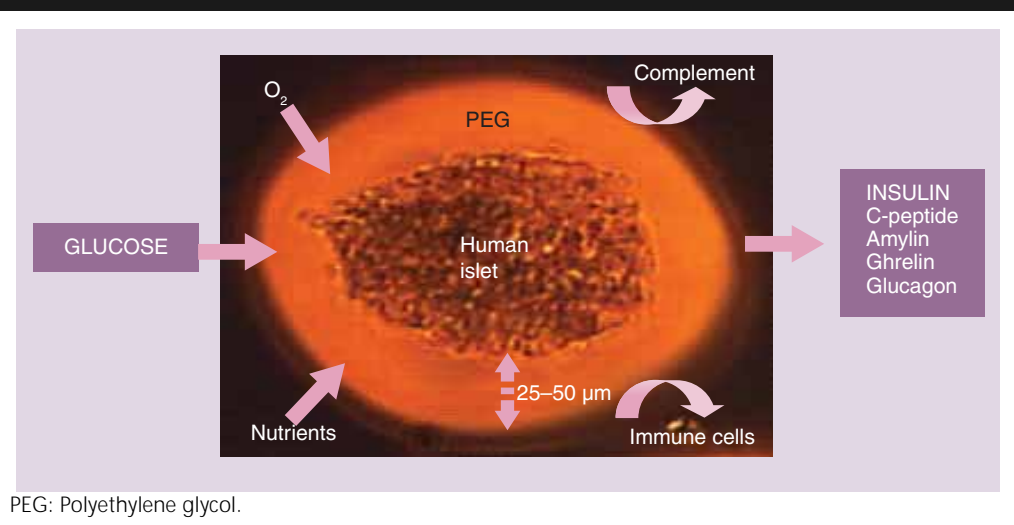
Novocell's conformally coated islet allografts make the technology clinically and commercially relevant [6]:

- Content of biodegradable-PEG
- Conformal coating (25–50 μm)
- Immunologically nonreactive
- Adjustable lifespan and permselectivity

The biocompatible substance, PEG, can be effectively and evenly applied to the surface of islets, allowing insulin and glucose to pass freely in and out of the capsule while preventing the body's immune system from destroying the islets within (Figure 2).

After definitive nonhuman primate studies evaluating encapsulated primary islets in experimentally induced diabetes were successfully completed, an Investigational New Drug (IND) was submitted to the FDA requesting a combined Phase I/II clinical trial. The FDA approved the IND and trial design and a Phase I/II trial began in patients with insulin-requiring diabetes in December 2005. The islets used in this trial were sourced from approved procurement agencies and donors in collaboration with other islet isolation facilities in the USA. A total of two Type 1 diabetic patients were transplanted and no significant adverse events have been observed to date (12 months post-transplant).

Figure 2. PEG encapsulated human islet is biocompatible, permeable to nutrients including glucose but not large molecules and immune cells



In summary, this study has shown:

- No safety concerns to date
- No evidence of autoimmune destruction or allograft rejection to date – no apparent induction of autoantibodies and no precipitous loss of islet function
- Evidence of function – C-peptide responses to oral glucose tolerance testing without immunosuppression for more than 12 months

Drug discovery Cancer stem cells

CSCs have recently been identified as self-renewing, immortal cells that are thought to be responsible for initiating cancerous tumor growth and promoting metastasis. CSCs have been isolated from a number of different tumor types and it is now thought that successful treatment of various cancers will require targeting of CSCs. While these CSCs have been isolated from a number of tissues, growing and expanding them *in vitro* has proved problematic. Novocell has expertise in the production of defined media and has developed two proprietary media for the growth of stem and progenitor cells [7]. One of these proprietary media has been licensed to Invitrogen, who launched the product in August 2007. Novocell is using its media development expertise to formulate media for the expansion of CSCs.

In addition, Novocell has isolated a stable hESC variant known as BG01v, which is trisomic for chromosomes 12 and 17. These same trisomies are found in many solid tumors.

Novocell has discovered a novel cell surface molecule on these cells that is believed to be involved in the self renewal of CSCs. This molecule represents a good target for future drug development for cancer treatment and Novocell is currently developing monoclonal antibodies to this cell surface molecule.

Novocell believes that CSCs represent novel targets for the development of cancer treatments and envisions a change in the way cancer is treated. This will involve both agents that target CSC in addition to chemotherapeutic regimens that target normal tumor cells making up the bulk of a tumor.

Hepatocytes & ADME/TOX applications for drug discovery

The liver hepatocyte is one of the principal epithelial cells arising from the ventral foregut DE. Currently, the supply of human liver hepatocytes required for drug metabolism and toxicology testing is quite limited. The pharmaceutical need for replenishable and reliable sources of normal human hepatocytes for drug metabolism and toxicity testing is considerable [8]. This market need could be further met with hepatocytes derived from hESCs having different genetic backgrounds (i.e., Caucasian European, North American, Asian, Indian, Arabic, and so on). Drug metabolism by liver hepatocytes occurs with different propensities in different genetic populations and therefore it would be of value to stratify the hepatocyte genotypes produced. We see the production of human hepatocytes as the most

practical way to leverage our know-how in the endoderm area to produce cells for product sales on a nontherapeutic R&D basis.

Drug screening & endoderm neogenesis

Neogenesis and survival assays for screening small molecules to identify compounds to regenerate pancreatic islet cells, liver cells, lung cells and other cell types are of paramount importance. Novocell is currently interested in developing assays to identify small molecules/drugs and biologics for cell survival and regeneration. In summary, in addition to the use of hESC-derived β cells for the treatment of diabetes, there are many potentially important products

that can result from expanding Novocell's platform technologies into new products for the treatment of diverse human diseases and disorders and for substantial R&D markets with specialized human cell sources for drug screening and toxicology testing.

Financial & competing interests disclosure

All authors are employees of Novocell, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Principal officers

Senior Executive Officers

Alan J Lewis, PhD	President & Chief Executive Officer
E Edward Baetge, PhD	Chief Scientific Officer
Allan Robins, PhD	Chief Technical Officer
Melissa Carpenter, PhD	VP of Research & Development
Xiaojie Yu, PhD	Sr Director of Biomaterials Science
Anne Sandan, CPA	Controller, Sr Director of Corp. Admin.
Liz Bui JD, PhD	Director of Intellectual Property

Board of Directors

Fred Middleton	Managing Director of Sanderling & Novocell's Chairman of the Board
Alan J Lewis, PhD	President & Chief Executive Officer
Donald J Elmer	Managing General Partner of Pacific Horizon Ventures
Franklin Johnson	Founding Partner of Asset Management Co.
Asish K Xavier, PhD	Vice President, Venture Investments, Johnson & Johnson Development Corp.
Orville G Kolterman, MD	Senior Vice President, Clinical & Regulatory Affairs of Amylin Pharmaceuticals

Scientific Advisory Board

Matthias Hebrok, PhD	Associate Professor, Diabetes Research Center, Department of Medicine, University of California, San Francisco, USA
Mike German, MD	Professor, Diabetes Center, University of California, San Francisco, USA
Jeffrey A Hubbell, PhD	Director of the Institute for Biomedical Engineering and Biotechnology, Lausanne, Switzerland
Marc R Montminy, MD, PhD	Professor, Salk Institute for Biological Studies (Affiliate Membership), Biomedical Sciences Graduate Program, University of California, San Diego, USA
Didier YR Stainier, PhD	Professor, Department of Biochemistry and Biophysics, University of California, San Francisco, USA
James M Wells, PhD	Assistant Professor Division of Developmental Biology, Children's Hospital Research Foundation, Cincinnati, OH, USA
Jeffrey A Bluestone, MD	AW Clausen Distinguished Professor, UCSF Diabetes Center, University of California, San Francisco, USA
Alberto Hayek, MD	Professor of Pediatrics, UCSD School of Medicine, Whittier Institute for Diabetes, La Jolla, CA, USA
James Shapiro, MD, PhD	Director, Clinical Islet Transplant Program University of Alberta, Canada

Bibliography

1. D'Amour KA, Agulnick AD, Eliazar S, Kelly OG, Kroon E, Baetge EE: Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol.* 23, 1534–1541 (2005).
2. Dalton S: It's endoderm...definitively! *Regenerative Med.* 1, 381–383 (2006).
3. Semb H: Definitive endoderm from embryonic stem cells. *Regenerative Med.* 1, 489–492 (2006).
4. Stanier D: No stem cell is an islet(yet). *N. Eng. J. Med.* 354, 521–523 (2006).
5. D'Amour KA, Bang AG, Eliazar S *et al.*: Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* 24, 1392–1491 (2006).
6. Scharp DW: Encapsulated human islet allografts: providing safety and efficacy. In: *Cellular Transplantation from Laboratory to Clinic*. Halberstadt CR, Emerich DF (Eds). Academic Press, 135–153 (2007).
7. Wang L, Schulz TS, Sherrer ES *et al.*: Self renewal of human embryonic stem cells requires insulin-like growth factor-1 and ERBB2 receptor signaling. *Blood* (2007) (In Press).
8. Pouton CW, Haynes JM: Embryonic stem cells as a source of models for drug discovery. *Nat. Rev. Drug Discov.* 6, 605–616 (2007).