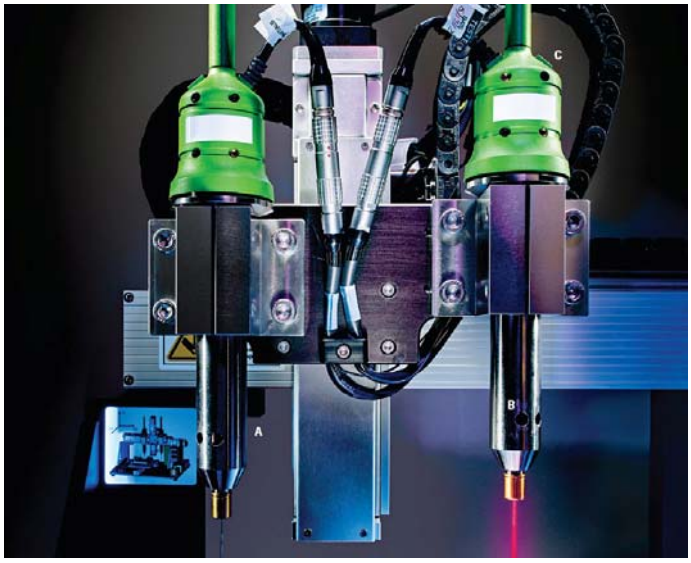

BIOPRINTING

Organs on Demand

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ASME SETTING THE STANDARD

WISE Washington Internships for Students of Engineering

UK UNIVERSITY OF KENTUCKY

EXECUTIVE SUMMARY

The amalgam of technological advancements in biology, chemistry, genetic engineering, mechanical engineering, micro-fluidics, medicine, computer programming, and other fields has resulted in a promising technology known as bioprinting. This technology has already redefined the limits of tissue engineering and regenerative medicine, and it has streamlined the drug-screening process during investigational new drugs trials for the FDA. (1)

Bioprinting technology has already been employed to administer an increasing quantity of stem cell-based therapies in the operating room. In addition, there are a growing number of bioprinted organs and tissue replacement therapies in-use today, including: skin grafts, wind pipes, bones, bladders, veins, and arteries. (2) Recently, a team in China reported that it successfully grew a kidney that lived for 4 months. (3)

In the future, bioprinting could eventually eliminate the need for organ donations altogether. (4) In addition, the technology could manufacture micro-organs that cure – among other diseases – Type 1 Diabetes. (5) Many years from now, bioprinted products could even be implemented to battle aging and senescence. (6) Understanding how certain therapies affect the body is obviously important, but implementing functional therapies that have been proven to work safely is far more beneficial to the public as a whole.

From a clinical perspective, what matters is not so much whether the active instrument in implanted tissue consists of stem cells, proteins, or genes, but whether that instrument is therapeutically effective. Bridging the gap between advancements made in the laboratory and the creation of safe and effective therapies may prove to be difficult. (7) Developing these technologies into products may require many years of work, and developing the manufacturing facilities to produce them is likely to require considerable capital investments.

Without federal intervention, bioprinted products may never reach the marketplace. From a policy perspective, there are a number of challenges to overcome, and they are not just limited to a lack of funding or safety protocols. This technology needs direction, solid leadership, and a competitive strategy for competing on a global scale. Additionally, the creation of educational programs and resources will be required to inform the general public about the benefits and limitations of this technology.

The purpose of this paper is to inform the reader about the current state of bioprinting technology. It provides a brief explanation of the materials and equipment platforms which make printed organs possible, and also touches on the relevant public policies already in place. It will address many of the strengths, weaknesses, opportunities and threats pertaining to bioprinting. Finally, this paper will attempt to make recommendations to the federal institutions capable of ensuring that this technology develops swiftly and safely.

PREFACE

ABOUT THE AUTHOR

James Gwinn is a rising senior at the University of Kentucky – Paducah Campus, where he will graduate with dual-degrees in Economics and Mechanical Engineering. He is an award-winning jewelry designer, and is certified as a gemologist through the Gemological Institute of America. James is active in the design/build/fly competition hosted by the American Institute of Aeronautics and Astronautics (AIAA). Outside of these pursuits, he enjoys snow-skiing, SCUBA diving, woodworking, and has competed in multiple marathons.

ABOUT THE WISE PROGRAM

Founded in 1980 through the collaborative efforts of several professional engineering societies, the Washington Internships for Students of Engineering (WISE) has become one of the premier Washington internship programs. The WISE goal is to prepare future leaders of the engineering profession in the United States who are aware of, and who can contribute to, the increasingly important issues at the intersection of science, technology, and public policy. (Description courtesy of the WISE program website; for more information, visit http://www.wise-intern.org/about.html)

ABOUT ASME

ASME helps the global engineering community develop solutions to real world challenges. Founded in 1880 as the American Society of Mechanical Engineers, ASME is a not-for-profit professional organization that enables collaboration, knowledge sharing and skill development across all engineering disciplines, while promoting the vital role of the engineer in society. ASME codes and standards, publications, conferences, continuing education and professional development programs provide a foundation for advancing technical knowledge and a safer world. ASME's mission is to serve diverse global communities by advancing, disseminating and applying engineering knowledge for improving the quality of life; and communicating the excitement of engineering*.

ACKNOWLEDGEMENTS

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* (Description courtesy of the ASME website; for more information, visit https://www.asme.org/about-asme/who-we-are/mission-vision-and-strategic-focus)

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INTRODUCTION

WHAT IS THE PROBLEM?

Breakthroughs in bioprinting are being made regularly, but there is currently no clearly defined regulatory framework in place to ensure the safety of these products. (8) Many of the best and brightest minds in the world are working to bring bioprinted products to the marketplace; however, the potential and functional limitations of bioprinting are not yet fully understood.

The technology, as a whole, is so new that public policy has not had the opportunity to catch up to the current state of the industry. (9) Products made via bioprinting technology span a number of product review divisions within the FDA due to the wide range of potential applications. (10) Additionally, FDA regulations for biosimilar biologics[†] do not yet address biosimilarity between human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPS cells).

The FDA evaluates all devices, including any that utilize 3-D printing technology, for safety and effectiveness, and appropriate benefit and risk determination, regardless of the manufacturing technologies used. In the US, a number of regulatory and legislative hurdles must be cleared before the first lab-printed kidney, liver, or heart implant will make it to market. As it is with all biologics, the critical regulatory challenges with bioprinted organs will revolve around demonstrating the safety of the final product and establishing consistent manufacturing methods. (11) There are also a number of technological advancements: software needs refinement; advances in regenerative medicine must be made; more sophisticated printers must be developed; and thorough testing of the products must be conducted. (12)

To ensure that bioprinted products reach the marketplace in a safe and timely manner, an effective game plan will need to be enacted. (13) This plan would include clearly identified goals, well-established short- and long-term expectations, and the creation of models and actions for linking investments to outputs. Additionally, the plan ought to clearly identify roles and responsibilities, milestones and metrics, and reasonable time frames.

WHO CARES?

Several constituencies will be directly impacted by the advancement and implementation of this technology, including:

Pharmaceutical Users: In the U.S., the FDA must deem a drug safe and effective before approving it for public use. Developing a new drug can be an expensive gamble, too. According to the Pharmaceutical Research and Manufacturers of America (PhRMA), roughly 250 out of every 10,000 different molecules make it past the initial screening process and into clinical trials. Of those 250 candidate drugs, only five might be suitable for testing in people. Only one of those five drugs may end up on the pharmacy shelf. (14) In the end, a company may use just 1 or 2 out of every 10,000 molecules they develop.

[†] **BIOLOGICS** are medications that have been created by a biological process. For more information, refer to the Appendix

Bioprinting can produce organ models which provide an opportunity to improve time-to-market for new drugs, decrease costs associated with drug development, and improve the safety of test subjects. Drugs account for around 10 percent of the country's $2.7 trillion annual health bill (14). A significant portion of this can be attributed to costs associated with clinical trials. Bioprinted organs could potentially help bring down the high cost of medicine. (1)

Organ Transplant Recipients: On average, 18 people die in the United States each day waiting in vain for organ transplants. There are currently over 134,000 individuals on the organ donor list; enough to fill two professional football stadiums. The majority of these people will die without an organ replacement. (15) For the first time in history, a potential cure for each and every one of these individuals looms ahead. This cure is made possible only through bioprinting technology. (16)

Type 1 Diabetics: The Centers for Disease Control estimates that 29.1 million US citizens (9.3% of the population) have diabetes. (17) A 2007 study estimated that direct medical costs for diabetics amounted to $116 billion. A bioprinted micro-organ – similar to a pancreas and engineered to produce the hormone insulin inside the body (in vivo) – could eliminate the need for insulin supplementation within the diabetic community altogether.

BACKGROUND

If a picture is worth a thousand words, a 3D object is worth a thousand pictures. Additive Manufacturing **(AM)** is a generalized term which encompasses a number of advanced manufacturing technologies. The unifying characteristic is that objects produced via AM are 'grown,' layer by layer, often from the bottom up. The technology was invented in 1983 by Chuck Hull. (18) Other names for this technology include: 3D printing, rapid prototyping **(RP)**, and more recently, bioprinting.

Bioprinting produces three-dimensional **(3D)** objects or parts from living tissue that are highly precise in shape and mechanical complexity. Using a computer-assisted design **(CAD)** program and computer-assisted manufacturing **(CAM)** blueprints, a bioprinter can deposit ultra-thin layers of living cells on top of each other. (19) The printer head follows a precise path that matches the desired shape of the intended product. Layer by layer, the organ is 'grown' vertically as its layers accumulate. Over a period of hours the final tissue construct is completed.

So far, the only way to accurately reproduce the architectural, physiological, and material properties of living tissue is through 3D bioprinting. (6) Bioprinters and advanced CAD/CAM software make it possible to design myriad biological tissues from scratch[‡]. These machines operate depositing droplets *(spheroids)* of biomaterials. (9)

The range of biomedical applications for AM is quite broad, but the holy grail of this technology is *de novo* organ printing. **DE NOVO** is a Latin phrase meaning *"from the new."* The creation of *de novo* organs requires contributions from a number of fields, including: biology, chemistry, genetic engineering, mechanical engineering, micro-fluidics, medicine, and computer programming. (12) Several of these disciplines are already capable of providing the accuracy and precision to create *de novo* organs. There are commercially available machines capable of depositing biomaterials accurately and precisely, and there are hydrogels that accurately mimic the strength

[‡] **For more information on the software used in the CAD/CAM process, see "Software" in the Appendix.**

and structure of natural human tissue.

BIOMATERIALS

Biomaterials are the fundamental building blocks of *de novo* organ fabrication. They consist of two components: *hydrogels* and a unique type of *patient-specific stem cells.* (20) These patient-specific cells have been reprogrammed and are used to produce different types of tissue. The hydrogels provide structural reinforcement to the reprogrammed cells until those cells have joined together. Then, the hydrogels dissolve within the human body. When they leave, only the patient's reprogrammed cells remain in place. (21)

HYDROGELS

Organ transplantation is not a new endeavor. Currently, the outcome depends largely on donor compatibility, and organ recipients are generally required to take immunosuppressant medications for the remainder of their lives.

In an effort to eliminate issues such as tissue rejection, one innovative method for manufacturing organs using stem cells has been attempted. The process is called *decellularization*, and it involves the use of cadaveric scaffolds. **SCAFFOLDS** are made up of collagen (and occasionally cartilage) because neither of these materials is rejected by the immune system. (22) Simply explained, scaffolds can be thought of as sponges which support stem cells while they merge together.

DECELLULARIZATION works like this: organs are harvested from cadavers and submerged in a chemical bath. In this bath, chemicals are used to strip away all of the organic material except for the scaffold. The scaffold is then rinsed clean of chemicals, sterilized, and placed into a bioreactor filled with a patient's stem cells. (23)

BIOREACTORS closely mimic the environment within the human body. Temperature, oxygenation, and nutrients required for organ generation are all present in quantities similar to those found in the human body. After a period of incubation, the stem cells merge together and form a simple organ. It should be noted that the human body is the best bioreactor for human tissue. The machines and environments designed by tissue engineers are intended to mimic the in vivo environment, but there exists an information gap which has yet to be overcome. It is likely that *de novo* organs will form more quickly inside the human body. (24)

Researchers have successfully implanted simple organs manufactured by this method since 2006, when Dr. Anthony Atala, a leader in the field of regenerative medicine, implanted an artificially-created bladder. The patient recently graduated from college and has had no adverse effects from the surgery. (4) With this method, the complications associated with tissue rejection have been largely reduced. However, the complexity of the organs being created is still limited to simple structures. In addition, these procedures still rely on organ donations.

The primary limitation to decellularized organs is their relative lack of vascularization. (6) **VASCULARIZATION** is the formation of blood vessels. In order for cells to become organs, they need four things: the proper environment, oxygen, nutrients, and waste removal. (6) Vascularization allows the last 3 of these requirements to happen. Making something complex (liver, pancreas, heart) cannot be accomplished using decellularized scaffolds because they do not allow for vascularization. (25)

Fortunately, this limitation has been overcome through the use of hydrogels. **HYDROGELS** have proven to be a versatile and indispensable medium for tissue engineering; both as a host for stem cells and as a building block

used in scaffold construction. Hydrogels are primarily water (75% to 90%). The other portion is made from a three-dimensional internal network which is employed to hold the water in place via surface tension effects. (26)

Hydrogels, stem cells, and bioprinters have made it possible to provide structural stability to stem cells while they form, while also providing channels that allow vascularization to the organ. These channels are literally printed into the scaffold. (12)

Hydrogels are essentially highly absorbent sponges; they are networks of synthetic or natural polymers similar to human tissue in both density and flexibility. Soft contact lenses, wound gels, and even the active ingredient in disposable diapers are all made from hydrogels. For a hydrogel to be worthy of consideration for use in bioprinted tissue, it needs to be *biodegradable*, *biocompatible*, and *bioresorbable.* (21) Other desirable qualities of hydrogels include: low cost, cross-linking capability, and a broad range of viscosities at room temperature. (20) Viscosity is important because hydrogels have tight requirements with regard to gelling speed (which is required for accurate printing).

STEM CELLS

De novo organs rely on a specific type of cell to function: the *stem cell*. Stem cells are uniquely capable of developing into many different cell types in the body during life and growth. All stem cells—regardless of their source—have three general properties: they are capable of dividing and renewing themselves; they are unspecialized; and under certain conditions, they can become tissue- or organ-specific cells with specialized functions. (27)

When stem cells divide, each new cell has the potential to remain a stem cell or it serve a different purpose, like a muscle cell, brain cell, or blood cell. **DIFFERENTIATION** refers to the ability of stem cells to change into different types of cells, and the number of differentiated states they can achieve is called potency. A stem cell is **PLURIPOTENT** when it can be made to differentiate into any type of cell in the body.

HUMAN EMBRYONIC STEM CELLS (hESCs) remain the gold standard for pluripotency and replicative potential, but the creation of patient-specific *(autologous)* hESC lines is not feasible. (28) In order to obtain patient-specific hESCs, the cells would have to be taken from the patient's embryo. As a result, cell replacement therapies utilizing hESCs are limited to applications in which the cells are taken from an individual other than the patient *(allogeneic)*.

When organs created from allogeneic tissues are implanted into patients, the body perceives the tissue as foreign, and initiates a response in the patient's immune system. The patient's immune system attacks the organ, and will kill it unless the patient regularly takes medications to suppress the immune system.

In 2012, the Nobel Prize was awarded to two scientists, Shinya Yamanaka and John Gurdon, who discovered that mature, specialized cells could be *reprogrammed*[§] to become immature cells. These reprogrammed cells are capable of developing into all tissues of the body. Their findings revolutionized our understanding of how cells and organisms develop. (29)

In 2006, Shinya Yamanaka discovered that, by introducing only a few genes, he could reprogram mature cells to

[§] **For more information about reprogramming, see section R in the Appendix.**

behave like hESCs. In other words, he induced pluripotency in cells. The result would come to be known as *induced pluripotent stem cells* **(iPS CELLS)**. iPS cells are somatic *("of the body")* cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. (Definition courtesy of NIH)

According to NIH, tissues derived from iPS cells will be a nearly identical match to the cell donor and thus avoid rejection by the immune system. The iPS cell strategy creates pluripotent stem cells that, together with studies of other stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body. (27) An added benefit is that they may reduce many of the ethical concerns normally associated with hESCs. The cells are collected directly from the patient; there are no fetuses involved.

BIOPRINTERS

Bioprinters offer a number of competitive advantages over traditional organ generation techniques (i.e. decellularization, transplantation, etc.). First, organs created via bioprinting do not require a donor. Second, bioprinters can use the patient's own cells to create an organ, reducing or eliminating the possibility of organ rejection. Third, bioprinters can build veins and arteries directly into the organ.

Bioprinters are capable of precisely controlling the spatial placement of the fundamental building blocks required to manufacture *de novo* organs, including: cells, genes, proteins, and growth factors.** This process deposits living cells together with hydrogel-base scaffolds for 3D tissue and organ fabrication. (12) In other words, these machines can deposit human cells in multiple dimensions. Bioprinting currently uses bioadditive manufacturing technologies, including laser based writing, (30) inkjet printing (31), and extrusion-based deposition. (32)

These machines are capable of maintaining a sterile environment, and they are able to gently and swiftly deposit cells and hydrogels in a fast, efficient manner. The first completely artificial organ, a trachea, was grown and implanted into a man with throat cancer in 2011. The cancer was progressing too rapidly for the patient to wait for an organ donor, so a trachea was 'grown' using a bioprinter, incubated while the cells merged together, and surgically implanted into the patient. So far, the patient has experienced no adverse reactions to this bioprinted tissue. (33)

One common question is: what do the cells do after they are printed? Amazingly enough, it seems that stem cells already know what to do. Anthony Atala said, "In much of our work, we have learned that 'nature knows best,' and this is certainly true with decellularized organs — they are ideal to support cells as they multiply and as tissue develops."

Together, iPS cells and hydrogels are capable of restoring damaged organs in the operating room. When used in tandem with bioprinting, these tools may eventually be used to grow complex organs *de novo*. One trend in organ printing is in situ printing, where living organs can be printed directly into the human body during operations. Currently, in-situ bioprinting has already been tested for repairing external organs such as skin [Atala].

** **For more information about growth factors, see Section G in the Appendix**

POLICY ASPECTS

CURRENT F.D.A. PRODUCT CATEGORY DESIGNATIONS

Bioprinting is relatively new, and therefore, the FDA does not have just one product category for bioprinted products. Due to their composition, the majority of bioprinted products will initially be regulated as either devices or biologics. Right now, products derived from stem cells are treated by the FDA as *somatic cellular therapies*. These are regulated as '*biologics'* under SECTION 351 of the PUBLIC HEALTH ACT. **BIOLOGICS** are medications that have been created by a biological process.

As cellular therapies, they are also subject to FDA guidelines for the manufacture of human cells, tissues, and cellular- and tissue-based products found in PART 1271 of the same act. Notably, Part 1271 establishes the requirements for donor eligibility procedures not found in the **CURRENT GOOD MANUFACTURING PRACTICES (cGMPs)** guidelines of PARTS 210 AND 211. These guidelines regulate the way stem cells are harvested, handled, and labelled.

Finally, stem cell based therapies are also defined as **INVESTIGATIONAL NEW DRUGS (INDs)**, and they are subject to TITLE 21 OF THE CODE OF FEDERAL REGULATIONS PART 312 (IND application), and applicable sections of PARTS 210 AND 211 cGMPs.

However, there are exceptions[††]. Bioprinted tissues used in research and education require no FDA approval during animal and *in vitro* testing. (1) **IN VITRO** is Latin for *"in glass"*, and refers to organic products that remain outside of the body. Organovo, a company in California, has developed a range of tissue and disease models for medical research and therapeutic applications which relies heavily on bioprinting technology. (1) These products help identify potential toxicity and efficacy issues before drugs enter clinical studies. They do not require FDA approval because they are not intended for use on humans. However, in TITLE 21 OF THE FEDERAL CODE OF REGULATIONS; PART 312; SUBCHAPTER D; SUBPART G, certain restrictions are defined with regard to shipping and disposal of these products.

Additionally, at the moment, FDA regulations insist that companies sponsoring an IND generate a sufficient amount of data to support clinical testing. They must prove that the drug is worthy of entering into clinical trials. In accordance with the BIOLOGICS PRICE COMPETITION AND INNOVATION (BCPI) Act, a **BIOSIMILARITY PATHWAY** exists which provides a way of streamlining this process. (34)

OMNIBUS APPROPRIATIONS ACT OF 2009

On March 9, 2009, President Obama removed President George W. Bush's restriction on federal funding for newer stem cell lines. Two days later, however, President Obama signed the OMNIBUS APPROPRIATIONS ACT OF 2009, which still contained the long-standing DICKEY-WICKER provision banning federal funding of "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death." This provision prevented federal funding from being used to create new stem cell lines by many of the known methods at that time.

Although scientists and engineers may not be free to create new stem cell lines through federal funding, President

[††] **The current FDA product category designations for all potential applications of bioprinting is available in the Appendix.**

Obama's policy allows funding into research using existing stem cell lines (there are hundreds). Additionally, federal funding can be provided to any other stem cell lines that have been created using private funds or state-level funding.

This ability to apply for federal funding for privately created stem cell lines is an expansion of the options allowed by President Bush. His executive order limited federal funding to the 21 viable stem cell lines which had been created at that time (2001).

Even today, the ethical concerns raised during the 1990s continue to restrict stem cell research. Dozens of stem cell lines have been excluded from funding due to ethical considerations raised from the method in which the cells are harvested.

AFFORDABLE CARE ACT

THE PATIENT PROTECTION AND AFFORDABLE CARE ACT (AFFORDABLE CARE ACT), signed into law by President Obama on March 23, 2010, amends the PUBLIC HEALTH SERVICE ACT (PHS ACT) to create an abbreviated licensure pathway for biological products that are demonstrated to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product. This pathway is provided in the part of the law known as the BPCI ACT. Under the BPCI ACT, a biological product may be demonstrated to be "biosimilar" if data show that, among other things, the product is "highly similar" to an already-approved biological product. (10)

Biologics are complicated drugs, and they require significant capital investment to develop and test. The development process for these drugs is very important because a substantial amount of a biologics' effectiveness is determined by how it is made. Once a biologic receives FDA approval, the developing company is issued a 12 year monopoly on that drug. This monopoly serves as an incentive for pharmaceutical companies to pursue new drug development.

After that 12 year period concludes, competing drugs that are biologically similar to the original drug may seek FDA approval. As long as these competing drugs can prove that they are biologically similar to the original, they should receive a streamlined approval process. In late July of 2014, the FDA accepted the first-ever application for a biosimilar candidate, but this application has nothing to do with stem cells.

Essentially, once hESC-based therapies pass clinical trials, the BCPI ACT would limit the scope and thoroughness of the trials to which iPS cells would be subject. Since iPS cells are similar to hESCs, there is a good chance that they will be subject to a majority of the regulations applicable to hESCs. Due to the additional processing required to create iPS cells, however, there is also a probability they will face novel regulatory concerns of their own.

The BPCI Act was created to relieve some of the costs associated with taking a new biological drug to market. The upfront costs of iPS cells will likely dwarf those of hESCs. Induced stem cells require more processing, more R&D, and are generally patient-specific (although not necessarily so). One possible approach through which iPS cells might circumvent the high costs of clinical trials is through the biosimilarity pathway for biologics.

Significant regulatory hurdles already exist for hESC therapies, but it is likely that the FDA will need to be even stricter with iPS cell therapies. This is because the additional steps involved in the reprogramming of iPS cells results in a predicted instability. It is well-documented that non-induced pluripotent stem cells (hESCs) accumulate mutations over time in culture – a number of which are cancer-related. (35) Since iPS cells are pluripotent and potentially unstable, the primary concern is an even greater risk of the generation of tumors and cancer.

REVITALIZE AMERICAN MANUFACTURING AND INNOVATION (RAMI) ACT

As noted in the National Academies' report, "Rising Above the Gathering Storm," the U.S. is losing its scientific and engineering competitiveness related to world competitors like China and India. Bioprinting is a cutting edge field, and the U.S. should be a leader. Given its manufacturing roots, bioprinting could be a part of President Obama's NATIONAL NETWORK FOR MANUFACTURING INNOVATION (NNMI).

In his 2013 and 2014 State of the Union Addresses, President Obama called for creating a full-fledged nationwide network devoted to innovating and scaling up advanced manufacturing technologies and processes. He has asked Congress to authorize a one-time $1 billion million investment—to be matched by private and other non-federal funds-to create an initial network of up to 15 INSTITUTES FOR MANUFACTURING INNOVATION (IMIs). Over the span of 10 years, he has proposed building out NNMI to encompass 45 IMIs. (Description courtesy of NNMI website.[‡‡])

This federal investment serves to create an effective manufacturing research infrastructure for U.S. industry and academia to solve industry-relevant problems. In the future, the NNMI will consist of linked IMIs with common goals, but unique concentrations (one of which could be bioprinting). In an IMI, industry, academia, and government partners leverage existing resources, collaborate, and co-invest to nurture manufacturing innovation and accelerate commercialization.

As sustainable manufacturing innovation hubs, IMIs will create, showcase, and deploy new capabilities, new products, and new processes that can impact commercial production. They will build workforce skills at all levels and enhance manufacturing capabilities in companies large and small. Institutes will draw together the best talents and capabilities from all the partners to build the proving grounds where innovations flourish and to help advance American domestic manufacturing.

The first topical focus of a manufacturing initiative was additive manufacturing. While bioprinting could fall in this category, this emerging field might merit an institute of its own. Currently, the NNMI is an Administration initiative that is funded by allowable funds. This funding exists for a limited time and will run out at the end of the current administration.

For this initiative to be continued post-Obama Administration, Congress would have to pass a law to authorize it. Recently, the HOUSE SCIENCE, SPACE AND TECHNOLOGY (SS&T) COMMITTEE marked up a manager's amendment to H.R. 2996, THE REVITALIZE AMERICAN MANUFACTURING AND INNOVATION ACT OF 2013[§§]. The amendment was approved by a unanimous voice vote. The legislation would establish a network of manufacturing innovation institutes with an authorized $600 million budget, focusing on commercialization of innovative advanced manufacturing technologies.

Under the amendment, for fiscal years 2015 through 2024, the institutes would be funded through the Department of Energy's advanced manufacturing research and development budget (for a total of no more than $250 million) and through the NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY (NIST)'s industrial

[‡‡] **For more information, see http://manufacturing.gov/nnmi.html)**

[§§] **For more information, H.R. 2996 may be read at: http://science.house.gov/sites/republicans.science.house.gov/files/documents/HR%202996.pdf**

technical services account (at $5 million per year throughout the period). The bill would also authorize an additional $10 million for fiscal years 2015 through 2019 to fund regional innovation strategies and clusters.

The companion bill in the Senate, S. 1468, was approved by the SENATE COMMERCE, TRANSPORTATION AND SCIENCE COMMITTEE in April 2013. However, during the Senate Committee's markup, the authorization amount was lowered to $300 million. Once H.R. 2996 and S. 1468 are approved, the authorizers will need to work with appropriators to secure program appropriations.

KEY CONFLICTS AND CONCERNS

Bioprinting could potentially produce any number of products, many of which may require regulatory considerations. Researchers at Gartner suggested that bioprinting facilities will advance human organ- and tissue-printing technologies faster than the public will be able to understand or accept them. (36) The study suggests that the availability of bioprinted organs and tissue will spark contentious political, ethical, and financial debate.

Presently, scientists and engineers have not yet been able to predict what long-term effects the implantation of reprogrammed cells may have on patients. Until long-term studies have been performed, the release of unproven stem cell therapies outside a carefully regulated clinical trial puts individual patients at risk. Furthermore, it may jeopardize progress in translational stem cell research. (37)

Demonstrating safety of the final cell product in a patient-specific setting will be the single greatest obstacle to advancing patient-specific iPS cell-based therapies to clinical trials. From a regulatory perspective, what is required is careful characterization of the cell lines at the time they are banked and at the end of the manufacturing process. (9) Essentially, the safety of the differentiated final cell product must be assessed.

To be considered safe, any bioprinted product ought to meet certain safety criteria for *purity, identity, and stability*. **PURITY** refers to the completeness with which iPS cells are reprogrammed.[***] The development of tests which identify poorly-reprogrammed cells will be important for establishing reproducible manufacturing processes. **IDENTITY** refers to the completeness of reprogramming throughout an entire cell population.[†††] Identifying the concentration of active cells for each specific iPS cell population will be a necessary step in bringing iPS cell-based therapies to clinical trials. **STABILITY** refers to the long term viability and efficacy of therapies and *de novo* organs.[‡‡‡] Human cells that have been harvested, turned into stem cells, and differentiated at some later point in time may lack stability. It is unclear what effects reprogramming will have on cellular stability. (8)

There are a few reasons to avoid reproducing the precise architecture of a failed organ. First, an identical anatomic replica of a failed organ may reproduce, identically, the flaw(s) responsible for that failure. Second, mapping and recreating a patient-specific virtual organ would be cost prohibitive, even with significant advances in medical imaging technology. Finally, the additional time may be required to accomplish a virtual reconstruction, which could easily result in the death of the patient waiting for the transplant. (6)

There are several differences between hESCs and iPS cells, due to the reprogramming process. The processes used to induce pluripotency present additional risks to patients that must be taken into consideration – at least until

[***] **Additional information about purity in the Appendix**

[†††] **Additional information about identity in the Appendix**

[‡‡‡] **Additional information about stability in the Appendix**

iPS cell-based therapies have been proven to be safe and effective methods of treatment.[§§§] Different iPS cells will likely demonstrate varying degrees of variability; therefore, it may be important to test each line individually. In short, additional research is necessary to determine the viability and efficacy of iPS cells. (38)

Biological properties of living cells can be difficult to assess and control. (39) Even mechanical properties of devices produced using inorganic 3D printing can vary significantly from those of conventional wrought or cast devices. It is unknown what effect the bioprinting process will have on the structural integrity of manufactured organs. (40)

POLICY ALTERNATIVES

The feasibility of creating bioprinted organs is no longer in question; several simple organs have already been manufactured using this technology. (24) It will likely be a decade or more before complex organs are commercially available, (20) but simple organs have already proven to be viable alternatives to traditional methods of organ replacement.

Ultimately, the fate of commercially-available bioprinted organs in the US will be determined by the American public with Congress and the Presidential Administration playing a large role. The fate of bioprinted organs may ultimately have nothing to do with the risks they pose, their long-term viability, or their ability to function adequately. (8) Political, ethical, and financial constituencies may also have significant influence over future laws relating to bioprinted organs.

Regardless, there is a lack of public policy issues applicable to bioprinting. (41) Before its products become commercially viable, a number of regulatory and legislative considerations may need to be addressed. Maintaining the status quo could eventually create a quasi-regulatory framework via the free-market economy. Without regulation, however, bioprinted organs and therapies could become commercially available before their risks are adequately quantified and mitigated. Alternatively, mandatory and stringent tests for purity, identity and stability may restrict the availability of bioprinted products. With over-regulation, bioprinted organs might never see commercial application. More likely, a balance will be found between the two options.

Whom should we select to determine standards for tissue quality, patient safety, and organ stability? The free market economy is one economic alternative, but it may present unacceptable risks to patients. Effectively, the free-market could allow companies to sell bioprinted organs before they are safe for consumption. The potential problem here is that recipients of dysfunctional organs may not survive long enough to seek restitution. On the other hand, 100% safe and functional bioprinted organs are virtually impossible to manufacture due to the aforementioned limitations. (42)

Who will be allowed to manufacture these organs? Bioprinting is clearly an advanced manufacturing technology, and those capable of producing bioprinted organs are likely to be few and far between. (42) However, just because an entity has the capacity to manufacture these products does not mean it should be allowed to. An established set of standards and certifications may the only way to ensure that those who manufacture bioprinted organs do so safely and responsibly.

The nature of bioprinted products is such that any errors made during the manufacturing process may have potentially fatal effects on those for whom they are intended. (43) To ensure that these products are manufactured safely and responsibly, technicians and operators will need education and training. Initiating

[§§§] **For an explanation about how reprogramming may affect a cell, refer to the Appendix.**

certification programs could ensure that this advanced training takes place. Additionally, further research and development may facilitate the education of these operators and provide them with valuable experience which could be carried into the marketplace.

Could there be unethical or inappropriate applications for bioprinted organs? It is possible that technology might eventually become capable of creating functionally superior organs. If and when this time comes, who will be allowed to receive these super organs? In an economy devoid of regulation, the obvious answer is: whoever can afford them. In a heavily regulated market, there may be significant restrictions with regard to form and function, among other things. Additionally, with the availability of printers and cybertheft of software and data, there is the possibility of a black market being created for bioprinted organs.

RESEARCH & DEVELOPMENT

By providing funding for research and development in STEM fields, the NATIONAL INSTITUTES OF HEALTH (NIH) has contributed billions of dollars to the indirect development of bioprinting. These contributions have provided tissue engineers with opportunities to advance stem cell research, regenerative medicine, and hydrogels. Thus far, it has been difficult for this researcher to quantify direct NIH contributions to the production and study of *de novo* organs. (27)

The NATIONAL SCIENCE FOUNDATION (NSF) has also taken a particular interest in bioprinting technology. (44) This foundation has tremendous influence over the development of nascent technologies. The lauded peer-review process utilized by this organization aims to direct funding to the most capable research institutions. The support of agencies like NIH and NSF will be of vital importance to developing viable organ replacements.

Ultimately, any viable artificial organ replacement will need to be of a design that has been clinically-proven to be both functional and safe. Right now, scientists and engineers are attempting to replicate the architecture of organs for two reasons. First, the architecture of a natural organ is already known to fit within the confines of the human body. Second, our understanding of how an organ's architecture influences its function is severely limited. We do know, however, that the current architectural models tend to work. (32)

NIH and NSF may want to consider investing in viability studies on bioprinted organs. Optimizing performance of these products will be a preliminary focus for tissue engineers. Early bioprinted organs may not function as well as typical healthy organs. Right now, the question we ought to be asking ourselves regarding function is: How good is good enough? For patients in the midst of congenital heart failure or advanced ventricular disease, any improvement in function may be a welcome alternative.

For example, an unfortunate truth about the *Total Artificial heart*, by SynCardia Systems, is that the implantation procedure requires cutting out the bottom half of the (patient's) heart, and there are risks of side effects like infection, bleeding, and stroke. Patients implanted with the device are tethered to a washing machine-sized power generator until they can receive a donor heart. (45) According to the company's website, it is still the safest and best-performing artificial heart on the market today.

This particular solution to congenital heart failure received FDA approval in 2004. Approximately 1 in 5 patients who use this device pass away before they are able to receive a transplant. In spite of these limitations, the artificial heart is still a far-preferred alternative to death. It has even sustained life in a patient for nearly 4 years, at which point that patient was able to receive a heart transplant. Long-term survivability of heart transplant recipients is slightly worse than that of artificial hearts; only about 3 in 4 heart-transplant recipients survive more than 5 years after surgery. (46).

It is impossible to predict the performance of FDA approved bioprinted organs because they have yet to be developed, created, or tested. There is no guarantee that *de novo* organs will function any better than a transplant, but bioprinted products do exhibit the potential for minimizing the risks of tissue rejection. A reasonable expectation for the performance of early bioprinted organs might begin with statistics taken from traditional organ transplantations. To this effect, is a bioprinted heart that does not perform as well as its natural counterpart so different than the Total Artificial heart? Neither alternative would be required if the patient's natural organ was functioning properly. Both alternatives would provide the recipient with an opportunity to extend his/her life. In either case, the fundamental architecture of the heart has been changed.

THE ROLE OF THE FDA

The FDA is tasked with evaluating all devices, including any that utilize 3D printing technology, for safety and effectiveness, and appropriate benefit and risk determination, regardless of the manufacturing technologies used. (47) Safety is paramount at the FDA with somewhat less emphasis placed on form and function.

Safety, form, and function are relative terms, though. Over 28,000 times last year, the FDA allowed organ donations because they made the difference between life and death in otherwise terminally-ill patients. (15) The agency allowed these transplants knowing full-well that virtually every organ transplant ever performed will fail without a near-constant stream of medication. It also approves these medications, which may vary in effectiveness, even though they present documented side-effects like increased cancer risk. (48)

Ultimately, the decision that must be made with regard to approving bioprinted organs will boil down to risk versus reward. The first patient-specific organs made via bioprinting may pose substantial risks to the patients. These patients will most likely have exhausted all other options before considering this method of treatment. In other words, bioprinting will be their last option. Regardless, the implications of the iPS cells these organs use - and the reprogramming risks associated with them - ought to be clearly outlined prior to patient consent.

Even before this is possible, safety studies will need to be performed by the FDA and other agencies. (49) These studies may include examining cell variability, reprogramming efficiency, and genetic variation in iPS cells. These studies may require animal testing, and these animal tests may require months or years to perform. (9) Ultimately, without predictive tests, it will be difficult to accelerate these studies for use in human trials. (48)

So far, the agency has not publicly commented about future clinical uses of genetically reprogrammed iPS cells. At the moment, these cells are considered biologics, and they are categorized as being biosimilar to hESCs. Someday, a hESC biologic will receive FDA approval. Due to the biosimilarity clause, an application for a streamlined approval process could get submitted by an iPS cell-based therapy of a similar nature. (48)

Even though these iPS cells may be biologically identical to hESCs, the reprogramming process required to create them may cause the iPS cells to vary in purity, identity, and stability. It is a possibility that these variations may even result in unanticipated complications. (8) To safeguard against this, protocols could be implemented which would ensure that poorly reprogrammed cells cause no undue harm. To *ensure* patient safety, iPS cells generated from every patient may need to be assessed for stability, differentiation, and tumor formation before being delivered to the patient.

STANDARDS & CERTIFICATIONS

As it is with all regulated biologics, the critical regulatory challenges with iPS cells will initially involve demonstrating the safety of the final cell product and establishing consistent reprogramming protocols. (42) The

strategy of individually testing each iPS cell line is not exactly practical – especially for acute applications – but this may not always be the case.

Currently, this limitation is due to a relative lack of sophisticated methods and equipment for testing bioprinted products. (42) Forcing IND sponsors to perform these tests frequently will persuade them to develop more cost-effective methods for assessing quality. In the meantime, it may still be possible to treat individuals or small groups. Unless an improvement in testing methods and equipment is developed, the amount of time required to analyze cell-based products and therapies may be prohibitive. (9)

Engineering societies like ASME and the AMERICAN SOCIETY FOR TESTING AND MEASUREMENTS **(ASTM)** have vast experience creating standards and certifications for products in a number of industries. Collectively, these two organizations have issued more than 3000 standards. ASTM has recently united a panel of experts in the biomedical field to investigate the development of standards and certifications in the field of biomanufacturing.

Additionally, the TISSUE ENGINEERING AND REGENERATIVE MEDICINE INTERNATIONAL SOCIETY **(TERMIS)** is a society comprised of leading figures in fields related to bioprinting. TERMIS has also started looking into regulatory considerations for stem cell products, and its members will be meeting with delegates from NIH, FDA, NIST, and NSF in December of 2014 in Washington, D.C.

There are experts in each of these organizations who are familiar with bioprinting technology; they have a unique and comprehensive understanding of its benefits and limitations. (50) These individuals are ideal candidates to direct the scientific advances being made in bioprinting into realistic therapies.

Obviously, mitigating short- and long-term risks associated with *de novo* organs is of paramount importance to the future of bioprinting technology. Developing safe and effective organs will require the development of inexpensive and accurate testing procedures. Until these technologies are tried and tested, there could be long-term safety concerns, including teratoma[****] and cancer formation. (51)

TRANSITIONING FROM THE LABORATORY TO THE MARKETPLACE

Compared to the alternative of certain death in the patient, the risks described above may prove to be relatively insignificant. Understanding how certain therapies affect the body is obviously important, but implementing functional therapies that work safely is far more beneficial to the public as a whole. From a clinical perspective, what matters may not be whether the active instrument used in implanted tissue consists of stem cells, proteins, or genes, but whether that instrument is therapeutically effective.

Additionally, another significant challenge to bringing bioprinted organs to market may be bridging the gap between laboratory advancements and economically competitive bioprinted products. The first experimental bioprinted organs may be paid for by the patient out-of-pocket. There is already a legal process in place for experimental applications of this nature.

Developing these technologies into products may require many years of work, and developing the manufacturing facilities to produce them will likely require considerable capital investments. Unfortunately, the acquisition of private funding to enable the construction of manufacturing facilities may prove difficult to accomplish. Angel investors generally want their investments returned in five years or less, and it may take longer than that just to

[****] **More information available on teratomas in the Appendix**

build a bioprinting facility.

Without federal intervention, bioprinted products may never reach the marketplace. There are a number of challenges to overcome, and they are not just limited to a lack of funding or safety protocols. Bioprinting technology needs direction, solid leadership, and a competitive strategy for competing on a global scale. We will need educational programs and resources to inform the general public about this technology.

Before the American public can be educated about the benefits and limitations of this technology, however, the risks will need to be better understood. Simply put, we need a roadmap. Fortunately, the PRESIDENT'S COUNCIL OF ADVISORS ON SCIENCE AND TECHNOLOGY (PCAST) has a history in developing roadmaps to facilitate the commercialization of new technologies. (52) A committee, selected by this council, could lay the groundwork for an initiative on bioprinting. Together, this committee, in conjunction with the NATIONAL ACADEMY OF SCIENCES (NAS), NATIONAL ACADEMY OF ENGINEERS (NAE) AND THE INSTITUTE OF MEDICINE (IOM), could enlist the aid of the nation's most knowledgeable scientists, engineers, health professionals, and other experts who volunteer their time to produce reports that have led to some of the most significant and lasting improvements in the health, education, and welfare of all the world's citizens. The National Academy would be a perfect instrument through which to investigate the state-of-the-art in stem cell-based therapies and bioprinted products.

Simultaneously, we could be using federal funds to enable the construction of a central manufacturing facility for technologies relating to tissue generation, regenerative medicine, stem cell research, and bioprinting. This facility could be built using funds provided by the Obama Administration's NNMI. It would serve as a hub from which scientists and engineers could promote the translation of knowledge into practical applications. The facility could also serve as an instrument through which to train a workforce and develop evaluation instrumentation to gauge long-term stem cell safety.

RECOMMENDATIONS

NON-GOVERNMENTAL ORGANIZATIONS (NGOS):

- NGOs should be working together to develop a set of guidelines advocating the cautious use of bioprinting. These could initially include voluntary guidelines which cover any product resulting from the application of bioprinting technology.
- Federal agencies should issue a request for information from the members in these NGOs. Educating government officials, agencies, and the general public about potential benefits and limitations of bioprinting technology is going to be of vital importance to the commercialization of bioprinting technology.

CONGRESS:

- Approve RAMI Act, then appropriate the authorized $300 million into the NNMI.
- Prioritize funding from NSF and NIH to advance stem cell therapies and *de novo* organ technologies.

NIH

- Fund studies relating to purity, identity, and stability of cellular reprogramming processes and procedures, which include:
  - Promote predictive in vitro assays for *de novo* organs made from iPS cells.

- Test *de novo* organs in large-animal trials.

NSF

- Fund additional exploration into chemical reprogramming of iPS cells.
- Fund the development of machinery and software to be used as bioprinting platforms.

FOOD AND DRUG ADMINISTRATION:

- Clearly define product categories for bioprinted organs.
- Differentiate between iPS cells and hESCs from a biological perspective, at least until the technology proves to be viable and safe.
- Require that each iPS cell line gets assessed for stability, its ability to differentiate, and the likelihood of tumor generation before patient-delivery.

PRESIDENTIAL ADMINISTRATION:

REVITALIZE AMERICAN MANUFACTURING AND INNOVATION (RAMI) ACT

- Seek to establish the United States' place as a leader in the field of global biomanufacturing.
- Use the RAMI Act as an instrument through which to develop an advanced manufacturing center for tissue engineering, regenerative medicine, biological fabrication, and bioprinting technologies.

PRESIDENT'S COUNCIL OF ADVISORS ON SCIENCE AND TECHNOLOGY (PCAST)

Bioprinting should be a priority for the Obama Administration, and a road map ought to be made by PCAST. The goals and visions implemented by this initiative would be similar to those made by the Office of Science and Technology of the President (OSTP) for the National Nanotechnology Initiative (NNI) during 2001 (13). A bioprinting-specific amended version of the NNI follows[††††]:

ADVANCE A WORLD-CLASS BIOPRINTING TECHNOLOGY RESEARCH AND DEVELOPMENT PROGRAM: Leadership in bioprinting technology R&D could be enabled by stimulating discovery and innovation. The Initiative would expand the boundaries of knowledge and develop technologies through a comprehensive program of R&D. Bioprinting technology has potential to significantly impact the fields of regenerative medicine and tissue engineering, but was born at the intersection of several fields of science and engineering.

FOSTER THE TRANSFER OF NEW TECHNOLOGIES INTO PRODUCTS FOR COMMERCIAL AND PUBLIC BENEFIT: A Bioprinting Initiative would contribute to U.S. competitiveness and national security by improving existing biological products and processes and also by creating new ones. Strategies could be implemented that maximize the economic and public benefits of investments made in bioprinting technology. Agencies or committees could be incorporated to foster a better understanding of the fundamental science behind bioprinting technology, and they could also promote the translation of this knowledge into practical applications.

[††††] **(For more information about the NNI, see http://www.nano.gov/.)**

DEVELOP AND SUSTAIN EDUCATIONAL RESOURCES, A SKILLED WORKFORCE, AND A DYNAMIC INFRASTRUCTURE AND TOOLSET TO ADVANCE BIOPRINTING TECHNOLOGY: A skilled science and engineering workforce, leading-edge instrumentation, and state-of-the-art facilities are essential to advancing bioprinting technology R&D. Educational programs and resources are required to inform the general public, decision makers, and other stakeholders (including regulators, managers, insurers, and financiers), and to produce the next generation of biotechnologists—that is, the researchers, inventors, engineers, and technicians who drive discovery, innovation, industry, and manufacturing.

SUPPORT RESPONSIBLE DEVELOPMENT OF BIOPRINTING TECHNOLOGY: Maximizing the benefits of the technology while, at the same time, developing an understanding of potential risks and the means to assess and manage them would be another objective for the Bioprinting Initiative. Agencies and committees created by this initiative would pursue a program of research, education, collaboration, and communication focused on the environmental, health, and safety (EHS) implications of bioprinting technology. Also, the responsible development of biotechnology would require engaging universities, industry, government agencies (local, regional, state, and Federal), nongovernmental organizations, and other communities.

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APPENDIX

BIOLOGICS

Biological products, or biologics, are medical products. (53) Many biologics are made from a variety of natural sources (human, animal or microorganism). Like drugs, some biologics are intended to treat diseases and medical conditions. Other biologics are used to prevent or diagnose diseases. Examples of biological products include:

- vaccines
- blood and blood products for transfusion and/or manufacturing into other products
- allergenic extracts, which are used for both diagnosis and treatment (for example, allergy shots)
- human cells and tissues used for transplantation (for example, tendons, ligaments and bone)
- gene therapies
- cellular therapies
- tests to screen potential blood donors for infectious agents such as HIV

SOFTWARE

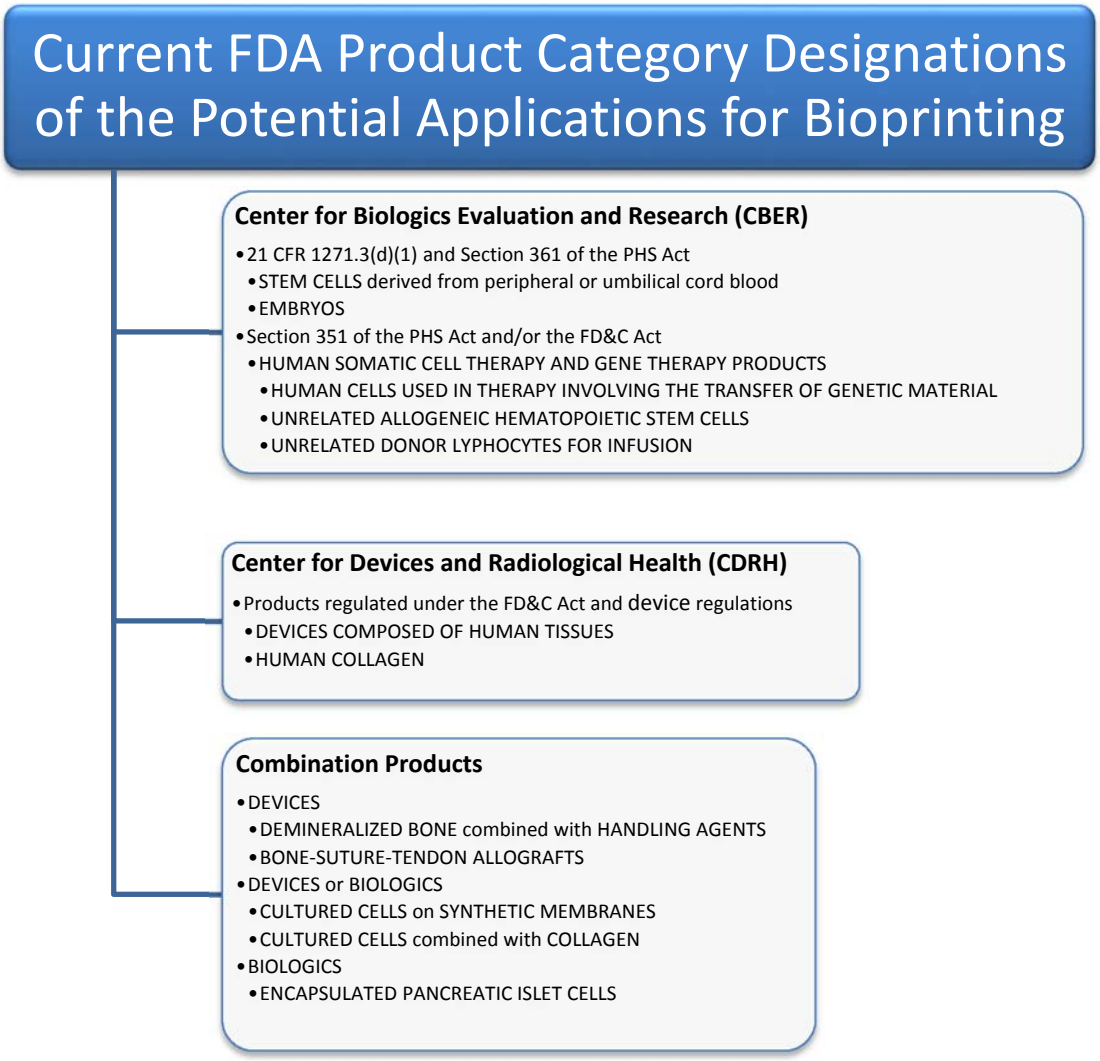
The first step in printing an organ is generating a three-dimensional (3D) virtual model of the organ using computer-aided design (CAD) software. From this computer generated model, a stereolithography (*.stl) file of the organ can be made. An *.stl is basically a polygonal mesh of lines and points which accurately mimic the geometry of the model. Effectively, *.stl generation turns organs into points and lines which can then be sliced into cross-sectional layers. These layers are essentially line-drawings, and they can be used to generate tool paths. These tool paths tell the machine tasked with creating the organ where to move. Once the virtual mesh of the CAD organ has been sliced and sectioned into pictures, the printer can systematically print each of these pictures; one atop the other – until an organ is made. (12)

The very first 3D printer was developed by Chuck Hall in 1983. The growth of the technology was initially limited to the software platform. Computing power has improved dramatically since the 1980's, but software is still incapable of exactly reproducing a functional 3D organ in CAD. It may, in fact, be impossible to achieve this. It may not be necessary to create an organ of identical architecture, though. It is important to remember, however, that the end game for organ manufacturing is function and safety, not necessarily form. (6)

GROWTH FACTORS

In order to be turned into another type of cell, stem cells require the presence of growth factors and proteins. According to Dorland's Medical Dictionary, growth factors are naturally occurring substances capable of stimulating cellular growth, proliferation, healing, and cellular differentiation. Growth factors are capable of regulating cellular processes. They function as signaling-molecules between two cells, and they are can stimulate cell differentiation and maturation. There are many different growth factors, and each performs a specific function. Growth factors, proteins, and genetic material all work together to promote differentiation and proliferation, ultimately resulting in new and specialized tissue generation. (27)

CURRENT FDA PRODUCT CATEGORY DESIGNATIONS OF THE POTENTIAL APPLICATIONS FOR BIOPRINTING (CHART BY JAMES S. GWINN, III)



REPROGRAMMING

To reprogram iPS cells, a small sample (about half the size of a postage stamp) of the patients' cells is collected surgically. (24) These cells are then carefully separated, and embryonic genes are injected into the cell. The introduction of new genes cause the cell to behave like a hESC; it induces pluripotency. Once the cell has been reprogrammed, millions of copies of the modified cell are cultured. (NIH) To date, human iPS cells have been generated from tendons, ligaments, skin cells, blood marrow, arterial cells, adult stem cells, and cord blood stem cells. (25)

Since the discovery of iPS cells in 2006, a number of concerns have manifested with regard to their potential use. Specifically, the industry is concerned that iPS cells might not be as functional as hESCs. (54) Reprogrammed cells meet the defining criteria for pluripotency, but it is not known if iPS cells and embryonic stem cells differ in clinically significant ways.

The methods by which iPS cells can be completely and reproducibly altered is still under investigation. Specifically, when the DNA in an iPS cell is altered, the cell can destabilize and become more susceptible to mutation. This is because the reprogramming process may affect unintended sections of the original cells' DNA.

Initially, reprogramming factors were introduced into adult cells through a virus. In animal studies, viral-modifications have been predicted to increase the risk of cancer. These risks may be avoided by using non-genetic methods – like the introduction of chemicals – to turn specific genes on or off. Researchers feel that genetically based iPS cell therapies will be subjected to considerably greater regulatory hurdles than, say, iPS cells reprogrammed without genetic modification. (55) Researchers have attempted to develop non-viral delivery systems with reduced cancer risks, but the experiments laying claim to these strategies, to date, have not been duplicated.

In 2013, Masahito Tachibana et al published a paper which claimed to have reprogrammed somatic cells into pluripotent embryonic stem cells (ESCs) by somatic cell nuclear transfer (SCNT). However, there were notable inconsistencies in the paper, and that no other institutions have yet been able to duplicate the experiment. (56)

There are also predicted differences between therapies and organs manufactured for certain patients. Cells from older or diseased patients may be more difficult to reprogram than cells from healthy younger ones; there may be differences in both reprogramming efficiency and completeness that depend on the patient.

In the case of patient-specific cell therapies, the potential for donor-associated reprogramming variability will make establishing a reproducible manufacturing process and demonstration of final product safety challenging. Addressing these concerns will likely require the development of additional characterization technologies, including predictive genetic and epigenetic analysis. (9)

PURITY

Purity refers to the completeness with which iPS cells are reprogrammed. Simply stated, cells that have been thoroughly reprogrammed will make organs and therapies of better quality. In order for engineers to understand the purity of the stem cells they have created, they must first be able to identify the reprogrammed cells efficiently.

The critical consideration is the degree of remodeling and 'completeness' of reprogramming required for an iPS cell line to be capable of differentiation. Preclinical studies will have to be made to analyze the effect

reprogramming has on the iPS cells' ability to change into other cells.

IDENTITY

Whereas purity refers to the completeness of reprogramming on a cellular level, identity refers to the completeness of reprogramming for the entire cell population. Thus far, researchers have had a difficult time producing iPS cell populations entirely made up of active iPS cells. (28) Despite advances made in reprogramming technology, the final product will most likely be a heterogeneous population (composed of active cells and inactive cells). Simply put, different cells respond to the reprogramming process differently.

Ultimately, defining acceptable limits for the concentrations of these inactive cells will be necessary. This task may be challenging due to the myriad variables which affect the efficiency with which iPS cells are programmed. Ideally, the majority of these tests could be performed in vitro.

Due to differences in active cell concentrations, there is a good chance the FDA will require certain tests for each patient-specific application of iPS cells. Developing testing equipment and methods to perform these tests accurately and quickly will be a major milestone in the development of this technology. (35)

STABILITY

Stability refers to the long term viability and efficacy of therapies and *de novo* organs. A concern from a regulatory perspective is how to determine that a cell product is capable of maintaining stable function after implantation. (57) Will these cells continue to replicate into the desired cell type, or will they revert back to their original cell-type? Will reprogramming cause them to mutate into some entirely different type of cell? Would this mutation be benign or malignant? The short answer is that iPS cells are so new that scientists have not yet had the ability to study these cells in the long-term.

Visually inspecting tissue products in vivo would subject the patient to additional risks and complications, so a method of verifying the validity of stem cell therapies is needed. (9) Acquiring this type of information is further disadvantaged by a lack of animal tests. Immunocompromised rodents are available for these types of studies, but they have a limited lifespan. Testing hESCs in larger animals will be necessary. (35) These creatures have longer lifespans and are more similar to humans, but these tests will require immunosuppression regimens. Similarly, undifferentiated stem cells that have been stored for long periods might react differently to reprogramming protocols.

NATIONAL NANOTECHNOLOGY INITIATIVE (NNI)

NNI today consists of the individual and cooperative nanotechnology-related activities of 26 Federal agencies with a range of research and regulatory roles and responsibilities. (58) Fifteen of the participating agencies have research and development (R&D) budgets that relate to nanotechnology, with the reported NNI budget representing the collective sum of these investments. Funding support for nanotechnology R&D stems directly from NNI member agencies, not the NNI. As an interagency effort, the NNI informs and influences the Federal budget and planning processes through its member agencies and through the National Science and Technology Council (NSTC). The NNI brings together the expertise needed to advance this broad and complex field—creating a framework for shared goals, priorities, and strategies that helps each participating Federal agency leverage the resources of all participating agencies. With the support of the NNI, nanotechnology R&D is taking place in academic, government, and industry laboratories across the United States.

TERATOMAS

Simply stated, teratomas are tumors. They are usually non-cancerous (benign), and are regularly formed when hESCs and iPS cells are injected into rats with no immune system. Teratomas may contain teeth, hair, bone, and sometimes even more complex organs when certain growth factors are present. These tumors are not necessarily bad things; they validate the efficacy and potency of iPS cells.

The issue with teratoma formation (tumorigenicity), when using iPS cells, is that the methods by which teratomas are evaluated (teratoma assaying) is inconsistent from lab to lab. The assays are evaluated to determine the potency of the reprogrammed cells, but no clearly defined standards exist for introducing the iPS cells into the test subject and no standards exist for analyzing the teratomas, themselves. Additionally, the rats used to test teratoma formation do not have competent immune systems, which should be a strong indication that the vast majority of these assays are clinically irrelevant. (59)

TIMELINE OF PERTINENT POLICIES RELATING TO STEM CELL RESEARCH AND BIOPRINTING (60) (61)

1996

- **The Dickey-Wicker Amendment:** Congress bans federal funding for research on embryos. The amendment prohibits the use of federal funds for the creation of a human embryo or embryos for research purposes, or research in which a human embryo or embryos are destroyed, discarded or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero.

2000

- **August 25: NIH guidelines for research using human pluripotent stem cells go into effect:** Human embryonic stem cells must be derived with private funds from frozen embryos from fertility clinics; that they must have been created for fertility treatment purposes; that they be in excess of the donor's clinical need; and that they be obtained with consent of the donor.

2001

- **June 7: President Bush prohibits federal funding of most human embryonic stem cell research:** President George W. Bush prohibits the federal funding of any research using stem cell lines derived after August 9, 2001, but his policy did not affect research in the private sector or research conducted with state funding.

2005

- **June: Congress passes the Stem Cell Research Enhancement Act (H.R. 810):** H.R. 810, which would have expanded federal funding for stem cell research to include stem cells derived from embryos created for, but subsequently not used in, the in-vitro fertilization process, passes both the House and the Senate in the 109th Congress, attracting bipartisan support. The bill is quickly vetoed by President Bush. The House votes 235-193 in favor of the bill, but the two-thirds majority needed to override the veto is not reached.

2007

- **July: Congress passes the Stem Cell Research Enhancement Act (S. 5):** In the 110th Congress, the Senate passes their version of The Stem Cell Research Enhancement Act (S. 5) with strong bipartisan support, 63-34. The House also passes the Senate's version of the bill 247-176. Again, the bill is vetoed by President Bush, and again Congress cannot override the veto.

2009

- **March 9: President Obama reverses 2001 executive order:** President Barack Obama issues an executive order, titled "Removing Barriers to Responsible Scientific Research Involving Human Stem Cells."
- **March 11: President signs the Omnibus Appropriations Act of 2009:** This provision facilitated the application process for obtaining federal funding for privately created stem cell lines. Effectively, this was an expansion of the options allowed by President Bush.
- **July 7: Sherley v. Sebelius, the most noteworthy court case regarding the government's funding of embryonic stem cell research, is filed:** A group of plaintiffs file a lawsuit against the NIH and the Department of Health and Human Services, arguing that federal funding of embryonic stem cell research is in violation of the Dickey-Wicker amendment.

2010

- **March 23: The Patient Protection and Affordable Care Act (Affordable Care Act) signed into law by President Obama:** Ammendment to the Public Health Service Act (PHS Act) creates an abbreviated licensure pathway for biological products that are demonstrated to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product. This pathway is provided in the part of the law known as the Biologics Price Competition and Innovation Act (BPCI Act). Under the BPCI Act, a biological product may be demonstrated to be "biosimilar" if data show that, among other things, the product is "highly similar" to an already-approved biological product.

2011

- **June 24: DeGette introduces H.R. 2376, the Stem Cell Research Advancement Act of 2011:** Introduction of H.R. 2376, a bill which calls for the Secretary of the Department of Health and Human Services to conduct and support research that utilizes human stem cells, including embryonic stem cells.
- **July 27: U.S. District Court for the District of Columbia rules in favor of embryonic stem cell research in Sherley v. Sebelius**

2012

- **August 24: U.S. Court of Appeals for the D.C. Circuit hears arguments about human embryonic stem cell research funding:** In a decision favorable to proponents of embryonic stem cell research, the U.S. Court of Appeals for the D.C. Circuit upholds a lower court ruling that dismissed a lawsuit challenging the Obama administration's expansion of federal funding for stem cell research.

2013

- **January 7: Supreme Court declines to hear *Sherley v. Sebelius:*** The Supreme Court announced that it would not hear *Sherley v. Sebelius*, thereby upholding the previous ruling of the D.C. circuit court's ruling.