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Advances in Tissue Engineering

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Abstract

Nearly 30 years ago, we reported on a concept now known as Tissue Engineering. Here, we report on some of the advances in this now thriving area of research. In particular, significant advances in tissue engineering of skin, liver, spinal cord, blood vessels, and other areas are discussed.

Keywords

tissue engineering; regenerative medicine; biodegradable materials

Introduction

Nearly 30 years ago, we wrote a paper entitled “Selective cell transplantation using bioabsorbable artificial polymers as matrices [1].” In this paper we described attaching cell preparations to bioerodable artificial polymers in cell culture and then implanting this polymer-cell scaffold into animals. Using techniques of cell harvest, single cells and clusters of fetal and adult rat and mouse hepatocytes, pancreatic islet cells and small intestinal cells were seeded onto a number of different biodegradable polymers. Sixty-five fetuses and 14 adult animals served as donors. One hundred fifteen polymer scaffolds were implanted into 70 recipient animals: 66 seeded with hepatocytes, 23 with intestinal cells and clusters and 26 with pancreatic islet preparations. The cells remained viable in culture and, in the case of fetal intestine and fetal hepatocytes, appeared to proliferate while on the polymer. After four days in culture the cell-polymer scaffolds were implanted into host animals, either in the omentum, the interscapular fat pad, or the mesentery. In three cases of fetal intestinal implantation coupled with partial hepatectomy successful engraftment occurred in the omentum, one forming a visible 6.0 mm cyst. Three cases of hepatocyte implantation, one using adult cells and two using fetal cells, also engrafted showing viability of hepatocytes, mitotic figures, and vascularization of the cell mass. We termed this concept “chimeric

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neomorphogenesis.” Over time, it provided a major basis for what is now called Tissue Engineering.

In 1993, we wrote a paper in *Science* entitled “Tissue engineering” where we defined tissue engineering 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” (see figure 1). This paper has been cited approximately 5200 times. Today tissue engineering is a thriving areas. As of 2011, there are over 100 companies involved in tissue engineering, employing 14,000 people and generating nearly \$4 billion in sales [2]. Here we discuss some of the progress that’s been made and the challenges ahead.

Liver and Vital Organs

In our initial paper describing what is now known as tissue engineering, we presented data on cell-scaffolding implantation of liver, pancreas, and intestine. This work pointed to the hope of creating vital organs on demand to solve the organ shortage worldwide. Through the years since presentation at APSA in May, 1987, vital organ tissue engineering has continued to be a major focus of our laboratories with a major emphasis on liver fabrication. After the initial work which demonstrated proof of principle, studies were performed to increase implanted liver cell mass. The small bowel mesentery was used as a large vascularized tissue bed for the placement of the liver cell scaffolding between multiple mesenteric leaves. Hepatocytes were obtained by collagenase perfusion of livers of co-genic Wistar rats and were seeded onto non-woven filamentous sheets of polyglycolic acid 1x3 cm. in size, 2 mm in thickness, at density of 500,000 cells/cm². They were implanted into 26 Gunn rats (UDP-glucuronyl transferase deficient). Eight sheets per animal were implanted. Moderate inflammation, neovascularization, and the presence of hepatocytes were seen in 96% of animals. Conjugated bilirubin was identified in 46% of animals implanted with normal hepatocytes and none detected in animals implanted with deficient liver hepatocytes [3].

We then studied implantation in a large animal canine model of hyperuricosuria. The Dalmatian dog is known to have an inborn error of metabolism in the hepatocyte which causes a decrease in the degradation of uric acid into allantoin. This leads to a rise in uric acid levels in blood and urine. Four male Dalmatian dogs were used as recipients of normal hepatocytes. Poly vinyl alcohol sponges measuring 250 cm.2×5 mm were implanted between the leaves of mesentery and 1.5×10(10) normal hepatocytes from donor beagles were implanted into prevascularized sponges after a portacaval shunt had been created for hepatotrophic stimulation. Excretion of uric acid decreased from 136.3 to 44.1 micromol/kg/day from week 2 to week 6. Control animals remained unchanged [4].

Although these results were promising, we concluded that the results were not adequate to produce a device for human testing. These approaches all relied on angiogenesis to form permanent vascularized new tissue and surviving, functioning cell mass was not sufficiently safe. Consequently, we developed a new approach in 1998 in which the vascular supply was designed and engineered as part of the cell scaffold implant. Using standard photolithography techniques (figure 2), trench patterns reminiscent of the branched architecture of vascular and capillary networks were etched onto silicon and Pyrex surfaces

to serve as templates. Hepatocytes and endothelial cells were cultured and subsequently lifted as single cell monolayers from these two dimensional molds. Both cell types were viable and proliferative on these surfaces. In addition, the hepatocytes maintained albumin production. The lifted monolayers were then folded into compact 3 dimensional tissues thereby demonstrated a new approach to large scale new tissue production (figure 3) [5].

In 1997, we described the use of a novel 3 D printer prototype to fabricate complex scaffolding for tissue engineering directly in three dimensions on the scale of hundreds of microns [6]. Since that publication, the technology has advanced to where extreme resolution approaching 25 microns can be achieved. Over the past several years, bioprinting of cells in hydrogels has emerged as a new tool for the field [7]. Organ decellularization in which biological scaffolding can be created by detergent washing of liver and other organs is now being developed to provide scaffolding which can be recellularized with organ specific cells as well as the cells of the vascular circulation since the vascular architecture is also preserved [8].

Spinal cord

Each year approximately 10,000 Americans sustain spinal cord injuries (SCI). Functional deficits following SCI result from damage to or severance of axons, loss of neurons and glia, and demyelination. SCI pathology is determined not only by the initial mechanical insult, but also by secondary processes including ischemia, anoxia, free-radical formation, and excitotoxicity that occur over hours and days following injury [9]. We sought to develop a tissue engineering approach that simulated the architecture of the healthy spinal cord through an implant consisting of a polymer scaffold seeded with neural stem cells (NSCs) modeled after the gray and white matter of the intact cord. The scaffold's inner portion would stimulate the gray matter via a porous polymer layer designed to be seeded with NSCs [10] for cellular replacement as well as trophic support. The outer portion would stimulate the white matter with long, axially oriented pores for axonal guidance and radial porosity to allow fluid transport while inhibiting ingrowth of scar tissue. The scaffold is designed to be tailored to fit into a variety of cavities. In our initial study, the scaffold was tailored to fit into the cavity created by a midline lateral hemisection in the spinal cord of an adult rat.

In a 50 animal study, implantation of the scaffold–neural stem cells unit into an adult rat hemisection model of SCI promoted long-term improvement in function (persistent for 1 year in some animals) relative to a lesion-control group. At 70 days post injury, animals implanted with scaffold-plus-cells exhibited coordinated, weight-bearing hind limb stepping. Histology and immunocytochemical analysis suggested that this recovery might be attributable partly to a reduction in tissue loss from secondary injury processes as well as in diminished glial scarring. Tract tracing demonstrated corticospinal tract fibers passing through the injury epicenter to the caudal cord, a phenomenon not present in untreated groups. Increased local GAP-43 expression was not seen in treated animals and these results suggest a possible regeneration component [11].

While rodent models exhibit high degrees of spontaneous recovery from SCI injury, animal care concerns preclude complete cord transections in non-human primates and other larger vertebrate models. To overcome such limitations a segmental thoracic (T9–T10) spinal cord hemisection was created and characterized in the African green monkey. Physiological tolerance of the model permitted behavioral analyses for a prolonged period post-injury, extending to predefined study termination points at which histological and immunohistochemical analyses were performed. Four monkeys were evaluated (one receiving no implant at the lesion site, one receiving a poly(lactide-co-glycolide) (PLGA) scaffold, and two receiving PLGA scaffolds seeded with human neural stem cells (hNSC)). All subjects exhibited Brown-Séquard syndrome 2 days post-injury consisting of ipsilateral hind limb paralysis and contralateral hind limb hypesthesia with preservation of bowel and bladder function. A 20-point observational behavioral scoring system allowed quantitative characterization of the levels of functional recovery. Histological endpoints including silver degenerative staining and Iba1 immunohistochemistry for microglial and macrophage activation, were determined to reliably define lesion extent and correlate with neurobehavioral data. These studies also showed efficacy and safety similar to the rat studies [12]. Most recently, this procedure was approved for human testing. At this time, the first 2 patients have been treated with no negative consequences and with initial promising results.

Blood vessels

We developed techniques to produce small-caliber autologous arteries *in vitro* from vascular cells grown on a biodegradable polymer matrix, by means of a pulsatile perfusion system for vessel culture. Ideal biological grafts should possess a confluent endothelium and differentiated, quiescent, smooth muscle cells (SMCs), as well as sufficient mechanical integrity and elastic moduli to allow suture retention and tolerance of systemic arterial pressures. Because vascular cells are exposed to pulsatile physical forces during most of vasculogenesis [13] and throughout life, we hypothesized that the development of arteries in the laboratory would be facilitated by the application of pulsatile physical stress to cultured vascular cells [14].

The biomimetic system we developed for vessel culture is composed of bioreactors containing engineered vessels assembled in a parallel flow system. In initial experiments, a suspension of cultured SMCs isolated from the medial layer of bovine aorta [15] was pipetted onto tubular biodegradable polyglycolic acid (PGA) scaffolds that were secured in reactors [16]. The surface of the PGA scaffolds was chemically modified [17] to increase hydrophilicity, increase adsorption of serum proteins, and improve SMC attachment. After an initial SMC seeding period of 30 min, the bioreactors were filled with medium and the SMCs were cultured under conditions of pulsatile radial stress for 8 weeks. Control vessels were cultured without pulsatile radial stress under otherwise identical conditions.

To assess the practical utility of these cultured arteries *in vivo*, we undertook initial implantation studies in miniature swine. Autologous arterial SMCs and ECs were cultured for each 6-month-old animal from small biopsies of the common carotid artery. *In vitro* graft controls were done without pulsatile flow. All animals underwent implantation into the right saphenous artery, which is a branch of the femoral artery and is the largest artery in the

distal half of the hind limb. Animals were followed for up to 4 weeks, after which time the grafts were explanted for assessment of histology and contractile function postoperatively as assessed by Doppler. A pulsed xenograft vessel was examined by digital subtraction angiography at 24 days after implantation and was patent, without evidence of stenosis or dilatation. The xenograft also had unchanged contractile responses to prostaglandin $F_{2\alpha}$ when explanted at 4 weeks. A pulsed autologous graft remained open for 4 weeks as assessed by Doppler [14]. This general approach is now being tested in humans using decellularized systems with initial promising results.

We developed the first approach for controlling the differentiation of human embryonic stem cells into vascular endothelial cells [18].

Additional areas

In other studies, we engineered cartilage, heart valves, bone, intestine, urological structures, tendons, and muscle. This work has also helped lead to the creation in humans of skin [19] now approved for burn victims and patients with diabetic skin ulcers (e.g., marketed by Shire), urinary bladder [20] and cartilage. Already over a million patients have received tissue engineered human skin (for burns or diabetic skin ulcers) based on our discoveries (e.g., Dermagraft, uses the exact polymer we originally used, polylactic glycolic acid, in this case with neonatal fibroblasts).

Coupled with these discoveries of the necessary biological principles of tissue formation, we developed new chemical approaches to produce scaffolding materials, which would specifically signal genetic cellular events including proliferation, provide attachment sequences for the cells, as well as augment angiogenesis, all in harmony to produce normal tissue [21]. We also created new biodegradable materials to further advance scaffolding technology for tissue engineering [22]. Other contributions include the first methods to create materials for controlling stem cell differentiation [23], methods of using stem cells to create muscle [24], methods of using materials to create heart tissue [25], and synthesizing the first surfaces for growing stem or iPS cells in a completely xenofree, serum free environment [26].

The future

This brief summary only touches on the progress that has been made since 1987 in the fields of tissue engineering and regenerative medicine. Almost every tissue of the human body has been studied for the possibility of replacement with living tissue engineered structures. Every year more clinical trials are showing success leading to clinical application. Progress will continue with collaborative approaches among clinicians, scientists and engineers.

References

1. Vacanti J, Morse M, Saltzman M, et al. Selective cell transplantation using bioabsorbable artificial polymers as matrices. *J Pediatr Surg.* 1988; 23:3–9. [PubMed: 2895175]
2. Jaklenec A, Stamp A, Deweerd E, et al. Progress in tissue engineering and stem cell industry “Are we there yet?”. *Tissue Eng.* 2012; 18:155–166.

3. Johnson L, Aiken J, Mooney D, et al. The mesentery as a laminated vascular bed for hepatocyte transplantation. *Cell Transplant*. 1994; 3:273–281. [PubMed: 7921632]
4. Takeda T, Vacanti J. Hepatocyte transplantation in the Dalmatian dog model of hyperuricosuria. *Tissue Eng*. 1995; 1:355–360. [PubMed: 19877899]
5. Griffith L, Wu B, Cima M, et al. *In vitro* organogenesis of liver tissue. *Annu NY Acad Sci*. 1997; 831:382–397.
6. Kaihara S, Borenstein J, Koka R, et al. Silicon micromachining to tissue engineer branched vascular channels for liver fabrication. *Tissue Eng*. 2000; 6:105–117. [PubMed: 10941206]
7. Bajaj P, Schweller R, Khademhosseini A, et al. 3D biofabrication strategies for tissue engineering and regenerative medicine. *Annu Rev Biomed Eng*. 2014; 16:247–276. [PubMed: 24905875]
8. Gilpin S, Ren X, Okamoto T, et al. Enhanced lung epithelial specification of human induced pluripotent stem cells on decellularized lung matrix. *Annu Thorac Surg*. 2014; 98:1721–1729.
9. Beattie M, Farooqui A, Bresnahan J. Review of current evidence for apoptosis after spinal cord injury. *J Neurotrauma*. 2000; 17:915–925. [PubMed: 11063057]
10. Yandava B, Billingham L, Snyder E. “Global” cell replacement is feasible via neural stem cell transplantation: Evidence from the dysmyelinated *shiverer* mouse brain. *Proc Natl Acad Sci*. 1999; 96:7029–7034. [PubMed: 10359833]
11. Teng Y, Lavik E, Qu X, et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc Natl Acad Sci*. 2002; 99:3024–3029. [PubMed: 11867737]
12. Pritchard C, Woodard E, Langer R, et al. Establishing a model spinal cord injury in the African Green Monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells. *J Neurosci Meth*. 2010; 188:258–269.
13. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995; 11:73–91. [PubMed: 8689573]
14. Niklason G, Gao J, Abbott W, et al. Functional arteries grown *in vitro*. *Science*. 1999; 284:489–493. [PubMed: 10205057]
15. Ross R. The smooth muscle cell growth of smooth muscle in culture and formation of elastic fibers. *J Cell Biol*. 1971; 50:172–186. [PubMed: 4327464]
16. Niklason L, Langer R. Advances in tissue engineering of blood vessels and other tissues. *Transplant Immunol*. 1997; 5:303–306.
17. Gao J, Niklason L, Langer R. Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells. *J Biomed Mater Res*. 1998; 42:417–424. [PubMed: 9788505]
18. Levenberg S, Golub J, Amit M, et al. Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci*. 2002; 99:4391–4396. [PubMed: 11917100]
19. Hansbrough J, Dore R, Hansbrough W. Clinical trials of a living dermal tissue replacement placed beneath meshed, split thickness skin grafts on excised burn wounds. *J Burn Care Rehabil*. 1992; 13:519–529. [PubMed: 1452585]
20. Atala A, Bauer S, Soker S, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet*. 2006; 367:1241–1246. [PubMed: 16631879]
21. Barrera D, Zylstra E, Lansbury P, et al. Synthesis and RGD peptide modification of a new biodegradable copolymer system: Poly(lactic acid-co-lysine). *J Am Chem Soc*. 1993; 115:11010–11011.
22. Wang Y, Ameer G, Sheppard B, et al. A tough biodegradable elastomer. *Nat Biotechnol*. 2002; 20:602–606. [PubMed: 12042865]
23. Anderson D, Levenberg S, Langer R. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat Biotechnol*. 2004; 22:863–866.
24. Levenberg S, Rouwkema J, Macdonald M, et al. Engineering vascularized skeletal muscle tissue. *Nat Biotechnol*. 2005; 23:879–884.
25. Engelmayr G, Cheng M, Bettinger C, et al. Accordion like honeycombs for tissue engineering cardiac anisotropy. *Nat Mater*. 2008; 7:1003–1010. [PubMed: 18978786]

26. Mei Y, Saha K, Bogatyrev S. Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. *Nat Mater.* 2010; 9:768–778. [PubMed: 20729850]

Biographies



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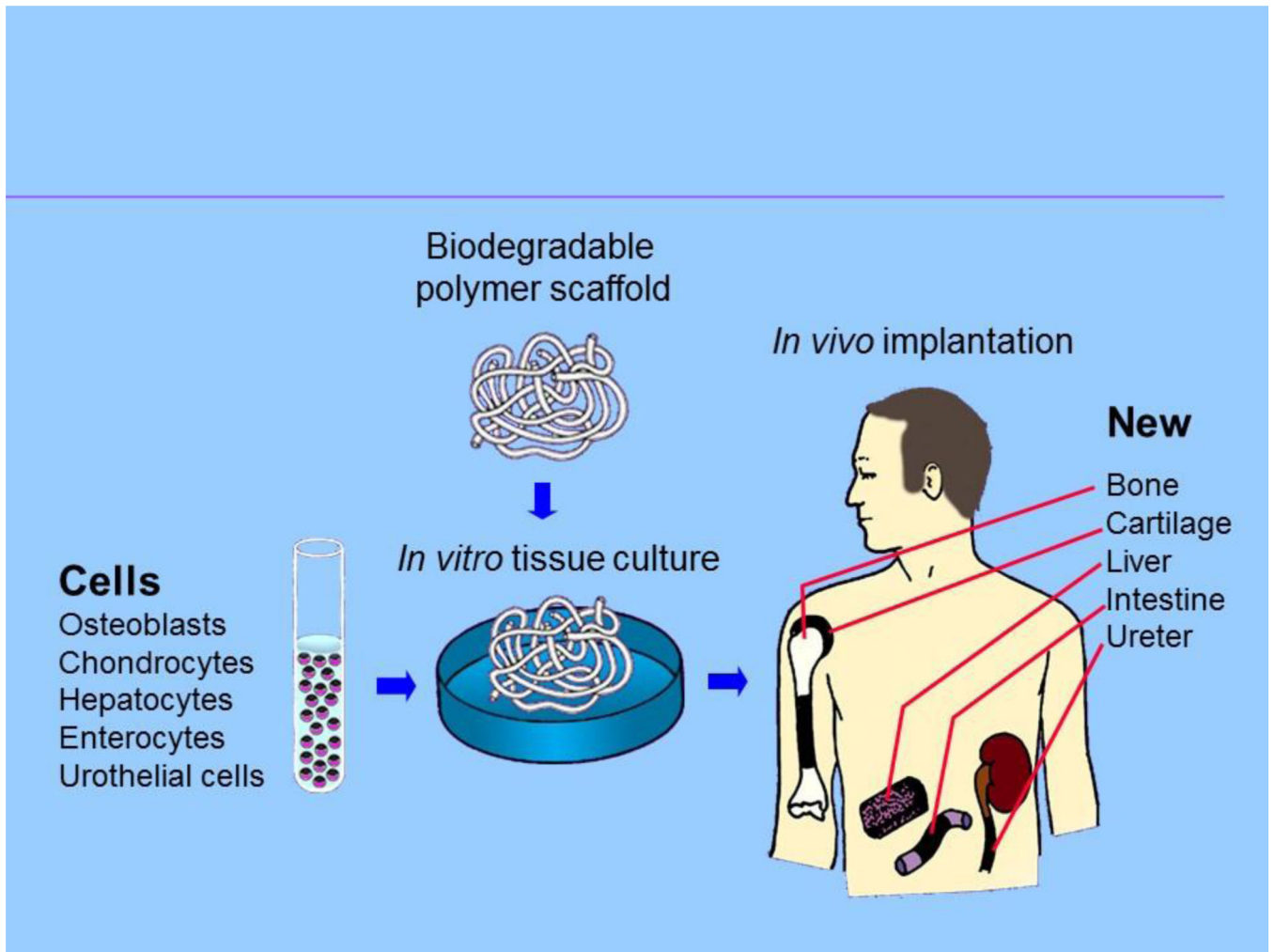
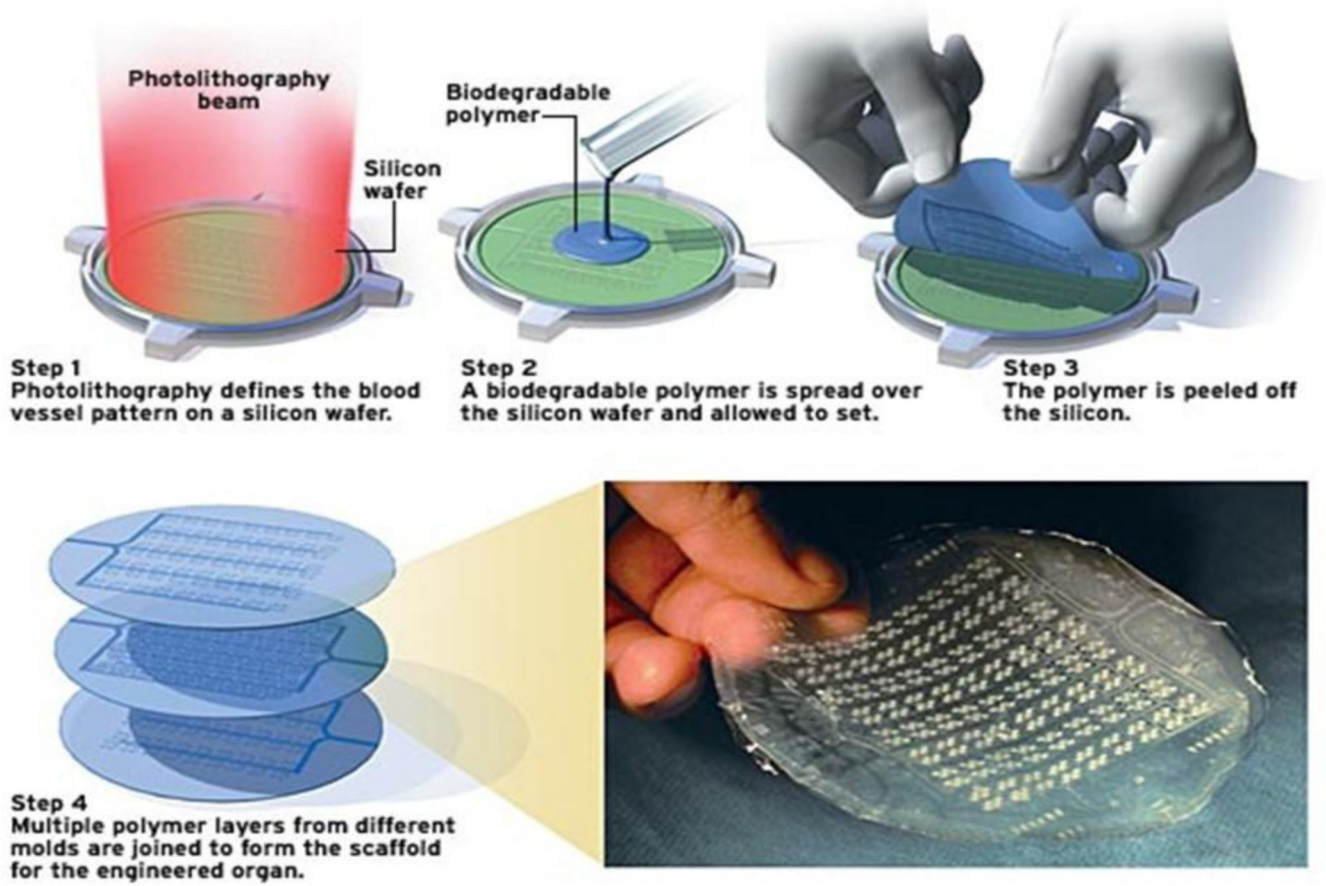


Figure 1.
Tissue Engineering Schematic



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Figure 2.
Photolithographic approach to create a vascular bed for tissue engineered liver.

Interconnected Lobule Network

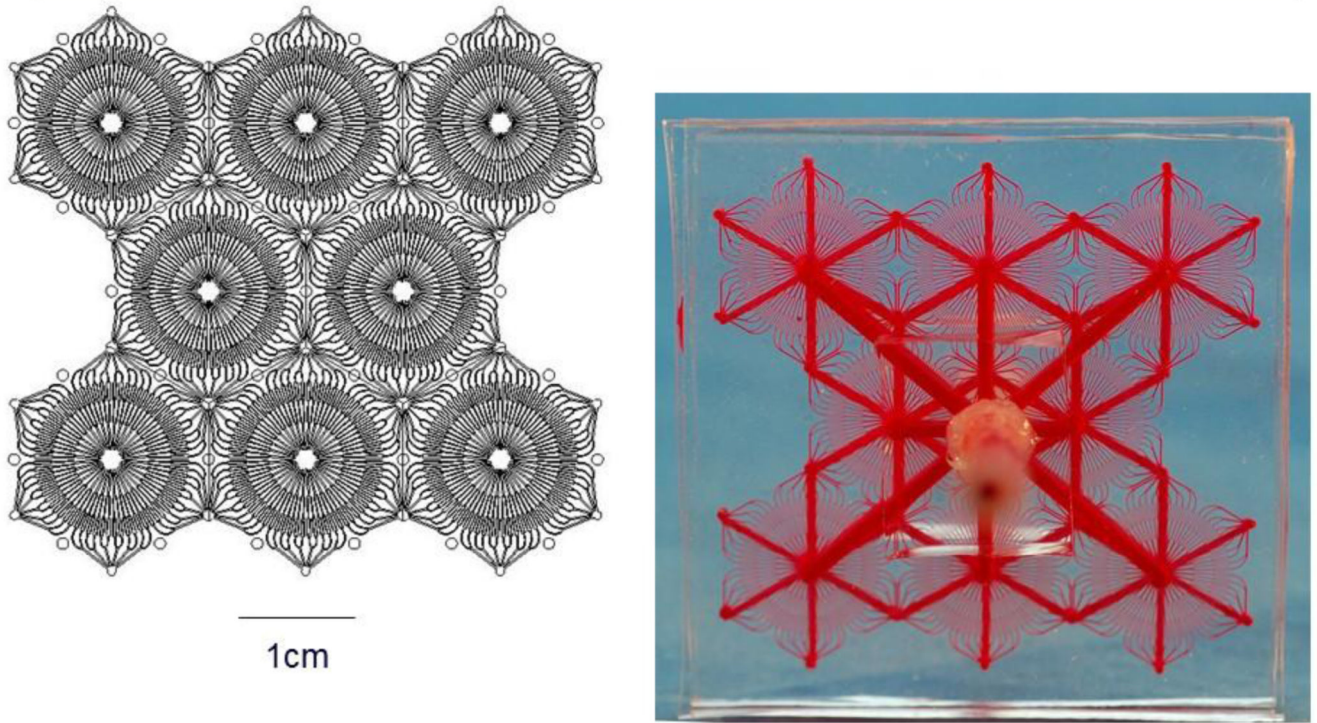


Figure 3.
A tiled lobular architecture of a vascular bed micro machined for tissue engineered liver.