

Solid Freeform Fabrication of a Multilayer Facial Tissue Scaffold

Pamela C. Duda
Rapid Prototyping Center
Milwaukee School of Engineering
1025 N. Broadway
Milwaukee, WI 53202. USA

Faculty Advisor: Dr. Larry Fennigkoh

Abstract

The idea of removing the face of one person to transplant it onto another may seem radical, but for severely disfigured people it may be the best chance they have at regaining a life with some semblance of normalcy. While surgeons are prepared to complete this complicated procedure, the current use of cadaver facial tissues presents many complex ethical dilemmas that are holding back this medical progress. This research uses rapid prototyping techniques to build a hollow scaffold on which a patient's own cells could be grown into the living tissues that are needed for a full facial transplant. The main tissues necessary for the transplant are skin tissue, muscle tissue, the corresponding fascia, vasculature, and some nerve tissue. Every tissue in the human body is considerably different and therefore requires slight differences in the scaffold used to grow it. This research combines the different types of scaffolds needed in the correct manner such that undifferentiated cells can be seeded onto the scaffold and the growth factors on each layer combined with the structural differences within the scaffold will cause the cells to differentiate into the proper tissues.

Keywords: tissue scaffold, skeletal muscle, skin, Tetralattice, hollow

1. Introduction

Only a few years ago, the idea of full facial transplantation was reserved for works of science fiction and blockbuster thrillers, yet surgeons in the U.S., France, and Britain have recently transformed this idea into a reality. The current procedure involves the removal of skin, muscle and some nerve tissue from a brain dead donor to be transplanted onto a severely disfigured patient. The procedure itself is feasible in as much as it is extremely similar to full hand transplants because of the tissues involved and has been successfully performed on mice in a laboratory setting. As of October of this year Dr. Maria Semionow, the director of Surgery research and training for the Cleveland Clinic Foundation, received permission to complete the world's first full facial transplant.¹ Yet even now, the current use of cadaver facial tissues presents many complex ethical dilemmas that are holding back this medical progress.

The main disputes with the procedure are ethical in nature. First of all, the procedure is not life-sustaining and is cosmetic in nature. This becomes an issue only because the patients would have to be on powerful immunosuppressive drugs for the rest of their lives.¹ The required drugs alone could lead to complications such as cancer, kidney failure, and liver damage.² Additionally, there are possible psychological difficulties for the patient his/herself and for the donor's family. If the transplanted face does not work properly, or if it looks strange or reminds either the recipient or others of the deceased donor, the effects could be very traumatic.^{1,2} Finally, there is a high possibility of an adverse impact on the public's willingness to support organ and tissue donation if facial tissue becomes a common transplant option.

The benefits of facial transplantation, however, are indisputable. After a facial transplant, the patient should theoretically regain most if not all normal movement allowing patients to regain a precious form of self-expression that currently is lost to immobile masks formed by facial skin grafts. Additionally, a facial transplant is one surgical operation which would replace the 120 or more separate major operations currently required for facial reconstruction. Surgeons predict that the transplanted face will resemble a cross between the donor and the recipient, but due to the key role that bone structure plays in facial structure, combined with the fact that the skull is

left untouched with the current procedure leads surgeons to believe that the patient should retain most of his/her original facial identity. Finally, the amount of muscle tissue that is required for the transplant will also influence the final outcome of the procedure because the less tissues that are replaced, the more likely the patient is to retain his/her former facial characteristics.¹ Surgeons in the United States, Britain and France are currently ready to complete facial transplants, but are being held back by the aforementioned ethical considerations.

This research combines the evolving field of tissue engineering with the idea of facial transplantation to overcome the dilemmas currently retarding this progress in medicine. The purpose of this research is to examine the needed microarchitecture of a multilayer tissue scaffold specially created to simultaneously grow all the tissues necessary for a full facial transplant and seek to identify the needed optimization parameters from cellular and functional perspectives.

1.1. overview of tissue scaffolds in engineering

The ultimate goal of all tissue engineering is to be able to grow a complete functional human organ so that organ donation would no longer be necessary. While this has not yet been fully accomplished, steps have been made toward this ultimate goal. Tissue engineering is basically the calculated and cultured growth of cells for a specific purpose. One method for tissue engineering includes the use of tissue scaffolds to guide the cell growth. Tissue scaffolds are necessary because experiments have shown that cells grown in a flat dish often act independently of one another, whereas cells grown on a three-dimensional scaffold tend to become more interdependent and develop the characteristics of the desired tissue.³ There are two main considerations that have to be made when creating a tissue scaffold: finding a suitable material and the macro- and microstructure of the scaffold.

1.1.1. material choice

The desired material characteristics for a tissue scaffold include biocompatibility and biodegradability. Biocompatibility is important in as much as the material should not provoke unwanted tissue response to the implant and its surface chemistry must promote cell attachment and function. Biodegradability applies to the facts that the scaffold must degrade into nontoxic products once implanted, it must leave the desired living tissue, and it must have the proper degradation rate such that the scaffold degrades at approximately the same rate as the tissue forms. Materials with these characteristics include natural and synthetic polymers, ceramics, metals, or combinations thereof.⁴ Table 1 shows a few of the many materials that would be appropriate for the creation of tissue scaffolds.^{5,9}

The materials recommended for this particular project are Poly (ether-ether-ketone) (PEEK) and hydroxyapatite (HA). These two materials have been proven to work well with the Selective Laser Sintering (SLS) process to create well-defined porous specimens with good interconnectivity.⁹

Table 1 materials for the creation of porous solid-state scaffolds

Material Name	Pros	Cons	Notes
Poly (glycolic acid) (PGA)	relatively hydrophilic nature; degrades rapidly in aqueous solutions or in vivo; loses mechanical integrity between two and four weeks		degrades through hydrolysis of the ester bonds; one of the most widely used polymers as of 2004
Poly(lactic acid) (PLA)	more hydrophobic than PGA; slower hydrolysis rate than PGA; Takes many months or years to lose mechanical integrity in vitro or in vivo		degrades through hydrolysis of the ester bonds
Poly(lactic acid-co-glycolic acid) (PLGA)	composite polymer of PGA and PLA		degrades through hydrolysis of the ester bonds
Poly(ϵ-caprolactone) (PCL)	slow degradation = great for long-term implants and controlled release applications	Slow degradation = bad for general tissue engineering	

Poly(propylene fumarate) (PPF)			degrades through hydrolysis of the ester bonds
Tyrosine-derived polymers	promising biocompatibility		
Segmented polyurethanes	allow structural variations needed to achieve a range of mechanical properties		
Collagen-glycosaminoglycan (GAG)		Potential pathogen transmission; immune reactions; poor handling ; and mechanical properties ; less controlled degradability	
Denatured collagen (gelatin)		Potential pathogen transmission; immune reactions; poor handling ; and mechanical properties ; less controlled degradability	
Silk	can degrade in vivo via enzymatic mechanisms; degradation rate is very slow	Concern over cytotoxicity	
Small Intestinal Submucosa (SIS)		Concerns over pathogen transmission and immune rejection	Contains type I collagen, GAGs, and some growth factors
Poly (ether-ether-ketone) (PEEK)	very good for SLS built scaffolds		average particle size = 25µm; marketed under the brand name Peek™ 150XP (Vitrex Plc, Lancashire, UK); not bioactive
Hydroxyapatite (HA)	very good for SLS built scaffolds		Sold under the brand name Camceram II HA (Cam Implants BV, The Netherlands); Particle size distribution with at least 90wt% below 60µm; average material density = 3.50 g/cm ³ ; bioactive

1.1.2. *micro- and macrostructure*

The scaffold architecture contributes significantly to the specification / differentiation of the stem cells into the correct cells to perform the desired biological functions. It also provides needed nutritional and spatial organization for cell growth. A large surface area and pore volume are both highly recommended in the design of tissue scaffolds. The large surface area favors cell attachment and growth and the large pore volume is necessary to accommodate and deliver cell mass sufficient for tissue repair. Additionally, high porosity allows for easy diffusion of nutrients to and wastes from the implant and aids in vascularization. Proper nutrient and waste flow is extremely important for tissue engineering because cells need to be no more than 200 µm away from a blood supply or they will become either metabolically inactive or necrotic due to low oxygen tension.⁴

As the desired amount of tissue growth rises, so too must the porosity to accommodate the extra cell growth unless some other form of nutrient and waste flow is created. Pore size is the most important aspect of tissue scaffold design because it is the pore size that determines most directly the type of cells that can grow on the scaffold.⁹ Minimum scaffold pore size is determined by the diameter of the desired cells in suspension, which varies

greatly from one cell type to another.⁴ Additionally, micro or even nanoporous scaffolds have been found to facilitate better cell growth and in vivo vascularization in some cases.⁹

1.1.3. scaffold formation

Throughout the past twenty years, tissue scaffolds have been created using many different techniques. Some of the more common techniques and their pros and cons are listed in Table 2.^{5,9} While all of these methods present viable options, the method chosen for this research is Selective Laser Sintering.

Table 2 techniques for the creation of porous solid-state scaffolds

Process Name	Pros	Cons	Notes
Textile Technologies : PGA nonwoven Scaffolds	used for Cartilage, tendon, ureter, intestine, blood vessels, heart valves and other tissues	low mechanical strength; fast degradation rate; difficulty in controlling pore shape; limited fiber diameter variations	
Particulate-leaching techniques	easy to carry out; pore size and porosity can be controlled	critical variables such as pore shape and inter-pore openings are not controlled	
Phase Separation		Pores have diameters on the order of a few to tens of microns and are often not uniformly distributed	Not suitable for tissue engineering applications
Paraffin Spheres	higher resolution; controlled interconnected spherical pores; cheap but effective		Spheres fabricated by a dispersion method
Three-Dimensional Printing [RP]		smallness of powder particles and binder drops are limited to a few hundred microns; accuracy of positioning printing nozzle is limited; preciseness limited; high shrinkage of scaffolds often encountered	often used to create negative molds for tissue scaffolds to be built in
Fused Deposition Modeling (FDM) [RP]		limited to simple geometric shapes; post processing steps are required to remove support material	not suitable for many Tissue engineering scaffolds
Stereo Lithography (SL) [RP]		Difficult to find biocompatible polymers for; tedious and complex process	
Rapid Prototyping Techniques [RP]		limited material selection; inadequate resolution; structural heterogeneity;	Reverse Fabrication technique = more homogeneous scaffolds (make a negative then pour in the polymer solution)
Selective Laser Sintering (SLS) [RP]	can build complex 3-D models without the need for support structures or the use of organic solvents;		The biomaterials PEEK and HA have been successfully used to make complex, viable porous interconnected scaffolds

1.2 selective laser sintering (SLS)

Selective Laser Sintering is a form of Solid Freeform Fabrication (or Rapid Prototyping). Solid Freeform Fabrication is a method of building objects from a .stl format computer file one very thin layer a time until the entire

object is formed. Selective Laser Sintering does this using a powdered polymer that is selectively sintered together layer by layer forming the desired object. The following is a description of the SLS process.¹¹

Selective Laser Sintering (SLS)

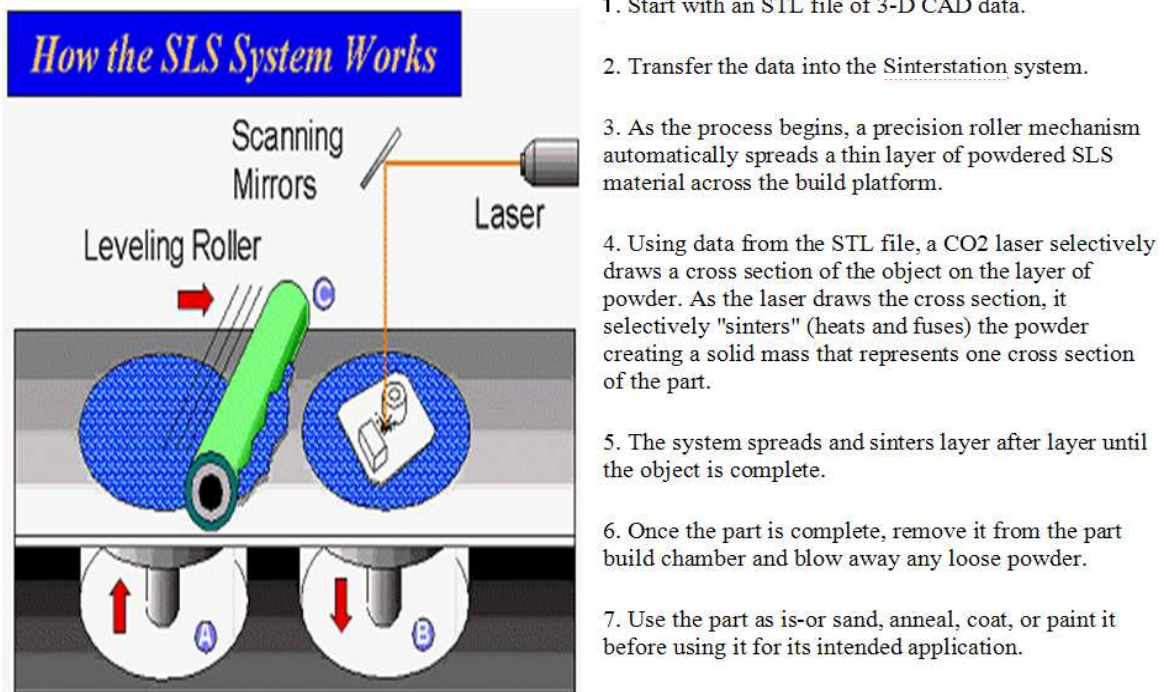


Figure 1 Image of part of the selective laser sintering apparatus and a description of its operation¹¹

2. Methodology

The tissues involved in facial transplantation were all thoroughly analyzed from anatomic and physiologic perspectives. The tissues that this scaffold was designed to facilitate the growth of are skin tissue and skeletal muscle tissue. Skin tissue is composed of two main layers: the epidermis and the dermis. The epidermis is made of four different cell types: keratinocytes, melanocytes, langerhans' cells, and merkel cells. The dermis is composed of fibroblasts, macrophages, occasional mast cells white blood cells, and a semi fluid matrix embedded with collagen, elastin and reticular fibers.⁶ Dr. Ioannis Yannas' research entitled "Discovery of induced regeneration of organs in adults. Synthesis of the first biologically active scaffold" has shown that a scaffold with the correct macro and microarchitecture to promote the growth of the dermis, when seeded with cells will simultaneously grow both the dermis and the epidermis.⁷ Based on this and the optimized range of pore size being 20 to 125 μm in diameter, the top layer of this multilayer scaffold was designed to have a pore size of 125 μm .⁴ Skeletal muscle cells have a diameter between 10 and 100 μm .⁶ The optimized range of scaffold pore sizes for skeletal muscle tissue is 225-500 μm .¹⁰ For this research, a pore size of 250 μm was decided upon.

2.1. scaffold architecture

Based on the thorough research that was completed, it appears that most tissue scaffolds are created with the proper pore size but remain rather disorganized in structure. Some scaffolds are formed with the concept of mimicking a textile appearance; others seem to have no logical order at all. This research uses the new concept of applying the

Tetralattice™ design created at the Milwaukee School of Engineering (MSOE) to tissue engineering. The Tetralattice™ structure is very complex yet ordered and should easily provide the large porosity required for the growth of the required mass of tissue.

To assist the tissue growth, the scaffold was designed to be hollow and microporous to allow for additional transportation of nutrients and wastes through the scaffold instead of just surrounding it. The hollow scaffold has an internal diameter of 340µm with a 500µm wall thickness and is based on research that successfully infiltrated a scaffold design longitudinally and radially with channels.⁸ Micropores are a natural by-product of the SLS process and should theoretically create inlets and outlets from the scaffold interior to the exterior environment for waste and nutrient transportation. Research has shown that the idea of creating a tissue scaffold such that the entire scaffold mimics vascularization is new.

3. Data

A hollow multilayer facial tissue was created using the SLS process as described in the *methodology* and *scaffold architecture* sections of this paper. The only difference between the design that was described and the scaffolds that were created is that the produced models are a scale of ten larger than the design specifications due to limitations of the Sinterstation 2500 Plus machine available at MSOE. The basic Tetralattice™ structure was obtained from a library at MSOE and was used to create the scaffold design. The scaffold was designed using the computer program RP Magics. The hollow nature was obtained through the careful Boolean Subtraction of an identical but offset scaffold from each layer of the scaffold such that the offset scaffold created the internal hollow dimensions desired. The file was saved in the .stl format and was created using both the Duraform PA and Duraform GF polymers present in the Rapid Prototyping Center (RPC). Five models were successfully created in this manner. Two of the models are enlarged unit cells from the skin scaffold and the skeletal muscle scaffold to illustrate the hollow nature of the scaffold (see figure 2) . The third and fourth models are scaled rectangular prisms of the skin scaffold and the skeletal muscle scaffold (see figure 3) . Finally, the fifth model is the multilayer facial tissue scaffold where both the skin scaffold and the skeletal muscle tissue scaffold are attached to one another and were created at the same time (see figure 4).

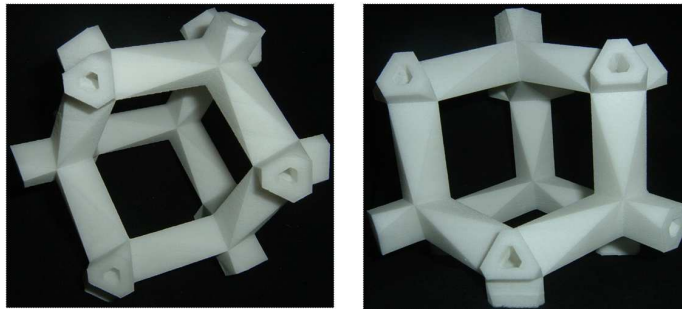


Figure 2 Picture of one unit cell from the skin scaffold (left) and the skeletal muscle scaffold (right)

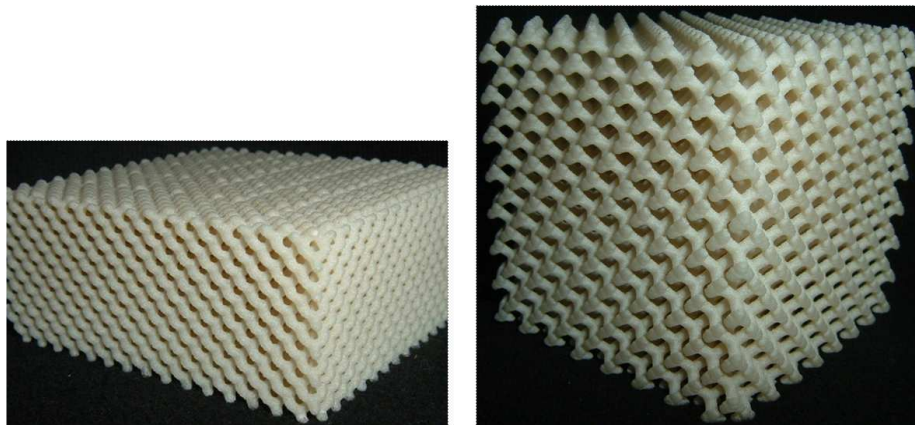


Figure 3 Picture of the skin tissue scaffold (left) and the muscle tissue scaffold (right)

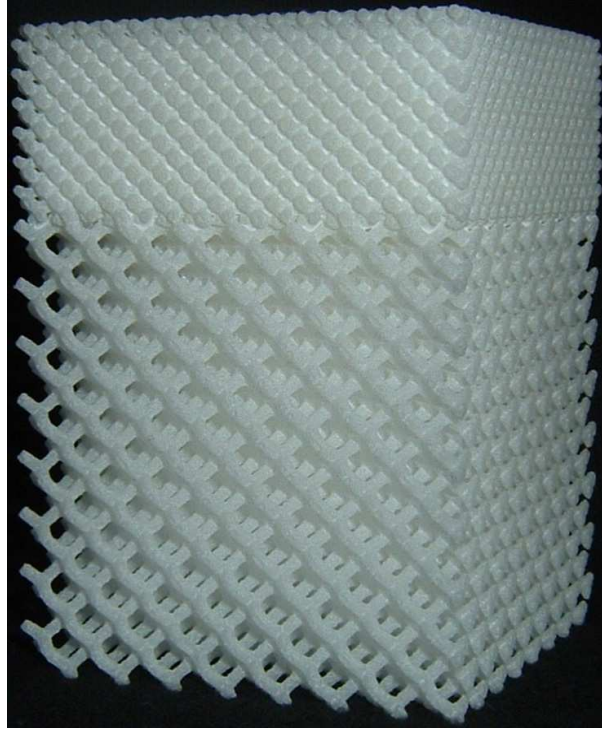


Figure 4 Picture of the final multilayer tissue scaffold

4. Conclusion

In conclusion, this research has shown that the creation of a hollow multilayer facial tissue scaffold is possible using the solid freeform fabrication technique of Selective Laser Sintering. Theoretically, this scaffold design should be producible at a usable scale on a micro-SLS machine. Additional research should be conducted to see if it is in fact possible to build a usable model of this scaffold with current SLS technologies. Also, the scaffold design should be tested by creating the scaffold using Poly (ether-ether-ketone) (PEEK) and hydroxyapatite (HA), adding the proper growth factors, and seeding the scaffold with stem cells to determine whether the hollow nature of the scaffold does in fact assist in the tissue growth and whether the Tetralattice™ structure truly does lend itself to the advancement of tissue engineering. Finally, the microporosity of the scaffold must be tested once the scaffold is created with the proper materials and in the proper size. This test can be done by submerging the scaffold in water that has been dyed with an indicator dye then using a small air pump to pump air through the hollow scaffold while observing any air bubbles created. If the natural microporosity caused by the SLS process is not effective at producing inlets and outlets from the inside of the scaffold to the outer environment, salt leaching should be used to create the micropores. This can be done by mixing popcorn salt into the powder used by the SLS machine so that it is incorporated into the scaffold as it is being sintered. The salt will then later be leached out of the scaffold using distilled water leaving micropores for nutrient and waste transfer. The idea of incorporating salt into a scaffold created using solid freeform fabrication to later be leached out has been shown to be effective in creating a microporous structure⁸ and should create micropores throughout the entire scaffold.

5. Acknowledgements

This project was funded by the National Science Foundation (NSF) and the Milwaukee School of Engineering (MSOE) Rapid Prototyping Center (RPC). Special thanks to Dr. Larry Fennigkoh (Advisor), Vito Gervasi, Gunner, Betty Albrecht, Ann Bloor, and to all of the MSOE and RPC staff who supported this project. Also, thank you to the other REU participants past and present for your input, ideas and support throughout the completion of this research.

6. Disclaimer

This material is based upon work supported by the National Science Foundation under Grant No. EEC-0139142. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

7. References

1. Peter Gorner, "Surgery's Next Step: Face Transplants," Chicago Tribune Early Edition, June 12, 2005, 1 & 14.
2. Arthur Caplan, "Face-off Over facial transplants," MSNBC.com, <http://www.msnbc.msn.com/id/5122174/print/1/displaymode/1098/>.
3. Gary Goettling, "The Art and Science of Healing," <http://gtalumni.org/Publications/magazine/win98/artheal.html>.
4. Shoufeng Yang, Ph.D., Kah-Fai Leong, M.S.E., M.S.M.E., Zhaohui Du, Ph.D., and Chee-Kai Chua, Ph.D., "The Design of Scaffolds for Use in Tissue Engineering. Part I. Traditional Factors," *Tissue Engineering* 7, no. 6 (2001): 679-689.
5. Peter X. Ma, "Scaffolds for Tissue Fabrication," *Materialstoday* (May 2004): 31-37.
6. Elaine N. Marieb, *Human Anatomy & Physiology*, 5th ed. (San Francisco: Benjamin Cummings, 2001), 148-152: 277-279: 313.
7. Ioannis Yannas, Ph.D., "Discovery of induced regeneration of organs in adults. Synthesis of the first biologically active scaffold," Research Focus, MIT Biological Engineering Division, <http://web.mit.edu/be/people/yannas.htm>.
8. E. Sachlos and J.T. Czernuszka, "Making Tissue Engineering Scaffolds Work. Review on the Application of Solid Freeform Fabrication Technology to the Production of Tissue Engineering Scaffolds," *European Cells and Materials* 5, (2003): 34 and 37.
9. K.H. Tan, C.K. Chua, K.F. Leong, M.W. Naing and C.M. Cheah, "Fabrication and Characterization of Three-Dimensional Poly (ether-ether-ketone) / - Hydroxyapatite Biocomposite Scaffolds Using Laser Sintering," *Journal of Engineering in Medicine* 219, no. H3 (2005): 184 and 192.
10. Shulamit Levenberg, Jeroen Rouwkema, Mara Macdonald, Evan S Gargein, Daniel S. Kohane, Diane C. Darland, Robert Marini, Clemens A van Blitterswijk, Richard C Mulligan, Patricia A D'Amore, and Robert Langer, "Engineering vascularized skeletal muscle tissue," *Nature Biotechnology* 23, no. 7 (2005): 879-880.
11. "How it Works," Selective Laser Sintering (SLS), <http://www.techok.com/slsworks.html>.