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Robert E. Gross lecture

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.

Dr. Robert Langer

Dr. Joseph Vacanti

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 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 Fig. 1). This paper has been cited approximately 5200 times. Today tissue engineering is a thriving area. As of 2011, there are more than 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.

1. 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

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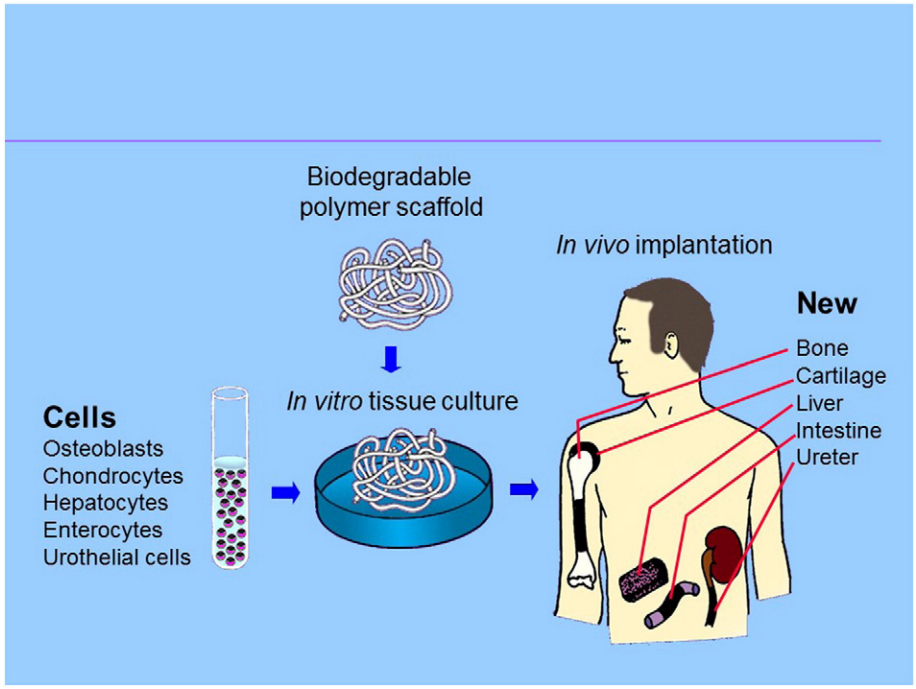

Fig. 1. Tissue engineering schematic.

scaffolding between multiple mesenteric leaves. Hepatocytes were obtained by collagenase perfusion of livers of cogenic Wistar rats and were seeded onto nonwoven filamentous sheets of polyglycolic acid 1 × 3 cm in size, 2 mm in thickness, at density of 500,000 cells/cm^2. 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 μmol/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 (Fig. 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 (Fig. 3) [5].

In 1997, we described the use of a novel 3D 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].

2. 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 more than 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 postinjury, animals implanted with scaffold-plus cells exhibited coordinated, weight-bearing hind