SMART CELLECTOR: A PROPOSAL FOR THE
DEVELOPMENT AND COMMERCIALIZATION OF A
CELLULAR IMAGING, ANALYSIS AND
PROCESSING TECHNOLOGY FOR APPLICATION IN
REGENERATIVE MEDICINE

By

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Smart Cellector: A Proposal for the Development and Commercialization of a Cellular Imaging, Analysis and Processing Technology for Application in Regenerative Medicine

Abstract

By

BRETT A. HOOVER

This thesis is a proposal for developing and commercializing a cellular characterization and processing technology (Smart Cellector™) for application in cell-based regenerative medicine, and includes an overview of stem cells, their therapeutic applications, the technology platform, and the commercialization strategy. Smart Cellector is a modular platform that will automate and standardize stem cell processing for use in cellular therapies to address the need to reduce the cost, time, labor and variability associated with manufacturing cells for clinical and research use. [REDACTED] This project is currently a top priority within the [REDACTED] pipeline with work scheduled to begin in January 2011. Both companies have solid intellectual property (IP) and expect to generate new IP as development progresses. The go-to-market plan involves partnering with local cell therapy stakeholders, manufacturers, and [REDACTED]. Initial target markets include research centers and autologous cell therapy manufacturers, followed by allogeneic cell therapy manufacturers and large hospitals.
1. Introduction

1.1. My work at Cleveland Clinic

At Cleveland Clinic I fill two roles. I function as a Project Manager for the Clinical Tissue Engineering Center (CTEC) and as a Commercialization and New Ventures Associate for the Armed Forces Institute for Regenerative Medicine (AFIRM). In these roles I manage multiple projects related to the fields of cellular therapy, including tissue engineering, and regularly work with Cleveland Clinic’s technology transfer office (Cleveland Clinic Innovations) to perform all forms of due diligence for commercialization projects for regenerative medicine technologies. This role also afforded me the opportunity to serve on the Cleveland Clinic / Parker Hannifin Joint R&D Steering Committee as a non-voting member. Part of my function is to raise non-dilutive funding for the projects I manage. To that end, I have been a lead writer on several commercialization grants submitted to the Ohio 3rd Frontier Program (3 applications), the Global Cardiovascular Innovation Center (1 application), Cleveland Clinic Product Development Fund (1 application) and the National Institutes of Health (1 application). Each of these grants was well received, making it to the final round of review. Of them, the most successful grant was an Ohio 3rd Frontier award for approximately $2.3M for work beginning in early 2011. This work will comprise adapting Cleveland Clinic’s Colonyze technology for the automated characterization and quality control of umbilical cord blood-derived stem cells for use in basic research or clinical transplantation.
1.2. Cleveland Clinic and Parker Hannifin Corporation Partnership

[REDACTED]

1.3. Scope of Thesis

This thesis is a result of some of my work at Cleveland Clinic, and with Parker Hannifin, as a Project Manager for CTEC. More specifically, this thesis represents the work associated with the $1M Smart Cellector Ohio 3rd Frontier Biomedical Program grant application which I defended in front of a panel of representatives from the National Academy of Sciences (NAS) and the National Venture Capital Association (NVCA) in 2010. Although this application was not awarded due to the Smart Cellector’s low relative score on the technology readiness level (TRL) scale, the application received top-marks for scientific approach, commercialization strategy and technical innovation. This document will provide an early product development and commercialization strategy for the Smart Cellector technology with an overview of stem cells, their therapeutic applications, and the Smart Cellector technology platform. This will include a detailed description of the medical importance of this technology, a definition of the market, the market need and target customers, and an overview of the go-to-market plan. Market drivers and barriers will be discussed, in addition to the competitive landscape. Finally, a product development road map spanning initial proof-of-concept testing through a market testable alpha-prototype and featuring several proof-of-concept stages with defined technical milestones will be presented.
2. Technology Description

2.1. Scientific Background

2.1.1. Regenerative Medicine

Regenerative medicine, which encompasses tissue engineering and cellular therapy, is a rapidly proliferating interdisciplinary field of study and practical application comprising life, physical and engineering sciences. It is the process by which new functional tissues are generated to repair or replace tissue damage or failure as a result of disease, age, injury or genetic defects. The goal of this field is to discover and apply clinical therapeutics to repair, maintain, replace or enhance biological function. Although scientists and clinicians have been studying Regenerative Medicine for many years in the form of cell culture, it has just recently experienced a boom of interest and innovation in the past 10 years, and is now advancing at a continually accelerating rate.

Regenerative Medicine empowers scientists and clinicians to generate new organs in a lab for future implantation into a body that is unable to heal itself. With the advent of this field scientist are now starting to consider the possibility of a replacement cell therapy for long thought incurable diseases like Huntington’s, Parkinson’s and Type-1 Diabetes. The thought of curing a disease as prevalent as Type-1 Diabetes is truly astounding. At its pinnacle of application, Regenerative Medicine could yield wholly organic blood substitutes and organs for banking and subsequent use. When this happens, the thought of being an organ or blood donor could be rendered obsolete.
A key aspect of Regenerative Medicine is the cells do the work: Stem Cells. As the workhorse of almost all Regenerative Medicine therapies, stem cells represent the key to harnessing the power to regenerate human tissue and cure intractable disease. As such, it is critical that scientists understand what stem cells are, what they do and how they work. With that said, there are three main types of stem cells: 1) Embryonic, 2) Induced Pluripotent and 3) Adult stem cells (e.g., Connective Tissue Progenitors). Over the next few pages I will briefly describe Embryonic and Induced Pluripotent stem cells, and then spend slightly more time on Adult Stem and Progenitor cells. In this context, and for simplicity, I will refer to adult stem and progenitor cells as Connective Tissue Progenitors (CTPs). The reason for this approach is because all proof-of-concept work associated with developing the Smart Collector technology will use adult stem cells, and virtually all current and near-future (next 10 years) cell-based Regenerative Medicine will focus on this type of stem cell. Clinical human applications involving Embryonic and/or Induced Pluripotent stem cells are least 10 years away, as there are significant mechanistic, safety and efficacy questions that remain unanswered for these two stem cell types.

2.1.2. Embryonic Stem Cells

Although Embryonic Stem Cells (ESCs) are beyond the scope of this Thesis, they are worth mentioning as scientific background. ESCs are totipotent cells with the capacity for indefinite proliferation and self-renewal. They also possess the ability to differentiate into any cell type within the three germ layers. This makes this particular stem cell a powerful research tool and cell source for Regenerative Medicine. Unfortunately, ESCs often form teratomas in vivo, which are cancerous masses comprising cells from all three germ
layers. This is one of the many reasons why the commercialization of ESC for use in humans has progressed so slowly. Another barrier to commercial translation is the mechanism by which ESCs are generated. Traditionally, they are derived from the inner cell mass of an embryo during the blastocyst stage of early development. When grown in a lab, ESCs require a “feeder layer” (e.g. mouse embryonic fibroblasts) of cells to support their continual self-renewal and maintain their undifferentiated state. The process of extracting ESCs often destroys the original embryo. As such, there is a great deal of political and social debate surrounding the use of this cell type for research or clinical applications [2].

Uses for ESCs range from models for studying congenital diseases to replacing animal models in pre-clinical studies for testing drug safety and efficacy. There is also the potential to cure currently incurable wounds like spinal cord injury. Evidence for this, and some commercial progress, is provided by the recent initiation of an FDA-approved phase I clinical trial by Geron Corporation (NASDAQ: GERN) for treating spinal cord injury with ESCs [3]. Unfortunately, the regulatory (safety and efficacy) and social issues surrounding ESCs will translate to a minimum 5 to 10 year timeline before a product will be approved for marketed use in humans. The next section will discuss an exciting alternative source of pluripotent stem cells that is not derived from human embryos, and offers a long list of potential applications as research tools and cell therapeutics.

2.1.3. Induced Pluripotent Stem Cells
Induced Pluripotent Stem Cells (iPS cells) are an exciting new addition to Regenerative Medicine’s cellular repertoire. iPS cells are formally somatic cells that have been transformed via called somatic reprogramming to yield cells that are virtually indistinguishable from ESCs in that they have a seemingly unlimited proliferative capacity and the ability to differentiate into virtually any tissue type. Our current understanding of iPS induction defines this process as a “de-differentiation” of a terminally-differentiated cell to a stem cell state. This is accomplished through epigenetic changes (e.g. chromatin repackaging, DNA demethylation, and histone demethylation) to the cell’s DNA without altering the cell’s genotype.

iPS cells were originally generated in 2006 by Dr. Yamanaka’s laboratory using retroviral vectors to introduce the \textit{Oct4}, \textit{Sox2}, \textit{cMYC} and \textit{Klf4} genes into mouse fibroblasts [4]. The successful induction of these genes led to a cellular genotype (epigenetic state) and phenotype like that of ESCs. Later, in 2007, iPS cells were generated from human fibroblasts [5-7]. With the excitement surrounding this new type of stem cell, research has continued and new advances are being made at an accelerated rate [8, 9]. Of particular importance is that iPS cells offer a pluripotent alternative to ESCs that can be sourced from virtually any tissue type without the need to destroy an embryo in the process. As such, there is a multitude of research and commercial applications for iPS cell as either tools or therapeutics.

As research tools, iPS cells offer scientists the ability to recapitulate disease states at the cellular level and study changes in epigenetic state and phenotype in correlation with the
stage of a given disease. Scientists can also use iPS cells to reiterate and study the redirection of epigenetic state (i.e. differentiation). For example, a dermal fibroblast can be converted into an iPS cell, then the iPS cell can be differentiated into a neural cell, and (conceivably) back again [10-12]. Other applications for iPS cells as basic and clinical research tools include, but are not limited to:

- deriving cancer iPS cells to study the effects of a patient’s genetic background on cancer development;
- conducting prospective studies to correlate human genotype with the probability of cancer onset;
- studying the correlation between epigenetic state and cancer development;
- studying and understanding tissue phenotype lineage progression;
- studying and characterizing the mechanism of tissue regeneration;
- studying and correlating the effects of specific teratogens or gene traits on cellular processes during embryonic development; and
- modeling and studying congenital diseases and gaining access to otherwise inaccessible cell type (e.g. cerebral neurons).
Figure 1 summarizes the process for generating an iPS cell-derived cellular therapy. As cell therapies, the applications for iPS cells are even more exciting. Such applications include, but are not limited to:

- cell replacement therapy for congenital diseases (e.g. Huntington’s);
- patient-specific screening for drug toxicity, side effects and dose regimen (e.g. iPS-derived hepatocytes);
- universal source of allogeneic cells that could be HLA-matched and then differentiation into virtually any cell type prior to use;
• cheaper, better, faster drug development programs thanks to drug candidate screening without the need for animal models, fewer pre-clinical studies and a reduction in “interspecies” toxicity or efficacy surprises; and
• application in preventative cell therapy by pre-banking iPS-derived cardiomyocytes from a patient at high risk for acute myocardial infarction.

Unfortunately, any human clinical application of iPS cells will not happen for at least a decade, as there are a significant number of science and commercialization barriers that exist. These range from needing a better understand of the subtle differences between ESCs and iPS cells, to significantly improving the induction efficiency of DNA-free reprogramming methods, and even figuring out how to control and standardize the reprogramming process such that it can be scaled for predictable commercial use [13, 14]. Until these discoveries are made, Regenerative Medicine will have to reply on Adult Stem and Progenitor Cells (e.g. connective tissue progenitors) as its primary source of material.

2.1.4. Adult Stem and Progenitor Cells

Stem cells and progenitor cells exist in adult tissues and are important for maintaining tissue health and responding to disease or bodily harm. These cells are the source of new tissue as a result of tissue repair and/or remodeling. The activation, migration, proliferation and differentiation of stem and progenitors cells is controlled by both chemical and physical signals of the dictated by the local micro- and macro-environment. A major difference between stem and progenitor cells is that stem cells beget progenitor cells and are able to replenish themselves (e.g. self-renewal/regeneration) by asymmetric
division (Figure 2). Progenitor cells differ by simply proliferating and expanding with little or no ability to self-renewal, as these cells are most often “committed” to differentiate into a particular tissue type [15, 16].

Figure 2. Cartoon Diagram Depicting Stem Cell Self-Renewal [17]

Stem and progenitor cells exist in a system of continuous cell death and rebirth in almost every human tissue type that requires a resident population of cells to regenerate and repair tissue. This takes place through a methodical evolution of cells from a “stem-like” state to a more terminally differentiated state. This turnover of cells is especially prominent in the epithelium of the gut and the skin, where complete cellular turnover takes place every three or fourteen days, respectively. However, cell turnover is not that rapid in other tissues. For example, musculoskeletal tissue turnover is significantly slower, as has been best characterized in bone. In bone there is a logical progression from stem cell to mature osteocyte through a series of transition stages whereby stem cells become osteo-progenitors, which become pre-osteoblasts and, finally, these cells
transition to osteoblasts and then osteocytes [16]. When one breaks a bone, this same predictable process of differentiation from stem cell to mature bone cells occurs in order to heal the wound, and the very same concept applies to all musculoskeletal tissues, including: cartilage, fat, muscle and tendon [18].

Another important feature of adult stem and progenitor cell is that they can possess the ability to differentiate into more than one tissue phenotype. This is particularly the case for stem cells and less-differentiated progenitor cells. For example, bone marrow, muscle and fat-derived stem cells have all been demonstrated to form bone, muscle, tendon, fat and nerve tissue [19-21]. This, coupled with the ability to self-renew themselves, is what makes stem cells the cornerstone of cell-based Regenerative Medicine because with the right knowledge and technology, we can convert a stem cell derived from one tissue into a cell useful for treating another tissue type. The most common source of adult stem cells is bone marrow, with alternative sources including: bone, muscle, fat, cartilage and the peri-vascular region (pericytes) [22-25].

An important consideration in cell-based Regenerative Medicine is that the number of stem cells available in a given tissue will vary across tissue phenotypes, and this difference can be assayed \textit{in vitro} by extracting cells from the tissue and culturing them to promote stem cell activation and proliferation. The gold standard assay for assessing stem cell concentration and prevalence in a given tissue type is the colony forming unit (CFU) assay (Figure 3, below) [20, 21]. This assay will be discussed in detail later in this Thesis, as the Colonyze technology was originally designed to be an automated,
standardized and quantitative version of this assay. The designation *Connective Tissue Progenitor* (CTP) refers to a heterogeneous tissue-resident population of stem and progenitors cells that possess the ability to form colonies (proliferate) and differentiate into at least one (often more) connective tissue phenotypes [15]. As mentioned later in this Thesis, human bone marrow-derived CTPs are the cell type chosen for use in the early proof-of-concept work to develop the Smart Collector technology because Cleveland Clinic is home to one of the foremost authorities (George F. Muschler, MD) in CTP biology, and their application in Regenerative Medicine.

CTPs can be grown *in vitro* through long-term culture for application in research and Regenerative Medicine – although their capacity for prolonged proliferation and expansion is relatively finite in comparison to ESCs and iPS cells, which possess the ability to proliferate indefinitely. As CTPs are expanded, the more highly proliferative cells dominate the less proliferative, and the overall population becomes more homogeneous over time in favor of these rapidly dividing cells. Thanks to this competitive advantage, long-term culture expansion generates naturally selective forces for increased rates of proliferation (i.e. shorter cell cycle). Expanded or culture manipulated cells have been given different names based primarily on the way in which they were expanded. Examples include, but are not limited to: mesenchymal stem cell (MSC) [19], bone marrow stromal cell (BMSC) [21] and multi-potent adult progenitor cell (MAPC) [26]. Although naming conventions do not necessarily reflect their biological functions, they all indicate that stem and progenitor cells can be harvested
from tissue and expanded \textit{in vitro}, without losing their ability to differentiate into multiple connective tissue phenotypes [15, 19, 21, 27].

Other functional variables associated with CTPs are their cellularity, prevalence and concentration (e.g. number of CTPs per unit tissue) across tissue types. Of the many tissue sources from which to obtain CTPs, bone marrow aspirated from mammals is the most commonly studied. According to several studies this source of CTPs contains approximately 2000 CTPs and forty million nucleated cells per milliliter. This means that for every one CTP, there are 20,000 other nucleated cells [28]. As mentioned earlier, CTP cellularity, prevalence and concentration vary with tissue phenotype. For example, at approximately six million cells per cc of solid tissue, muscle and adipose tissue contain far fewer cells. However, these same tissue types actually yield a higher prevalence of connective tissue progenitors with adipose recorded as having up to one CTP for every 4,000 nucleated cells – a 5-fold increase over bone marrow. As such, cellularity and prevalence are two of many factors that must be taken into account when selecting a tissue source for stem cells to be applied in Regenerative Medicine. Other factors include:

- stem cell harvest site (e.g. bone marrow from spine versus hip);
- the health and histological aspects of local tissue;
- site-specific stem cell function and kinetics; and
- patient age, disease state and gender [15, 28].

As an example, several studies have shown that the cellularity of human bone marrow decreases as one ages, while another study demonstrated that (in women) connective
tissue progenitor prevalence decreases in a age-dependent fashion [28]. It has also been shown that since age and gender do not account for all of the variance in tissue to tissue cellularity and prevalence, connective tissue progenitors can be obtained from virtually anyone, regardless of age or gender [28, 29].

Lastly, connective tissue progenitors from different tissue types can vary in function and biological potential. This fact has very important connotations relative to selecting an ideal tissue from which to source CTPs for application in Regenerative Medicine. As stated previously, human bone marrow-derived connective tissue progenitors comprise cells with the ability to differentiate into a multitude of tissue phenotypes such as fat, bone, muscle, tendon, nerve, liver, cardiac and fibrous tissue [30-32]. However, CTPs derived from adult articular cartilage are often found to be singularly restricted and only able to differentiate into cartilage. However, this “fact” may only be defined by the dearth of literature focusing on exploring the multi-potency of cartilage-derived CTPs because rarely is this cell type used for anything other than cartilage repair (or the study thereof). Like articular cartilage-derived connective tissue progenitors, similar multi-potential restrictions may also apply to adipose and muscle tissue phenotypes [33-35]. What all this variation tells us is that the importance of proper characterization and validation of tissue sources and cell types used in Regenerative Medicine cannot be overstated.
Over 30 years ago, the Colony forming unit (CFU) assay was introduced and has since been heavily utilized in clinical and academic research. This assay takes advantage of the fact that stem and progenitor cells have a high tendency to attach to cell culture surfaces and proliferate into distinct colonies (Figure 3). In this technique, the number of stem and progenitor cells is enumerated by manually counting and classifying individual colony types [36]. Applications of this assay include, but are not limited to, evaluation of inhibitory or stimulatory effects of a variety of compounds on stem cells; quantifying the stem and progenitor cell content of various tissue types; end-stage drug toxicity testing; diagnosis of hematological disorders; predicting marrow or cord blood transplantation efficacy; and assaying stem cell functional potency (biological performance and potential), a requirement of phase II clinical trials [37]. Mononuclear cells from bone marrow and cord blood are routinely plated, and it is well recognized that the CD34+ stem cell population yields the progeny cells for the colony forming unit granulocyte-macrophage (CFU-GM), the colony forming unit granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM), and the burst forming unit erythrocyte (BFU-E) colony.
types. Significant variations in the intra-laboratory enumeration of CD34+ derived colonies accounts, in part, for the use of mononuclear cells, whose colony enumeration tends to yield a much lower coefficient of variation. A robust assay that would allow the prediction of the time frame for the transplant engraftment of stem cells as reflected by neutrophil and platelet formation would be invaluable to assist in the planning of supportive care for the patient [38, 39].

![Figure 4. CFU Prevalence in Human Bone Marrow](image)

Figure 4. CFU Prevalence in Human Bone Marrow

Most CFU assays limit themselves to counting colonies formed after plating a known number of cells, and therefore only provide data related to the prevalence (CFUs per million cells plated) of CFUs in the original sample (Figure 4). However, even small changes in proliferation rate are associated with large and significant changes in the total number cells contributed by the colony progeny of a single stem cell, and it is widely recognized that individual stem and progenitor cells generate progeny with widely
different biological properties [40]. This is manifest in tissue culture by differences in colony size (proliferation rate and cell migration), cell density, morphology and the expression of differentiation markers. However, until recently, collection of colony specific data has traditionally been highly subjective, as well as cost, labor, and time intensive [41]. As a result, few investigators have attempted to characterize the diversity among CFUs or to utilize these data to explore differences in the biological functional potency between early hematopoietic stem cells and committed progenitor cell populations. To address this challenge, Cleveland Clinic (i.e. George F. Muschler, MD) developed quantitative automated methods for stem cell colony and cell specific analysis by combining high resolution 2D microscopy and large field-of-view (FOV) motorized image acquisition with object recognition, segmentation, and cluster-based image analysis software. This system is termed Colonyze™ [42].

2.2. Technology Platform

2.2.1. Colonyze™: Cellular Imaging and Analysis System

The Colonyze system comprises image acquisition hardware (automated high-throughput bright-field and fluorescence microscopy) and proprietary image processing and analysis software, in an adaptable, user-friendly interface. The platform converts large field-of-view (FOV) images of cells, colonies, and tissues on 2D surfaces or in 3D media into quantitative data detailing cell type and in vitro functional potency. The outputs define the concentration and prevalence of colony forming progenitors and characterize their heterogeneity within a sample. Statistical metrics include, but are not limited to: colony forming efficiency, cells per colony or per unit area (proliferation), cell density and
distribution (migration), object morphology, nuclear size, nuclear morphology, and area fraction of expression for a given cellular marker at the colony, cell, and nuclear level. The system also outputs a quantitative characterization of the proliferation, migration, differentiation, and survival of the progeny of individual tissue- or culture-derived progenitors destined for therapeutic use. Further, this technology has demonstrated a significant improvement in inter- and intra-observer variability and accuracy in bone marrow-derived CTP cell and colony enumeration [42]. Figures 5 and 6 below demonstrate the Colonyze analysis system applied to bone marrow and umbilical cord blood samples, respectively.

**Figure 5. Summarized Colonyze Automated CFU Analysis in Bone Marrow samples**
To accomplish this, a technician harvests cells from tissue (marrow, blood, bone, cartilage, etc.), and seeds them on a culture surface and cultures them for several days. Once the cells have proliferated and formed colonies, a single, high resolution image of the entire culture surface is generated (0.74 microns/pixel resolution) by stitching and montaging hundreds of raster-scanned image tiles. Image processing and analysis by the Colonyze software utilizes proprietary object recognition, segmentation, and clustering algorithms to provide a highly robust, reproducible, and accurate method for identification of individual colonies and determination of a wide range of colony- and cell-level metrics.
Although Colonyze was originally developed and validated for use in human bone marrow-derived CTPs, it has been tested and validated for colony and cell identification and characterization in several other cell types including (Figure 7; clockwise order):

- Connective Tissue Progenitors (CTPs)
- Cartilage stem cells
- Umbilical Cord Blood (UCB)-derived stem cells;
- Multi-potent Adult Progenitors Cells (MAPCs; Athersys, Inc.);
- Embryonic Stem Cell (ESC) embryoid bodies (not shown);
- Engineered Skin Substitute (ESS): epidermal fibroblasts and melanocytes; and
- Engineering Skin Substitute (ESS): dermal endothelial cells and keratinocytes.

Figure 7. Multiple Cell-types identified and Analyzed by Colonyze
The ability to customize the Colonyze system to specific analysis applications, and its automated, large field-of-view imaging, and high-throughput approach to cellular validation is unconventional and exceptionally innovative in nature. As a result, this technology will play a significant role in the Smart Cellector’s ability to reduce the cost, time, labor and lack of reproducibility associated with traditional manual methods of cell therapy manufacture.

2.2.2. **Smart Syringe™: Automated Pneumatic**

[REDACTED]

2.2.3. **AutoSampler™: Robotic Fluidic Handling System**

[REDACTED]

2.3. **Product Concept Description**

[REDACTED]

3. **Commercialization Plan**

Smart Cellector will be designed to characterize, isolate and expand cells in a quality controlled and automated fashion to reduce the time, labor, cost and end product variability associated with the manufacture of cells for use in cell-based Regenerative Medicine. [REDACTED] Since this Thesis is largely based on one of my Ohio 3rd Frontier Grants, much of the commercialization plan is described in terms of the benefits and impact on the State of Ohio.
3.1. Definition of the Need

Despite uniform recognition of the potential for cell-based therapeutics, there are still significant barriers to realizing their potential. Principal among them are relatively crude processing and manufacturing methods, characterized by:

- No accepted industry-wide standards for validating cell type and functional potency – Current standards are either lacking or highly variable
- Open bench top assessments that are vulnerable to contamination
- High inter-production run, inter-observer and inter-institutional variability
- Slow, and extremely costly, cell source & end-product validation – technicians currently spend hours on microscopes and in front of incubators manually assessing cell identity/function and processing cells of interest.
- Low production yields, and high production costs – Manufacturing and Quality Control currently comprise up to (and sometimes more than) 50% of the cost of goods sold for currently commercialized cell therapeutics
- Difficulties in establishing an appropriate product audit trail for regulators

Taken together, these issues contribute to a currently slow and unclear FDA and European Community regulatory pathway for cell therapies, and still present a significant barrier to commercialization [44, 45].

In order to address these needs and lower these barriers to entry, the cell-based therapeutics industry needs:
- A standard means of validating cell type and functional potency
- A more robust & reliable product audit trail for regulators
- A closed production process that is less vulnerable to contamination
- An automated production process that offers inter-run, inter-observer and inter-institutional consistency for cell source & end-product validation
- An ability to individually process multiple cell source samples in parallel
- A high-throughput process that can lower costs through economies of scale
- Lower manufacturing and processing costs

A manufacturing and production system that met these needs would provide the basis for an industry-wide standard, substantially reduce the current regulatory uncertainty, and increase the likelihood of profitable investment in this nascent industry [46-49].

3.2. Value Proposition

[REDACTED]
3.3. Cell-based Regenerative Medicine Market

3.3.1. Overview

Much of the promise and growth potential of cell-based Regenerative Medicine stems from its broad applicability in treating many serious diseases. Figure 11 summarizes many of these applications and diseases.

The cell therapy market contains these basic partitions: 1) basic and clinical research; 2) autologous products; and 3) allogeneic products. Cell therapy companies and researchers face many challenges when managing the journey from early discovery to clinical trials to commercial production. Apart from satisfying regulatory requirements and demonstrating that the therapy is efficacious and safe, commercializing such therapies also requires the therapy to be cost effective and scaleable. These requirements are present across the board, and are particularly challenging where the therapy is
manufactured in small batch sizes (e.g. specific to an individual patient such as autologous cell therapies). Traditional small volume cell processing is largely manual and only partially enclosed. In many cases the manual labor cost associated with manufacturing a cell therapy 67% of total cost [51]. Whilst arguably this approach is appropriate for basic research and early stage clinical trials, it introduces several significant commercialization challenges including:

- Long culture expansion periods to generate pure, homogeneous cell populations;
- Poor utilization of clean-room facilities;
- Dependence on high-cost, high-skilled labor;
- Processes are not readily scaleable or standardized;
- Regulatory approval of early stage clinical trials based on a manual process may not be readily transferable to late stage clinical trials or commercial scale production using a different process;
- Difficulties in satisfying quality requirements (cGMP) due to open processes and operator dependence; and
- The process may not be readily transferable across multiple sites.

As a consequence, the cost of these therapies is often very high and achieving the reimbursable cost of therapy is, for the most part, not obtainable. As a result, the cell-based regenerative medicine market is desperately in need of automated technologies to integrate and streamline the characterization, manufacture and quality control processes [45, 46, 51].
3.3.2. Market Size and Forecast

The cell therapy manufacturing market is very attractive from the perspective of potential customers, dollar market size, market growth, and future prospects. A recent review of commercial activity in the cell therapy market suggests approximately 344 products in varying stages of clinical development as shown in Figure 12 [47, 52].

<table>
<thead>
<tr>
<th>Phase of Development</th>
<th>Products</th>
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<tr>
<td>Pre-clinical</td>
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<td>Phase I</td>
<td>77</td>
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</tbody>
</table>

Figure 12. Cell Therapy Product Breakdown by R&D Phase (2008)

These products are either autologous treatments or allogeneic products. In the former, cells are removed from an individual patient; specific cells of interest are identified, isolated, cultured and expanded, and returned to the same patient for therapeutic benefit. In the latter, cells are removed, identified, isolated, cultured, expanded and administered to a patient other than the source for therapeutic benefit. Several other recent reviews of the cell therapy industry collectively estimate the 2008 global cell therapies product market at $4 billion, with the USA accounting for 60% of the 2008 market turnover. Here, the autologous cell therapy segment accounted for 37% share of the total 2008 market. The allogeneic cell therapy market accounted for 63% share of the 2008 market (Figure 13).
Figure 13. Forecasted Near-term Growth of Cell Therapy Market by Segment

Approximately 250 companies and institutions are estimated as engaged in the fully integrated manufacture and marketing of cell-based therapies around the world. Manufacturing and Quality Assurance/Quality Control (QA/QC) service providers are considered to be among the 700 or so stakeholder companies engaged in the industry. Due to the inefficiencies of current manufacturing methods, Manufacture (MFR) & QA/QC costs are currently estimated to be up to 50% of the total market. This is often the case for autologous cell products and small lot sizes. This segment is currently dominated (~80% market share) by a large contract manufacturing organization (CMO). Growth in this segment is rapid due to increased out-sourced manufacture of cell therapies in an attempt to reduce costs [47-49, 52, 53].

Forecasts of the development of the cell therapeutics market suggest a compound annual growth rate of 27 - 39% during the period 2005 - 2020 and forecasts by segment as
shown in Figure 13, above [47, 49, 53]. Thus, cell-based therapeutics as an industry, and the cell therapy manufacturing market as a specific business segment within this industry, represents a particularly attractive long-term target.

3.3.3. Market Drivers

Near-term (2011 – 2020) growth of this market is expected to be fueled by a number of factors, including:

- Increases in R&D funding;
- Technological innovations;
- Regulatory developments within some countries;
- Aging population escalates prevalence of diseases treatable by stem cells;
- Increased strategic alliances (academic and commercial);
- Applications in drug development and discovery;
- Ineffectiveness of traditional medications to treat certain illnesses; and
- High amount of unmet medical needs and an aging population [44, 52, 54-57].

3.3.4. Market Barriers

In spite of the large projected growth of the cell-based Regenerative Medicine market, there are several barriers which, if not addressed, could significantly stymie innovation and growth. These barriers include:

- Society is still elucidating and defining the mechanisms by which stem cells operate;
- No currently approved allogeneic cell therapy on the market;
- Regulatory and reimbursement pathway through FDA and CMS is largely undefined and untested;
- No accepted industry-wide standards for validating cell type and functional potency;
- Slow and extremely costly and labor-intensive cell source and end-product validation due to extensive use of non-automated processes;
- Low production yields, and high production costs makes scale-up either difficult, costly or both; and
- Difficulties in establishing an appropriate product audit trail to meet FDA safety and efficacy requirements [44, 48, 51, 52, 55, 56].

3.4. Competitive Analysis

<table>
<thead>
<tr>
<th>Company</th>
<th>Pre-Processing Modules</th>
<th>Imaging &amp; Analysis</th>
<th>Selection</th>
<th>Harvest</th>
<th>Expansion &amp; Scale-Up</th>
<th>GMP &amp; GTP Compliant</th>
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</table>

![Figure 14. Current Competitive Product Landscape](image)

As shown in Figure 14, no single currently available device platform in the market offers the same array of needed features as Smart Collector. Cyntellect’s LEAP® System represents the closest competitor to the Smart Collector platform. However, unlike Smart
Cellector, LEAP “purifies” desirable cells by employing laser ablation to destroy all the non-desirable cells and colonies in a zone of interest. This methodology leaves behind considerable cellular debris on the plate along with the “isolated” target cells, and, as such is considered less than optimal for manufacturing and production purposes. Alternatively Genetix’s ClonePixFL® uses sterile pins to “pick” (scrape) and transfer colonies, however this technology is not geared to the commercial market and is not validated for use with human stem cells. With the exception of the Cytori system, all products are currently marketed solely to the academic research sector because they are not fully-enclosed (sterile) and thus cannot meet GMP/GTP specifications. Only Cytori’s system meets the latter specification, but it lacks the planned functionality of Smart Cellctor.

[REDACTED]

3.5. Business Model

3.5.1. Which Model: Product Sales versus Fee-for-Service?

[REDACTED]

3.5.2. Customer Segments and “Readiness”
Within the three market segments, there are five distinct customer groups. As shown in Figure 15, each has slightly different requirements for cell therapy characterization and production, and their degree of readiness to purchase the Smart Collector platform will vary according to their needs. Each of the target customer segments is defined below.

**Segment #1: Academic research institutions; Tissue Procurement Banks**

This includes a group of some 180 academic research institutions worldwide--including Cleveland Clinic, CWRU, MIT, Rutgers, Wake Forest, UVA, and the Cleveland Cord Blood Center--with leadership positions within the cell therapy community. These institutions currently lack cost-effective methods for cell/colony enumeration (QA/QC testing), and this is an impediment to research progress. [REDACTED]

**Segment #2: Autologous Cell Therapy Companies**
This includes companies like Dendreon, BioHeart, TiGenix, Becton Dickenson, Genzyme, and Progenitor Cell Therapy, which are currently producing commercially available autologous cell products. Relative to profit margins for traditional pharmaceuticals and biotechnology products, these products have higher manufacturing costs and lower profitability due to labor, cost, time intensive production and QA/QC processes [48, 49, 51]. [REDACTED]

Segment #3: Allogeneic Cell Therapy Companies

This represents an emerging customer-base, and includes companies like Athersys, Osiris, Geron, Celgene, J&J, GSK, and Pfizer – each of which have allogeneic products moving through the development pathway. The demand for a technology like Smart Collector in this customer segment is significant insofar as they are exposed to the same scale-up, QA/QC and production cost challenges experienced by companies with autologous products in the market. [REDACTED]

Segment #4: Contract Manufacturing Organizations

This particular customer base is currently small, currently consisting of companies like Lonza – which holds the dominant position in this marketplace – Covance and Progenitor Cell Therapy. Outsourcing of manufacturing is common throughout the pharmaceutical industry, however, and the cell-based therapeutic sector is expected to follow suit. [REDACTED]
Segment #5: Large Hospitals and Advanced Out-Patient Treatment Centers

This customer group includes leading clinical centers like the Cleveland Clinic, Mayo Clinic, Mass General, etc. As cell-based therapies progress into the physicians’ armamentarium over the next decade, its considered likely that they will progress from R&D programs to commercialized products and, ultimately, will yield personalized medicine applications at the point of care. [REDACTED] Mayo Clinic CEO Dr. John Noseworthy stated that regenerative medicine is a “top priority” for his institution, and one of four of its “major institutional directions.” He also commented on how individualized medicine, which allows a patient to be treated with their own cells, has profound potential. Dr. Noseworthy concluded his interview by stating, "Oh, it's [Regenerative Medicine] breathtaking. This is really big [58].”

[REDACTED]

3.5.3. Intellectual Property

[REDACTED]

3.5.4. Regulatory and Reimbursement Strategy

Smart Cellector is a cell therapy manufacturing platform and not a medical device, drug or biologic which will have direct contact with a patient. As such, there is no defined or required regulatory or reimbursement pathway that this product must follow. That said, it is important that Smart Cellector be developed in such a way as to help cell therapy manufacturers meet the requirements defined in their products’ Drug Master Files
(DMF). A Drug Master File is a non-mandatory submission to the Food and Drug Administration (FDA) that may provide confidential and detailed information regarding the facilities and processes (e.g. manufacturing, processing, packaging, storage, etc.) associated with a particular drug or biologic [59]. [REDACTED]

3.5.5. Pathway to Market and Success

[REDACTED]

These companies, and others that will follow, represent potential management and skilled workforce for the Smart Collector project as well as a potential market for its products. Moreover, Smart Collector will create a need for trained cell-base therapeutic products manufacturing beyond what currently exists in Ohio. To support this need, CSCRM and Case Western Reserve University offer four courses, an undergraduate Summer Program and a Business Seminar-- all specific to cell-based therapeutic research development and production. Through the involvement of the Cleveland Clinic, CTEC, etc. students in these classes will have the opportunity to intern on this Wright Projects grant, and work side-by-side with skilled clinicians, researchers, engineers and designers. This continuous supply of employees will be yet another reason that Smart Collector will lead to Ohio being recognized as the pre-eminent cell-base therapeutics production center of excellence.

3.5.6. New Venture Financial Forecast

Assumptions and Sales Forecast
Project Risks

As with any product development program there exist various technical and market-based risks. These are outlined below. Many, if not all of these, will be addressed during prototype research and development ("Prototype Road Map", next section). Technical risks associated with developing the Smart Collector technology include, but are not limited to:

[REDACTED]

Investment Comparables

[REDACTED]

4. Product Development Timeline (Prototype Road Map)

[REDACTED]

4.1. Proof-of-Concept 1 (PoC-1)

Action Items (3 months)

[REDACTED]

Milestone 1

[REDACTED]
4.2. Proof-of-Concept 2 (PoC-2)

Action Items (4 months)

[REDACTED]

Milestone 2

[REDACTED]

4.3. Proof-of-Concept 3 (PoC-3)

[REDACTED]

Action Items (5 months)

[REDACTED]

Milestone 3

[REDACTED]
Methods

[REDACTED]

4.4. Proof-of-Concept 4 (PoC-4) and Beyond

[REDACTED]
5. References


51. Hoover, B., *Interview with Steven T. Boyce, Ph.D.* Oct. 10, 2010 at 12:30pm: Cincinnati, OH.


