Advances in Plastic & Reconstructive Surgery

Applis Publishers

Review Article ISSN: 2572-6684

A Revolution of Plastic and Reconstructive Surgery: Biofabricating Customized Tissues, Organs, and Human Parts

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Abstract

The biomedical burden of treating patients with damaged tissues and organs continues to grow at an unprecedented rate [1]. Yet, in spite of current state-of-the-art surgical and medical treatments, clinical outcomes are persistently sub-optimal, in part due to a lack of biological components for transplantation [2]. This paper proposes the use of regenerative medicine and tissue engineering to biofabricate patient-specific tissue and organs to alleviate this burden. We specifically examine the tissues: skin, fat, and bone; as well as the organs: kidney, liver, and pancreas to illustrate the future possibility of creating entire customized human parts. We outline a method for biomanufacturing in which organs and tissues would be customized with 3D models of the patient's vasculature to allow for easier and improved surgical re-anastomosis. Fabrication would be performed with induced pluripotent stem cells (iPSCs) derived from a patient's somatic cells, negating the issue of rejection. Industrial software that integrates liquid handling robotic hardware and Design-of Experiments (DoE) mathematics to create "recipes" for the development of distinct cell types, can be combined with automation and robotics to bio-print customized tissues and organs on an industrial scale. Also, we outline some of the major ethical issues concerning biofabrication. Yet, with this system the field of plastic and reconstructive surgery would shift from the practice of repairing defects to replacing deficient tissues and organs. More significantly, the production of replacement biological components has great potential to improve the quality of life and clinical outcomes for millions of people as well as reduce the tremendous biomedical costs currently associated with transplant rejection and immunosuppression.

Background: History

The modern origins of tissue and organ replacement can be traced back to mid-twentieth century Holland. There, Dr. Willem Kolff created the first artificial kidney from old car and washing machine parts, orange juice cans, and sausage casings in 1943 [3]. This marked the beginning of new era of medicine, one in which science can be used to supplement a vital body function. The first attempts to create a surplus of organs focused on artificial devices, using primarily plastics and metals. These devices demonstrated that organ function could be restored: however, the artificial materials led to protein absorption, inflammation, and fibrous encapsulation. Also, the body is a harsh environment for inorganic materials, eventually degrading them overtime [4]. The result, ultimately, was progressive failure and life-threatening patient complications [5]. Since then, the field of replacement tissues and organs has made great strides. In 1954, the first successful living-related kidney transplant, led by Dr. Joseph Murray and Dr. David Hume at the Brigham Hospital in Boston, was was performed. In this case, a kidney was transplanted from a man named Ronald Herrick into his identical twin, Richard. The procedure was a success partly because it took place between two identical twins, allowing the recipient's immune system to recognize the new organ as non-foreign [6]. Other transplantations between non-related individuals were later performed; however, these procedures resulted in donor graft rejection [7]. The next big step occurred in 1962 in the U.S, when Dr. Roy Calne and Dr. Murray used the immunosuppressant drug azathioprine to inhibit donor graft rejection in a deceased donor renal transplant between unrelated individuals, which resulted in long term recipient survival [7]. In 1983, the Food and Drug Admiration (FDA) approved cyclosporine, the most successful anti-rejection medication developed to date [7]. These advances paved the way for the implantation of tissues and organs, which have become part of key practices within plastic and reconstructive surgery.

The latest progress in the history of tissue and organ replacement involves tissue engineering and regenerative medicine. Tissue engineering is defined as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [8]. Similarly, regenerative medicine is defined as "the process of replacing, engineering, or regenerating human cells, tissues or organs to restore or establish normal function" [9]. The critical functions of tissue engineered constructs include structural,biochemical and secretory, and barrier- and transport-related [10]. While evidence indicates that skin grafting dates back hundreds of years, the first modern tissue

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Received: November 14, 2017; **Accepted:** March 11, 2017; **Published:** March 15, 2017.

engineering based therapies developed were skin grafting techniques produced in the late 1970s and early 1980s [10]. Another major development in this field was the creation of induced pluripotent stem cells (iPSCs) in 2006 by Shinya Yamanaka and his team at Kyoto University in Japan. Their work advancement unlocked tremendous possibilities for the fields of regenerative medicine and tissue engineering, and earned Shinya Yamanaka the Noble Prize for Physiology or Medicine in 2012 [11, 12]. With these breakthroughs and others in the last decade, we now have the capacity to build three-dimensional constructs or tissues with controlled cellular interfaces [1]. Advances in micro- and nano-fabrication as well as microfluidic technologies now enable cell-cell and cell-material interactions to be engineered and controlled at unprecedented levels. Bio-printing technologies improve the scale through automation and reproducibility of engineered tissues in real time assays [1, 13]. These advances have the potential to cause a paradigm shift in plastic surgery and modern medicine as a whole.

Current Paradigm

Today transplantation is the standard of care for severely damaged tissues and organs [14]. Transplantation or grafting is the removal of diseased or injured tissue and subsequent implantation of a corresponding healthy replacement. There are four main types of grafts: autograft (from the same individual), allograft (from an unrelated donor of the same species), cadaveric (from a deceased donor), and xenograft (from a different species) [15]. Each of these graft types can be utilized in the transplantation of skin, fat, bone, or organs.

Skin

For cutaneous transplants, the grafts may be further classified into four main subtypes: split thickness (STSG), full thickness (FTSG), composite, and artificial skin grafts. Skin grafts are used to replace damaged or missing skin and temporarily provide wound coverage [16]. When determining the appropriate skin graft, a surgeon must consider availability of skin at the donor site, likelihood of wound infection, sites of wound, likelihood of contracture, aesthetic outcome, relative cost, and time consumption [17]. STSGs include the epidermis and a portion of the dermis, and are typically used to cover larger areas and are more likely to heal than full thickness skin grafts [15]. FTSGs include the epidermis and the entire dermis. The advantages of the FTSGs include good contour, natural coloring, and little contraction at the grafted site. Therefore, FTSGs are often used on more visible areas of the body such as the face [16]. For each type of graft, skin is removed from a harvesting site and implanted at a recipient site. A dermatome is often utilized to remove a uniform section of skin with a desired thickness. Also, in many cases a mesher is employed to expand the area a skin graft can cover, by cutting small incisions in the skin at regular intervals [18]. However, if more than 50% of a patient's skin is damaged, there will not be enough donor material available for an autograft, even with a mesher. In such cases, an allograft, xenograft, or artificial skin substitute is employed to cover the wound [19, 20]. Yet, in such cases the patient must be placed on an immunosuppressive regime [17]. Composite grafts are a combination of skin, fat, and cartilage, and are used in areas that require three-dimensionality, such as the nose. Artificial skin grafts usually consist of a synthetic epidermis and a collagen based dermis, whose fibers are arranged in a lattice [16]. Skin substitutes are alternatives to STSGs and FTSGs when these stan- dard therapies are not desirable or possible [17].

Bone

Bone grafting is involved in many reconstructive surgery procedures. Autografts are the most common form of bone transplant and are currently the gold standard for managing defects [21]. When performing an autologous bone graft, the surgeon must first choose the harvest site. This will depend on the amount of bone needed and the nature of the defect [21]. A second operation must also be performed to implant the harvested bone at the recipient site [21]. Allografts represent the second most common bone-grafting technique performed in the United States (U.S) and involve transplanting donor bone tissue from a cadaver. The main advantages of allogeneic bone grafts are that more bone can be harvested and it does not require a second operation [22]. There are several bone substitutes on the market in place of a bone graft. However, bone is unique in that it is a composite of fiber and mineral [23]. As a result, grafts are the preferred and more common treatment for bone defects and disorders [22].

Fat

Fat grafts are used in many cosmetic and reconstructive surgeries. The most common form of fat graft is lipo-injection of autologous adipose tissue (i.e. fat tissue from the patient [24]. In this procedure the surgeon must first select a harvest site. The site is chosen based on the level of invasiveness and ability to provide sufficient amounts of fat for the procedure [25]. Harvesting is often performed through suction with a blunt cannula of varying size depending on the procedure. Viable adipocytes are then separated from blood, serum, and damaged adipocytes [26]. Finally, the fat is injected with multiple passes in the area of augmentation using a blunt-tipped infusion cannula [24].

Organs

The first step in organ transplantation is determining a recipient'seligibility. To be eligible a candidate must be 70 or younger, have no active infections, and no neoplasms. If a person meets these criteria, the next step is to find a compatible organ donor. To determine compatibility, demographic and health information is considered, as well as cross-matching, human leukocyte antigens (HLA) histocompatibility, and ABO blood type [27]. Once a suitable donor has been found, the recipient must undergo induction therapy for a short period preoperatively to help prevent rejection. Next, a transplant procedure is performed, in which the surgeon places the donor organ in the appropriate position and reattaches it to the patient's vasculature. Following the transplant procedure, the recipient must adhere to a long-term immunosuppression regimen [28].

Current Problems

The current paradigm for skin, bone, fat, and organ transplantations share several major problems. Although specific tissue types and current tissue engineered products face unique individual challenges, there are two overarching limitations in the transplant field: namely severe scarcities of organs for transplantation and issues associated with rejection and immunosuppression [28, 29].

Skin

An autologous skin graft is possible only if the patient has enough undamaged skin available [30]. If more than 50% of a patient's total

body surface area (TBSA) is damaged, then the amount of harvestable skin is insufficient to provide wound coverage, even with the use of a mesher to expand the harvested skin [30]. Many patients with extensive burns die each year because of this problem and could be saved with the transplantation of large patches of skin [20, 30]. These patients are treated with allografts, xenografts, and synthetic skin substitutes, but these grafts are only temporary [1]. The patient's immune system recognizes the transplanted skin as foreign and launches an immune attack, causing the graft to fail. To combat rejection, patients who receive an allograft must also be placed on immunosuppressants. These have serious side effects including infection, avascular necrosis, and susceptibility to neoplasms [1].

In addition to issues of rejection, skin substitutes face the additional challenges of the production time and associated costs to create a viable cell based skin substitute. A scaffold must first be made and then seeded with cultured fibroblasts [31]. In the case of synthetic skin substitutes, the materials used to compose the structure of the substitute must be pain stakingly engineered and arranged in order to produce a viable product for surgical wound coverage. The average time required to produce one square meter of such skin substitute is three to four weeks, and can be as long as three to twelve weeks for cultured skin substitutes [32-34]. Also, the cost of such substitutes can be extremely high. For example, it is estimated that every one percent body surface covered with Epicel™, a common skin substitute, costs thirteen thousand dollars [33].

Furthermore, even if a patient has no significant skin damage, a very limited number of potential donor sites will result in proper graft coloring and quality [35]. Ideal harvesting sites for FTSG to produce optimal pigmentation and quality in facial reconstructions or cosmetic procedures are limited to the retroauricular region and above the eyebrows. For hand reconstructions both wrist and elbow crease skin can be considered. However, these donor areas should be avoided in cultural settings where scarring in these areas can result in stigmatization of the patient, and even in related suicides [35]. In total a small number of potential donor sites exist for ideal FTSGs.

Bone

The global incidence of bone disorders and conditions has risen sharply and is expected to double by 2020, especially in populations where aging is coupled with chronic comorbidities and inactivity, like the U.S [22]. Bone grafts are widely used to treat these conditions. In the U.S. alone, over half a million patients receive bone defect repairs each year, at a cost of more than $2.5 billion. These figures are expected to greatly increase by 2020 [22]. The current gold standard treatment for bone disorders is autologous transplantation. Yet autologous bone grafts require two surgical procedures: the first to harvest bone, and the second to implant it. Having two procedures doubles the invasiveness of the operation as well as the risks related to surgery. Also, harvesting bone from a donor site can result in patient deformities [36]. The largest challenge facing autologous bone transplants is a scarcity of harvestable bone. In cases where large amounts of bone are needed, harvesting from the patient is not feasible. In such cases allogeneic bone transplants are performed, often from a cadaveric donor [37]. However, the increase in bone grafting procedures annually performed in the U.S. has created a shortage of cadaveric donors [38].

Fat

Fat does not present the same sort of shortages as skin and bone. Fat is relatively abundant in many patients and is not particularly invasive to harvest. Furthermore, the vast majority of fat grafts do not elicit an immune response, since the tissue is often autologous [39]. The primary problem with fat transplantation is that the fat must be separated from blood, blood tumescent anesthesia, and other substances collected in harvesting [39]. Currently there is no consensus on the best way to filter adipose tissue, resulting in high variability in the amount of undesirable substances being injected along with fat [40]. If fat is not adequately separated from other materials, then graft failure and other mal-effects may occur, such as necrosis and tissue reabsorption [39]. One common mal-effect is fat absorption. Grafted adipose tissue survives via plasmatic imbibition until revascularization occurs, but often neovascularization does not occur quickly enough throughout all the grafted tissue, resulting in unprecedented reabsorption and subsequent volume loss [41].

Furthermore, the Food and Drug Administration (FDA) has recently begun regulating autologous fat grafts. The regulations state that autologous graft should be homologous with the tissue it is replacing, minimally manipulated, and cannot be combined with anything except water, crystalloids, sterilizing agents, preserving agents, or storage agents [42, 43]. These regulations particularly restrict autologous abdominal fat grafts for breast augmentation. The main concern is that abdominal fat is not breast tissue and can stimulate cell propagation, which may cause malignant cell growth [44].

Organs

Today there is a major organ scarcity because donors that have damaged organs or systemic diseases are ineligible, thereby making organ transplantation increasingly dependent on patients who have suffered rare fatal car accidents or isolated brain injuries [45]. The World Health Organization (WHO) conservatively estimates that organ transplants are meeting less than 10% of global demand [1]. Also, such organs are often not an exact match to a patient's vasculature. The transplantation operation itself is an open morbid procedure that takes many hours and is technically difficult. Even if an allograft donor is well matched, the recipient will need long- term immunosuppression which has serious side effects of infection, avascular necrosis, susceptibility to neoplasms, and many more [1]. Yet, even with immunosuppression there is still a significant risk that the transplanted organ will be rejected. Because of these potential complications, only the healthiest patients are selected as waitlist candidates. Therefore, the number of people who actually need an organ transplant is far greater than the 119, 820 people on the current U.S. transplant waitlist. Furthermore, wait times for those on the list for an organ transplant can range from five years for a kidney to eleven years for a liver [1].

Biofabrication: The Solution

Biofabrication is the production of 2D or 3D tissue constructs using a combination of biomaterials and living cells [46]. One of the most promising methods for biofabricating tissues and organs is bioprinting. Bioprinting applies additive manufacturing (AM) and computerized models to appropriately lay down living cells and biomaterials layer by layer to create tissue and organ constructs [47].

The fabrication process is devised into three stages: preprocessing, processing, and post-processing. In the preprocessing phase, a computerized model of the desired tissue or organ construct is created as a blueprint. The processing step is the actual printing of the organoid according to the computerized model. Lastly, post-processing is the tissue remodeling ad maturation in a bioreactor [47, 48]. Prior to the bioprinting process, the required cell types must be developed and placed in printable biomaterial inks. These bioinks are stored in cartridges and are the materials printed in accordance to the computerized model [49]. Three main categories of bioprinter systems exist, namely laser based, extrusion based and inkjet based, each with its associated advantages and disadvantages [see Figure 1]. These technologies have already been adopted by industry leaders and have opened the potential for a new era in medicine [47-51].

	Laser-based	**Inkjet-based**	**Extrusion-based**
Accuracy	High	Medium	Medium-Low
Materials	Cells in Media	Liquids, Hydrogels	Hydrogels, Cell aggregates
Commercial Availability	No	Yes	Yes
Fabrication Time	Long	Long-Medium	Short
Cell Viability	Medium	High	Medium-High
Throughput	Low-Medium	High	Medium
Hydrogel Viscosity	Medium	Low	High
Gelation Speed	High	High	Medium
Overall Advantages	High accuracy and single cell manipulation	Affordable, versatile, high cell viability, high throughput, and commercially available	Multiple compositions, short fabrication time, and commercially available
Overall Disadvantages	Cell damaging, low scalability, low viscosity prevents build up in 3D, and not commercially available	Low viscosity prevents build up in 3D	Sheer stress on nozzle tip wall, limited biomaterials used, and relatively low accuracy
References	52, 53, 54, 55	55, 56, 57, 58, 59, 60, 61, 62, 63, 64	55, 65, 66, 67, 68, 69, 70

Figure 1: This table shows the three main types of bioprinting systems and their key features relevant to biofabricating tissues and organs. Additionally, the table summarized the overall advantages and disadvantages associated with each system.

In the last decade, significant breakthroughs in regenerative medicine, tissue engineering, biofabrication, and materials science have occurred. These laboratory advances have created a the foundation that can now be built upon for the large-scale manufacturing and commercialization of engineered autologous tissues and organs, which could provide an unlimited, undamaged supply of patient-specific tissues and organs for transplantation [71, 72, 73]. Not only would this eliminate the current scarcities of these materials, but also it would also enable physicians to order a specific tissue or organ in advance while planning a procedure [74]. Perhaps even more significantly, all of these tissues and organs would be derived from a patient's own cells, therefore negating the need for immune suppression and risk of rejection.

How Do We Go There? - Our Platform

Our technology platform consists of a series of methods involving cell culture and differentiation and bioprinting technologies. Various researchers around the world have been working on ways to create highly specialized human cells from stem cells of a variety of different origins, with the ultimate goal of making functional tissues and organs. Those involved in regenerative medicine place great emphasis on the use of stem cells. Stem cells are undifferentiated cells capable of giving rise to a diverse range of specialized cell types [75]. Our platform utilizes iPSCs derived from an individual patient's cells. Through the process of induction and refinement, iPSCs can be differentiated into distinct cell types [75, 76]. Both the genetic makeup and phenotype of cells must be considered because unique environmental factors (biofactors) influence cell expression and function [77]. Biofactors give cells a combined set of instructions that direct the cell to have specific functionality. By combining all of these techniques, we can create the unique cells and surrounding environment necessary to form functional organs and tissues [77-79]. A partner company has developed an industrial software that integrates liquid handling robotics and Design-of Experiments mathematics to create "recipes" for developing distinct cell types [79-81]. These manufactured specialized cells can then be used as "ink" for 3D organ printing onto biocompatible scaffolds [82].

Our platform utilizes the above tissue engineering and biofabrication techniques in combination with imaging technologies to manufacture tissues and organs. The use of 3D imaging and modeling enables the customization of tissue and organs to a patient's own unique vasculature. Matching vasculature between patients and transplantable tissues and organs can make the implantation procedure technically much easier as well as reduce surgical complications [45, 72]. Furthermore, quality control will be maintained by high content imaging, flow analyses, and molecular identification [83]. Cell-type specific functional analyses will also ensure that each manufactured tissue and organ functions properly in vivo.

How Close Are We Now?

We are closer to obtaining an unlimited supply of autologous tissues and organs than many may suspect. Our collaborative teams expect the technically simpler tissues of skin, bone and fat to be available for clinical use first. Some crucial subunits of solid organs, such as pancreatic islets of Langerhans, may be available for implantation long before entire biofabricated solid organs [8, 9, 10, 13, 78]. However, we believe that fabricated tissues and organs will be commercially available in the not so distant future.

Skin

An ideal substitute for a patient's natural skin would re-vascularize quicklyand contain important structures and cell types such as sebaceous glands, sweat glands, melanocytes and dendritic cells [10]. Products that are based on autologous cultured keratinocytes and

and fibroblasts derived from iPSCs can fulfill these requirements to create a true skin substitute. In this method, epidermal keratinocytes and dermal fibroblasts are attached to collagen-glycosaminoglycan substrates which are used to synthesize skin substitutes [10]. These tissue-engineered skins then stratify and keratinize in vitro to initiate the formation of an epidermal barrier. Proliferating keratinocytes then attach directly to dermal fibroblasts on the surface of the biopolymer sponge and initiate the development of a base membrane, which inhibits blistering after healing [20]. By incorporating complex structures such as isolated neonatal dermal cells, the biofabricated skin can contain components necessary for normal epidermis and dermis function [20, 84]. In addition, such skin replacement techniques can expand the harvested skin by 65-fold, meaning that less than 2% of TBSA is required for complete body resurfacing [20]. In the not-so-distant future it may be possible to biofabricate autologous skin for a variety of applications ranging from cosmetics to reconstruction.

Bone

Anatomically appropriate bone can be biofabricated to fit a patient's specific needs. Our platform uses 3D imaging techniques to create a digital model of a desired tissue. Other studies have applied similar techniques to create large bone grafts in shapes based on a model of the patient's anatomy [85]. Decellularized scaffolds can be sculpted based on these models with additive manufacturing (AM) techniques to produce the desired shape for each patient [86]. With AM, three-dimensional objects are produced in a computer-controlled layer-by-layer fabrication process, based on a digital model [87]. This allows scaffolds to meet macro-level anatomical requirements for a specific patient's condition, as well as micro-level requirements such as implanted cell differentiation and proliferation related to desired tissue characteristics. Also, AM techniques have relatively few steps, allowing scaffolds to be economically manufactured in a matter of days or weeks instead of months [87]. Once a scaffold is made, iPSCs derived from the patient's native cells can be differentiated into bone-specific cell types such as osteoblast, osteocyte, and immune cells [88]. These manufactured cells can then be printed onto the scaffold with a variety of bioprinting technologies to create viable autologous bone in any desired anatomical shape and size.

Fat

Adipose tissue engineering commonly involves scaffolds of polymer materials. These polymer scaffolds are made with components like polyester based absorbables, hyaluronic acid, and cologne [89]. These materials are chosen because of their biocompatibility and their ability to degrade overtime as the fat tissue develops. A common goal of biofabricating adipose tissue is the restoration of tissue volume rather than function. Therefore, scaffold shape and rigidity are important factors to provide appropriate softness and geometry specific to each patient's needs [89]. 3D imaging and modeling are combined with AM techniques to create an ideal scaffold. These scaffolds are then seeded or bio-printed with pre-adipocytes (precursor cells committed to adipocyte lineage) derived from iPSCs [89, 90]. These cells then proliferate and accumulate triacyglycerol and lipid vacuoles as they become adipocytes, forming viable autologous fat tissue [89]. Soon, it may be common clinical practice to simply "order" patient specific adipose tissue from biofabrication companies, rather than having to harvest and filter fat as part of a procedure.

Organs

Currently we are conducting studies in collaboration with several other institutions to biofabricate viable livers, kidneys, and pancreases. In regard to the liver, it has been shown that rat and pig de-cellularized and re-cellularized liver grafts can be assembled by stepwise infusion of hepatocytes, endothelial, and biliary cells, ex-vivo [91, 92]. Furthermore, protocols have been developed for generating functional and regeneration-responsive human iPSCs hepatocyte-like cells (iPSC-Heps) [93, 94]. Yet, ultimately a great number of liver cells are needed to achieve an autologous liver for clinical implantation. To this end some studies have shown that huge numbers of human iPSC-Heps can be produced with 3D micro-scale culture systems [95]. Also, many researchers have used immune-compromised animal livers as in-vivo bioreactors [91, 94, 96]. In such models human iPSC-Heps can proliferate and mature in immune compromised animals when exposed to regenerative stimuli [92]. One study went so far as to de-cellularize a rat liver and proceed to re-cellularize it with human iPSC-Heps and transplant the cellularized scaffold into immune-compromised rats. The grafts expressed hepatocyte markers and functioned properly without rupturing [91]. In the future these methods of creating large numbers of iPSC-Heps could be combined with biofabrication techniques to produce fully functional livers. This could greatly reduce costs. Current efforts to bioengineer a functional liver cost $9.7M, and only produced 35% of a liver with suboptimal hepatic function. It is expected that the models utilizing iPSC-Heps and in-vivo bioreactors could produce a fully functional liver for as little as $150,000 [97].

We have also made significant progress with fabricating kidneys. Design-of Experiment mathematics and robotic handling technologies are used to create a collection of protocols for cells within renal lineages [6, 80, 81]. This protocol could potentially be applied to manufacture any renal tissue. As an example, a new protocol utilizing renal progenitor cells was designed and implemented to develop posterior intermediate mesoderm. This is a key step in developing a functional bio-fabricated kidney. Ongoing work is focused on downstream lineage control of the renal progenitor cells, into the formation of nephron [98-100]. The ultimate goal of this project is the creation of a functional artificial kidney, and to this end the National Institute of Health (NIH) has sponsored the rebuilding the kidney consortium (RBK) [81].

At the Massachusetts Institute of Technology (MIT), Langer and Anderson labs have developed biomaterials and drug delivery systems for the successful in lab-scale manufacture and transplantation of tissue engineered pancreases. This system was accomplished by encapsulating human stem cell derived and xenogeneic islets in biocompatible materials such as triazole-thiomorpholine dioxide (TMTD) [101]. The capsules create an engineered microenvironment that constrains islets in the peripheral layers of the capsule, causing long-term controlled in vivo drug delivery [102]. MIT researchers and their associates demonstrated this by implanting immune competent mice with human stem cell derived B-cells encapsulated in alginate derived devices, capable of mitigating foreign-body response invivo. The mice were treated with streptozotocin, which chemically induces type I diabetes in animal models. The implants caused glycemic correction without causing an immune response until their removal at day 174 [102]. Furthermore, the current lead formulation has shown

to dramatically lower levels of fibrosis in non-human primates (NHPs), compared to the current gold standards. This was achieved due to triazole modification distribution forming unique hydrogel surface that inhibits recognition by macrophages and fibrous deposition [103].

Future Implications

This paper has restricted its focus to skin, bone, fat, liver, kidney, and pancreas, but other studies have reported engineering tissues such as muscles, nerves, cartilage, vessels, lungs and hearts [104]. Yet, in the future, it will become possible to create cosmetic organs and entire human parts such as a hand, foot, face, eye, etc. It may also become feasible to change their shapes into long cylinders. This would enable solid organs to be implanted using minimally invasive methods, such as robotic surgery with intuitive DaVinci robots. Although most solid organs are 5 to 10 years away from being commercially available, breasts could be biofabricated for clinical use much sooner, depending on future advances in fabricating blood supply. Breasts could be created by combining the relatively easily fabricated tissues of skin and fat [105, 106].

With advances in automation and robotics it may also soon be possible to manufacture human tissues, organs, and parts on an industrial scale. These parts could then be used to replace damaged or missing biological components in clinical settings, or to replace aging tissues for cosmetic purposes. All of these engineered human components would be monitored using embedded sensors with telemedicine try to ensure quality and function in a minimally invasive manner [107, 108]. The enormous real time data feed from these sensors would be monitored semi-autonomously with big data systems, ensuring that every patient is well monitored to reduce human error.

Challenges: Biomaterial Inks

A major challenge inhibiting advancement of biofabricating tissues and organs for clinical use, is the current lack of diverse biomaterial inks. Biomaterial inks may or may not contain living cells, but for the purposes of tissue and organ fabrication it is a requirement [109, 110]. Cell-encapsulating, or bioinks, are the class of biomaterial inks that consist of living cells. They are composed of specific cell types mixed within aqueous polymer solutions, and chemically or physically cross-linked to form hydrogels [111]. To be effective bioinks must maintain a balance between structural strength and biocompatibility, which will vary depending on the tissue type being fabricated [110]. Also, different bioinks are better suited for specific cell types. Since solid organs consist of many cell types arranged in a specific manner, a large library of bioinks is required to create a functional organiod [111, 112]. Additionally, stresses form the physical strain of bioprinting; ultra-violet light, chemical cross-linking, and high temperatures negatively affect cells in bioinks. Thus, a major bottleneck of past several decades has been a lack bioinks capable of striking a balance between structural requirements and biocompatibility for many cell lineages while protecting the embedded cells [109-112]. Although, this challenge will need to be overcome to make biofabricating replacement biological parts for implantation a common clinical practice, the field of bioprinting has developed rapidly and continues to do so [see Figure 2] [47]. Recently, significant progress has been made with bioinks being developed and utilized to successfully biofabricate bone, heart, blood vessel, liver, and skin tissues [109, 113]. Also, funding and collaborative efforts have been made to further advance this technology. For example the Advanced Regenerative Manufacturing Institute (ARMI), is a consortium of eighty-seven partners, forty-seven of which are industry based and twenty-six are academic. This consortium and others like it are committed to developing bioinks and other regenerative medicine technologies for tissue and organ fabrication [114, 115]. With such progress and considerable research

Year	Major Development
1996	Observation that cells stick together and move together in clumps
1996	First recorded use of natural biomaterial for human tissue regeneration
1998	Creation of cell sheet technology
2001	Tissue engineered bladder created using a synthetic scaffold seeded with a patient's own cells
2002	Inkjet bioprinting is enabled
2003	Inkjet printer generated cell viability
2004	Inkjet printer is modified to accurately dispense cells
2004	3D tissue construct was developed without the use of a scaffold
2006	3D cellular assembly of bovine aortal was biofabricated
2007	Bioprinting based on digital models was developed
2008	The concept of using tissue spheroids as building blocks in biofabrication was developed
2009	Advent of first commercial bioprinter, the Novogen MMX
2009	Scaffold free vascular constructs were biofabricated
2010	In situ skin printing accomplished
2010	Hepatocytes are partnered in collagen using laser direct write (LDW)
2012	Inkjet bioprinting is applied to repair human articular cartilage
2012	Bipolar wave-based drop-on-demand jetting bioprinter system is developed
2012	An engineered liver is biofabricated using an extrusion-based bioprinter system
2014	Integrated tissue fabrication with bioprinted vasculature was achieved using a multi-arm bioprinter system.
2016	Bioprinting of large scale perusable tissue constructs was achieved
References	47, 116

Figure 2: This figure gives a brief timeline of the major developments in biofabrication. It illustrates just how quickly bioprinting technologies have advanced. In the past twenty years, the field has moved from simply observing cells form and move in clumps to being able to biofabricate large scale pursuable tissue constructs.

research efforts, a bioink library diverse enough to enable large scale biofabrication may exist in the not so distant future.

Future Scenario in the Year 2050

Imagine a middle-aged woman named Sally in the year 2050 with end stage renal disease (ESRD), who requires a new kidney. Instead of searching for a compatible donor, Sally's doctors can access molding with 3D imaging technology, carefully analyzed by a team of plastic surgeons, tissue engineers, and biofabrication specialists. The model would then be altered so that the kidney becomes long, narrow, and cylindrically shaped; allowing it to be implanted through a small incisionusing intuitive DaVinci robots. While this modeling process is taking place, some of the patient'ssomatic cells would be harvested. These cells and the model of 3D model of the optimized kidney would be sent to a biofabrication company. The company would convert the somatic cells into iPSCs, and then differentiate them into distinct renal cell lineages through the process of induction and refinement [75]. These cells would then be exposed to the appropriate biofactors in accordance with the "recipes" for renal cell lineages to develop the desired cell functions [77-80]. These specialized manufactured cells would then be used as ink to 3D bioprint a new kidney. The bioprinters would be highly automated with advanced robotics to quickly and accurately biofabricate the new solid organ in accordance with the optimized digital model of Sally's kidney [117]. The patient's doctors would be notified several weeks before the completion of the manufactured organ, to plan and prepare for the transplant procedure. Once finished, Sally's new kidney would have sensors with telemetry capabilities embedded in it for in-vivo monitoring. The data from these sensors would be monitored semi-autonomously using big data analytics, and any abnormities automatically reported to Sally's physicians [107, 118]. Then the manufacture dorgan would be quickly shipped to the operating site. Once on site the manufactured cylindrical kidney would be transplanted into the patient using intuitive DaVinci robots. The narrow engineered kidney would be implanted through a small incision only a few inches long. This would allow implantation and attachment of the patient and new organ vasculature in a minimally invasive fashion. Sally's recovery time is reduced to approximately 4 to 5 days, rather than the standard 7 to 12 days most kidney transplant patients endure today [119].

Ethical Considerations

As with any revolutionary technology, the ethical implications must be considered. One of the greatest ethical dilemmas facing issue engineering and biofabrication is the source of cells used to create the tissue construct [120]. Central in the debate over what cells are ethical to use in tissue engineering is privacy of the donor, informed consent of the donor, invasiveness of the cell obtaining procedure, and ownership of donated cells [120]. These ethical concerns are valid and merit discussion. However, they rest on the premise that a third party donor will provide cells for a patient's procedure [121]. Our method of biofabricating tissues and organs negates this issue by utilizing the patient's own cells, specifically iPSCs. Thus, provided that the patient is willing and gives well informed consent, the source of the cells to create a replacement tissue or organ for the patient is non-problematic.

Another ethical concern in biofabrication is the safety of the enginered products. Though hydrogel-laden cells can be successfully printed using various bioprinting processes, little data is available regarding the metabolism of cells before, during, and after the biofabrication process [121]. If the cells become mutagenic after the bioprinting, they could lead to cancer or various other diseases [122]. Critics raise the concern that biofabricated tissues and organs are not sufficiently tested and would use humans as "guinea pigs" [121, 122]. This is a legitimate concern and further research is needed to show that cells retain their normal characteristics after the bioprinting process. Equally important is that such research is carried out in accordance to Food and Drug Administration (FDA) regulations.

Additionally another concern is distributive justice, in which only those with resources would benefit from the clinical use of biofabricated tissues and organs [120-122]. This too is an issue that merits further debate as the fields of tissue engineering and regenerative medicine continues to advance. Historically new medical technologies do not reach impoverished and under developed nations for many years, if at all [123]. However, this apparent inequality should not stop the development of tissue engineering and biofabrication technologies. Such innovations still have the potential to greatly improve and save millions of lives [1, 10]. Instead, we encourage discussion of the ethical issues raised by biofabrication, as the technology continues to advance and an era of replacing rather than repairing tissues and organs rapidly approaches.

Conclusion

We are on the cusp of a revolution in plastic and reconstructive surgery from scar to regenerative paradigm. Current standards for tissue and organ transplants have resulted in a severe scarcity of viable biologic comments. Even people who are fortunate enough to receive a transplant must still endure the challenges of adhering to long-term immunosuppression regimens as well as the associated side effects. Millions of lives can be saved or greatly improved with biofabrication and tissue engineering to produce customized autologous tissue and organs. With recent advances in these fields and 3D medical imaging, a physician in the future could order a biological part for clinical use that perfectly conforms to a patient's anatomy and is composed of a patient's own cells. We would simultaneously produce enough biological components to satisfy global transplant demand, while irradiating the risk of graft rejection and the need for immunosuppressant regimes. After one hundred years of the scar paradigm, the field of plastic and reconstructive surgery is shifting to a new era of tissue engineering and biofabrication. In this new age, plastic and reconstructive surgery practices will evolve from repairing and augmenting pieces of the body to replacing them.

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