2.0 Precursors of Tissue Engineering

Tissue engineering represents the confluence of a complex array of pre-existing[2] lines of work from three quite different domains: the worlds of clinical medicine, engineering, and science. This section offers a brief overview of the range and character of these different contributing elements, to provide a context for the discussion in later sections of intellectual and institutional developments in TE.

2.1 Roots in Clinical Medicine

Perhaps the most obvious precursors to TE lie in the clinical domain. These are best understood as specific examples of general problem-solving strategies employed by physicians.

Consider, for example, the basic dilemma faced by a surgeon. While the removal of organs or body structures that are damaged beyond repair by disease or trauma can be life-saving, the patient must cope with the functional effects of tissue loss and, in some cases, the psychological impacts of disfigurement. And for those vital organs whose complete removal is incompatible with life, the surgeon's hard-earned skill is, by itself, of no avail: the procedure cannot be done unless there is some way of replacing or reconstituting essential functions.

The resourceful application of virtuosic craft through the tools and materials at hand to meet the distinctive needs of each patient is central to the ethos of surgery. Thus, surgeons have sought to reconstruct anatomic structures using the patient's own tissues as raw material; they have pressed artificial materials into service as prostheses; and, most spectacularly, they have brought patients back from the brink of death by transplanting an ever-wider range of vital organs – primarily living organs, but in a few cases, with only very limited success to date, prototype artificial organs as well.

However, with experience, surgeons have come to understand in detail not only the benefits of such measures, but their limitations as well. Anatomic reconstruction using the patient's own tissues can cause substantial morbidity at the donor site; the improvised structures are usually functionally inferior to the natural organs they replace, and less durable as well. Poor compatibility between artificial materials and mechanical systems and the internal environment and physiologic requirements of the human body can lead to dysfunctional interactions and new failure modes. Transplantation of living organs brings with it profound immunologic complications, and the number of patients who can be treated in this way will always be severely constrained by the limited supply of organs suitable for use.

For a surgeon, then, the development of engineered tissues is a logical next step in the ongoing effort to improve the match between the surgeon's various reparative and reconstructive contrivances and the requirements of human anatomy and physiology.

Physicians in a range of internal medicine specialties as well have found themselves impelled to explore clinical solutions that incorporate living cells. Generally, internists seek to identify therapies that can repair or reconstitute physiologic functions with sufficient effectiveness to enable patients to avoid surgery. Often these therapies are pharmacologic – physicians may use small molecules, or, increasingly, complex, genetically-engineered biological macromolecules, to replace critical molecular species that are in short supply within the body, to counter the effects of molecules that are in harmful oversupply, or to intervene in more subtle ways in regulatory pathways that control critical functions. Other therapies rely on physico-chemical effects – sometimes implemented through external artificial devices – to replace

[2] For present purposes, a substantively relevant line of work is considered to have been "pre-existing" if it was underway prior to 1987, the point at which the conscious involvement of NSF in tissue engineering began.

critical filtering functions that maintain electrolyte balance and remove metabolic wastes from the body. Yet the metabolic functions of human organs are so complex and interrelated that simple pharmacologic or physico-chemical approaches, while they can be life-saving, nevertheless often constitute highly imperfect solutions whose limitations become increasingly apparent in chronic use. In such situations, the notion of leveraging the kinds of complex, integrated physiologic functions that are accessible only at the level of intact cells or tissues becomes compelling.

2.2 Contributing Research Domains in Engineering and Science

The clinical perspective on tissue engineering is strongly applications-oriented. Viewed the "other way around", in terms of enabling knowledge and technologies, TE is remarkable for the breadth of its "footprint" in fundamental and applied biomedical research. Table 2.1 identifies a range of fields and subfields that have played important roles in TE.[3] Research in all of these areas substantially predates the emergence of a generalized concept of tissue engineering, and continues in parallel with TE.

Table 2.1: Research Fields and Subfields that have Contributed to Tissue Engineering

Cell and developmental biology
- Cell differentiation, morphogenesis and tissue assembly
- Cell-cell and cell-matrix interactions
- Growth factors
- Cell isolation and selection
- Cell culture
- Angiogenesis
- Stem cells

Basic medical and veterinary sciences
- anatomy
- cytology
- physiology and pathophysiology

Transplantation science
- Applied immunology – immunosuppression, immunomodulation and immunoisolation
- Organ preservation

Biomaterials
- Natural and synthetic, biodegradable and non-biodegradable polymers
- Polymer chemistry
- Ceramics
- Cell interactions with biomaterials
- Controlled release of bioactive molecules
- Microencapsulation
- Microfabrication techniques
- 3D fabrication techniques
- Surface Chemistry

[3] The list of fields presented here is not intended to represent a definitive taxonomy of the knowledge underlying tissue engineering; the intent is simply to provide a qualitative appreciation for the breadth, depth and character of the "inputs" to the field.

Biophysics and biomechanics
- Molecular and cell transport
- Micro- and macrocirculatory dynamics
- Cell and tissue mechanics

Biomedical engineering
- Bioreactors
- Membranes and filtration
- Musculoskeletal joint engineering
- Biomedical sensors
- Biomedical signal processing, feedback and control
- Electrical and mechanical engineering of biohybrid systems
- Engineering design and systems analysis
- Quantitative tissue characterization
- Biosensors and biolectronics

2.3 Examples

Landmark conceptual developments prerequisite for a concept of tissue engineering have emerged over a period of decades within the problem-solving traditions of clinical medicine. In some clinical domains, physicians and non-clinician researchers reached the stage of incorporating living cells into prototype tissue-engineered clinical solutions years before the emergence of a generalized concept of tissue engineering. The following examples illustrate the depth and breadth of activity prior to 1987:

Vascular grafts The earliest explorations by surgeons of the possibility of transplanting blood vessels took place more than a century ago; the renowned surgical researcher Alexis Carrel was awarded the 1912 Nobel prize in physiology or medicine for his demonstration of successful techniques for the anastomosis of blood vessels and the extension of these techniques from the transplantation of vessels to the transplantation of entire solid organs.[4] Over the succeeding decades, experimental use of rigid glass and metal tubes as vascular grafts yielded disappointing results.[5] In the early 1950s, Voorhees demonstrated the first use of tubes of synthetic fabric as arterial prostheses. With the expanded use of a range of synthetic grafts in clinical practice and ongoing research into the characteristics of a range of alternative materials, surgeons and biomaterials researchers gained a deeper appreciation of thrombogenesis and other problems arising from the interaction between synthetic materials and the blood and perigraft tissues with which they came in contact.[6] The concept of a resorbable vascular graft was introduced in the 1960s, and the first fully-resorbable graft was reported in 1979. Improvement in the healing process of Dacron vascular grafts via pre-seeding with endothelial cells was reported in 1978. Finally, the first attempt to create entirely biologic vascular structures *in vitro*, using collagen and cultured vascular cells, was reported in 1982.[7]

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4 Alexis Carrel – Biography and Nobel Lecture, http://www.nobel.se/medicine/laureates/1912/carrel-bio.html and http://www.nobel.se/medicine/laureates/1912/carrel-lecture.html (URL verified July 13, 2002).

5 Voorhees AB, "How it all Began", pp. 3-4 in Sawyer PN, Kaplitt MJ, *Vascular Grafts* (New York: Appleton-Century-Crofts, 1978).

6 Callow AD, "Historical Overview of Experimental and Clinical Development of Vascular Grafts", pp. 11-25 in Stanley JC, Burkel WE, Lindenauer SM *et al., eds.*, *Biologic and Synthetic Vascular Prostheses* (New York: Grune & Stratton, 1982).

7 Xue L and Greisler HP, "Blood Vessels", pp. 427-446 in Lanza RP *et al., eds., Principles of Tissue Engineering*; Burkel WE, Ford JW, Vinter DW *et al.*, "Endothelial Seeding of Enzymatically Derived and

Skin grafts For centuries, physicians have attempted to cover severe wounds with grafts from a variety of sources, including cadavers and living humans. By the early decades of the 20th century, researchers were investigating the immunologic basis for rejection of skin allografts, though there was no apparent progress toward any practical solution.[8] The marked increase during World War II in the number of burn victims for whom a skin allograft was not feasible provided a renewed impetus for research on skin replacement. During this era, the distinguished immunologist Peter Medawar made important contributions both to further progress in the understanding of the immunology of graft rejection,[9] and to the *in vitro* culture of epithelial cells drawn from a patient. By 1953, Billingham and Reynolds demonstrated in animal models that the products of a brief culture of epidermal cells could be applied to a graft bed to reconstitute an epidermis.[10]

Despite these early successes, more efficient means of cultivation were needed to provide enough cells to sustain transplantation. Overgrowth of certain cell types, such as fibroblasts, suggested that existing culture techniques would not be effective in producing large quantities of cells. Research in the 1960s and 70s identified growth factors that could be added to culture medium to induce greater proliferation of epidermal cells.[11] Starting in the mid-1970s, three research groups working independently at MIT reported a series of milestones in the development of skin replacements. In 1975, Green and Rheinwald described the co-culture method, a technique for serial cultivation of human epidermal keratinocytes from small biopsy samples.[12] Using the technique, one sample of cells was sufficient to generate enough thick, multilayered skin to resurface the entire body of a burn victim. Some differentiation among epidermal cells to mimic that of the epidermis was also visible. In 1979, Green and colleagues, building on the work of Bellingham and Reynolds, demonstrated that cultured cells can be grown in sheets in a petri dish and transferred intact, rather than as disaggregated cells, to a graft wound bed.[13]

That same year, Bell and colleagues described the use of fibroblasts to condense a hydrated collagen lattice to a tissue-like structure potentially suitable for wound healing.[14] These findings led to the first functional living skin equivalent (LSE) in 1981, consisting of fibroblasts suspended in a collagen-glycosaminoglycan matrix.[15] Yannas identified the components of the underlying matrix structure of skin

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Cultured Cells on Prosthetic Grafts", 1982. pp. 631-651 in Stanley JC *et al., eds., Biologic and Synthetic Vascular Prostheses, 1992.*

8 Duquesnoy RJ, "History of Transplant Immunobiology (Part 1 of 2)", http://tpis.upmc.edu/tpis/immuno/wwwHISTpart1.htm (URL verified December 31, 2002).

9 Duquesnoy RJ, "Early History of Transplantation Immunology (Part 2 of 2)", http://tpis.upmc.edu/tpis/immuno/wwwHistpart2.html (URL verified December 31, 2002).

10 Billingham RE, Reynolds J, "Transplantation Studies on Sheets of Pure Epidermal Epithelium and on Epidermal Cell Suspensions", *Br J Plast Surg* 1953;6:25-36.

11 Cohen S, "Epidermal Growth Factor", Nobel lecture, 8 December 1986, http://www.nobel.se/medicine/laureates/1986/cohen-lecture.pdf (URL verified December 31, 2002).

12 Rheinwald JG, Green H, "Serial Cultivation of Human Epidermal Keratinocytes: the Formation of Keratinizing Colonies from Single Cells", *Cell* 1975 Nov;6(3):331-43.

13 Green H, Kehinde O, Thomas J, "Growth of Cultured Human Epidermal Cells into Multiple Epithelia Suitable for Grafting", *Proc. Natl. Acad. Sci. U.S.A.* 1979 Nov;76(11):5665-68.

14 Bell E, Ivarsson B, Merrill E, "Production of a Tissue-Like Structure by Contraction of Collagen Lattices by Human Fibroblasts of Different Proliferative Potential In Vitro", *Proc. Natl. Acad. Sci. U.S.A.* 1979 Mar;76(3):1274.

15 Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T, "Living Tissue Formed In Vitro and Accepted as Skin Equivalent Tissue of Full Thickness", *Science* 1981 Mar 6;211:1052-54.

and used this knowledge to develop a dermal regeneration template which, when implanted and seeded with autologous basal cells, stimulated re-growth of functional skin.[16] All of these lines of research, continuing into the 1990s, proceeded to the development of commercial products.

Kidney A number of investigators experimented sporadically with kidney transplantation during the early part of the 20th century, but planned renal transplantation efforts began only in the late 1940s. During the war years in the Netherlands, Kolff developed the first dialysis machine; its design was refined at the Peter Bent Brigham Hospital in Boston and used in patients for the first time in 1948. In turn, the availability of effective short-term dialysis facilitated progress on transplantation, culminating in the successful transplant of a donated kidney from a twin by Murray and colleagues at Brigham and Women'a Hospital in 1954.[17] Subsequent advances in immunosuppression for transplantation and further development of Kolff's dialysis machine made both techniques practical for widespread, routine use, transforming the management of end-stage renal disease. However, the limited supply of organs suitable for transplantation, combined with recognition of the therapeutic limitations of the dialysis machine, motivated the formulation of the concept of the bioartificial kidney, which would mimic more faithfully the physiologic functions of the kidney and thus avoid the debilitating side effects of chronic dialysis.

During the late 1960s and early 1970s, Wolf experimented with combinations of kidney cells with hollow synthetic fibers as conduits for nutrients and waste. Subsequent work on growing liver cells on the outside of hollow fibers by Wolf and independently by Knazek led to the demonstration of hollow-fiber bioreactors.[18] In the mid-1980s Galletti and colleagues furthered development of the bioartificial kidney concept through their research on hollow-fiber bioreactors employing renal epithelial cells.[19]

Pancreas / islet cells Although the introduction of insulin more than 70 years ago had a miraculous impact on the lives of diabetes patients, with prolonged survival it became apparent that the highly imperfect glycemic control typical of routine insulin therapy was associated with severe long-term complications for a variety of organ systems. The first insulin pumps appeared in the 1960s, but the sensor and control technology required for a complete "closed loop" system to mimic the adaptive character of physiologic glucose control was not available. The first pancreas transplant, in conjunction with a simultaneous kidney transplant, was performed by Lillehei in 1966.[20] Lacy reported a method for isolation of intact islets in 1967, and isolated islet cells were first transplanted in 1970, though without a solution to the problem of immune rejection. The use of microencapsulated islets as artificial beta cells was proposed by Chang as early as the mid-1960s.[21] During the 1970s, Chick and colleagues, building on the work of Knazek, developed a "hybrid artificial pancreas" consisting of beta cells cultured on synthetic semipermeable hollow fibers, and demonstrated the ability of this device to restore glucose homeostasis

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16 Yannas IV, Burke JF, Orgill DP, Skrabut EM, "Wound Tissue Can Utilize a Polymeric Template to Synthesize a Functional Extension of Skin", *Science* 1982 Jan 8;215:174-76.

17 Alexis Carrel – Nobel Lecture, see note 2; Joseph E. Murray – Nobel Lecture, http://www.nobel.se/medicine/laureates/1990/murray-lecture.html, accessed July 20, 2002.

18 Lewis R, "A Compelling Need", *The Scientist* 1995 Jul 24;9(15), http://www.the-scientist.com/yr1995/july/tissue_950724.html, accessed July 20, 2002.

19 Aebischer P, Ip TK, Panol G, Galletti PM, "The Bioartificial Kidney: Progress Towards an Ultrafiltration Device with Renal Epithelial Cells Processing", *Life Support Syst* 1987 Apr-Jun;5(2):159-68.

20 United Network for Organ Sharing, "Milestones", http://www.unos.org/Newsroom/critdata_milestones.htm, accessed July 21, 2002.

21 Chang TMS, *Artificial Cells* (Springfield, IL: Charles C. Thomas, 1972).

in rats when connected to the circulatory system via shunt.[22] Sun and colleagues reported similar work in the 1970s, followed by studies of implanted microencapsulated islets beginning in 1980.[23] Investigation of different ways of "packaging" islet cells to provide effective and durable glycemic control continued through the 1980s and beyond.

Liver The first successful liver transplant was carried out by Starzl in 1967,[24] but in the absence of an adequate supply of transplantable organs, researchers and clinicians have continued to pursue alternative approaches to the replacement of hepatic function. Over more than four decades, clinicians have attempted in a variety of ways to provide extracorporeal support to patients suffering from liver failure. Nonbiological approaches that have been explored include hemodialysis, hemoperfusion over charcoal or resins or immobilized enzymes, plasmapheresis, and plasma exchange. However, these approaches have met with limited success, presumably because the complex synthetic and metabolic functions of the liver are inadequately replaced by these systems.[25] Work on cell-based therapies and bioartificial systems has reflected the same themes observed in the case of pancreatic islet cells. Wolf and Munkelt reported in 1975 on a bioreactor containing a rat hepatoma cell line cultured on the surface of semipermeable hollow fibers within a plastic housing.[26] Sutherland and colleagues reported in 1977 on the use of transplanted hepatocytes in the treatment of drug-induced liver failure in rats,[27] and Sun and colleagues in the mid-1980s explored approaches to microencapsulation of hepatocytes.[28]

Bone and cartilage A variety of materials generally perceived as chemically inert, such as various metals and alloys, have been used for many years to replace damaged bone or to provide support for healing bones. With experience, however, it has become clear that non-biologic materials do not remain biologically inert in the environment of the human body, but rather elicit reactions whose intensity is related to a variety of factors such as implantation site, the type of trauma at the time of surgery, and the precise material in use. Beginning in the 1970's, bioactive materials, such as porous glass and hydroxyapatite ceramic, were examined as alternatives, as they were shown to elicit the formation of normal tissue on their surfaces.[29]

Aside from novel biomaterial development, the growth and regenerative capacities inherent in bone have also been intense topics of scientific and clinical study for decades. In a 1945 publication in *Nature*, Lacroix hypothesized that osteogenin, a substance in bone, was responsible for its growth. Twenty years later in 1965, Marshall Urist proved that there was, indeed, some substance or combination of substances

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22 Chick WL, Like AA, Lauris V *et al.*, "A Hybrid Artificial Pancreas", *Trans Am Soc Artif Intern Organs* 1975;21:8-15; Whittemor AD, Chick WL, Galletti PM *et al.*,"Effects of the Hybrid Artificial Pancreas in Diabetic Rats", *Trans Am Soc Artif Intern Organs* 1977;23:336-41.

23 Lim AF, Sun AM, "Microencapsulated Islets as Bioartificial Endocrine Pancreas", *Science* 1980 Nov 21;210:908-10.

24 UNOS, "Milestones", see note 19.

25 Allen JA, Hassanein T, Bhatia SN, "Advances in Bioartificial Liver Devices", *Hepatology* 2001;34(3):447-55.

26 Wolf CF, Munkelt BE; "Bilirubin Conjugation by an Artificial Liver Composed of Cultured Cells and Synthetic Capillaries", *Trans Am Soc Artif Intern Organs* 1975; 21:16-27.

27 Sutherland DE, Numata M, Matas AJ *et al.*, "Hepatocellular Transplantation in Acute Liver Failure", *Surgery* 1977 Jul;82(1):124-32.

28 Sun AM, Cai Z, Shi Z *et al.*, "Microencapsulated Hepatocytes: an In Vitro and In Vivo Study", *Biomater Artif Cells Artif Organs* 1987;15(2):483-96.

29 Ducheyne P, El-Ghannam A, Shapiro I, "Effect of Bioactive Glass Templates on Osteoblast Proliferation and In Vitro Synthesis of Bone-Like Tissue", *J Cell Biochem* 1994; 56: 162-167.

present in demineralized bone, which when transplanted, could induce growth of new bone.[30] Urist's landmark finding encouraged many to investigate the precise factors which trigger bone induction. During the 1970s and 80s, research demonstrated that the process is mediated by a category of growth factors, termed bone morphogenetic proteins or BMPs, which act in a multistep cascade highly reminiscent of embryonic bone morphogenesis.[31] Reddi and colleagues developed techniques to isolate these proteins from the extracellular matrix of bone.[32] The findings are promising, too, for induction of cartilage as BMPs initially induce a cascade of chondrogenesis and might just as easily be called cartilage morphogenetic proteins.[33] Work on isolation, purification, and proliferation of BMPs continued throughout the 1980s.

[30] Urist, MR, "Bone Formation by Autoinduction", *Science* 1965; 150: 893-899.

[31] Reddi, AH, "Morphogenetic Messages are in the Extracellular Matrix: Biotechnology from Bench to Bedside", *Biochem Soc Trans* 2000; 28: 345-349.

[32] Sampath TK, Reddi AH, "Dissociative Extraction and Reconstitution of Extracellular Matrix Components Involved in Local Bone Differentiation", *Proc Natl Acad Sci USA* 1981 Dec; 78(12): 7599-603.

[33] Reddi, AH, "Morphogenetic Messages", see note 30.