Journal of Cellular Biochemistry 45:239–241 (1991)

VIEWPOINTS

Tissue Engineering: A Perspective

In one sense, tissue engineering is a long-standing practice engaged in by plastic and reconstructive surgeons who continue to meet the challenge of repairing defects and remodeling body parts by using the body's own tissues with or without the help of non-biologic devices. Similarly, the transplantation of organs based on the art and science of installation is largely a surgical matter. While tissue typing and matching have been practiced, organ transplantation has gained its present prominence less because of them than because of advances in immunosuppression.

What is emerging as a new discipline is the science and technology of tissue engineering that has as its goal the reconstitution of tissues and organs in vitro for use as model systems in basic and applied research or for use as grafts to replace damaged or diseased body parts or body functions. It is perceived that the products of tissue engineering, whether developed for research or grafting, must be defined, standardized, and quality-controlled. Regulatory issues governing their use must be formulated and resolved. These necessities, although burdensome, will greatly magnify their usefulness and breadth of application.

Tissue engineering is a nascent field that draws support from multiple disciplines beginning with cell culture and extracellular matrix biochemistry since the principal raw materials required for reconstituting tissues are the cells and the structural matrices in which or on which cells normally exist. Limitations and challenges lie in the kinds of cells that can be propagated and the degree to which cells under cultivation can be helped to maintain their phenotype (see Jauregui and Grann, to be published in *Journal of Cellular Biochemistry*). The requirements for continuous, scaled-up production of recoverable cells for use in tissue and organ equivalent products and for the establishment of cryopreserved banks are still only minimally met. Approaches to the propagation of cells that are proving refractory to cultivation consist of the manipulation of growth substrates, of the classical manipulation of media, including the addition of newly discovered growth factors, of the search for stem cell populations, and the insertion or deletion of mitotic gene regulators that govern the cell's cycling state.

Providing cells in vitro with structural molecules with which they can interact readily and which they can organize into tissues is an essential aspect of tissue engineering (see Parenteau et al., this issue). The structural molecules include primarily the family of collagens, proteoglycans, elastin, and cell attachment proteins. There are good sources and extraction methods for harvesting some collagen types, but ones that are less good for other collagens and other structural molecules. Essential questions of what to provide in a matrix cocktail, as distinguished from what cells resident in matrices can be stimulated to provide through their own biosynthetic activity, are largely unanswered. While cells will enrich simple matrices with their biosynthetic products, stimulating specialized cells to recapitulate more precisely what they do developmentally in the embryo, fetus, or in healing is part of what the tissue engineer must know how to do by chemical or physical induction.

If the job of the tissue engineer is that of imitating nature, studying how nature works in creating tissues and organs is a critical assignment. It is now commonplace to say that understanding genetic readout and the sequential appearance and usefulness of gene products will yield only part of the story of development. The *spatial* organization of regional tissue and organ components and the physical constraints imposed on them as they grow and differentiate are fundamentally directive in themselves. Only little attention has been given to how tension, compression and shear, as examples, contribute to normal differentiation and pattern formation. In tissue engineering, there is a need to provide appropriate mechanical configurations for developing tissues in vitro so that whole embryo physical forces can be imitated. Here the tissue engineer leans on the biomechanical engineer for collaboration.

Knowing that cell-cell interactions and cell-matrix interactions lie at the heart of developmental events, there are several major issues for the tissue engineer; first, whether the CAMS, integrins, and other critical attachment molecules are expressed and are functionally available among cells used for tissue building; second, whether cells can be stimulated to express them if given the appropriate chemical and physical signals; and third, if they are not expressed, whether there is value in adding them to the starting matrix provided for cells asked to make a tissue (see Emerson et al., this issue). The chemical signals may be autocrine, paracrine, or endocrine secretions needed for morphogenesis, differentiation, and the expression of specific receptor sites that can bind the chemical messengers. The physical signals I have alluded to above. Tissue engineering will call upon the cell biologists and biochemists for the chests full of probes to evaluate how well or how poorly laboratory or in vitro reconstitution progresses by measuring what markers of differentiation are expressed and what kinds of assist molecules or mechanical conformation might help.

In engineering model histiotypic and organotypic systems for in vitro use, there can be no reliance on undefined factors that drive differentiation in vivo. On the other hand, in preparing a tissue or organ equivalent for grafting, the undefined local and humeral contribution of the host to the graft is a new subject. The development of the graft can be thought to consist of two phases: the first is the endowment of the engineer that is expressed in vitro and the second is what happens in vivo (see Solursh et al., this issue). The answers of how much remodeling, morphogenesis, and additional differentiation occur in vivo will be learned from implantation experiments with species-specific cells used for the engineered constructs.

The in vitro reconstitution of engineered tissues and organs for grafting will be guided by growing evidence that engineered tissues may be made immunologically neutral by cell selection and purification strategies. Eliminating immune system cells from isolated tissues or tissues fabricated in the laboratory has already made possible successful allogeneic grafting of certain human cells. Other approaches, such as the use of antisense molecules, are being considered for neutralizing the immune response of the host to foreign cells. Understanding the basis for T-cell anergy or unresponsiveness is an important part of what tissue engineering asks of cell immunologists as the possibilities for generic grafting are tested.

In fabricating replacement parts for grafting, there are approaches in tissue engineering that do not depend on the use of tissue cells but on the use of their structural proteins and other products. Organized in the appropriate form and enriched by other molecules, collagen structures in particular have already been shown to induce complex tissue remodeling. The capacity of the body to remodel itself under planned direction from within or without is a significant tissue engineering interest that calls for much fundamental work. Most significant is the realization that the body's ability to remodel may be much more extensive than has been appreciated so far. The elongation of skeletal elements by forces from the outside applied through external devices attached to them is an example of engineering at a distance that unexpectedly results in the growth and elongation of soft tissues as well. Still to be understood are body building through exercise regimens, hormone treatment, and diet—alone or in combination.

The fabrication of acellular matrix constructs may in itself be insufficient for creating a device capable of inducing, directing, and sustaining coherent events of tissue or organ remodeling in vivo. An approach that appears to hold promise is that of enriching matrices fabricated in vitro by cultivating cells in and on them. After some period of residence on the substrate, cells, but not their products, can be removed. The addition of cell products to matrices through cell biosynthesis may achieve the conditioning required for recognition and sensible remodeling in vivo.

The uses of living engineered model systems for research are broad. There is much evidence that cells organized in three dimensions in vitro in modes similar to those that prevail in vivo express morphological and biochemical profiles that are very different from those expressed by cells in monolayered cultures but similar to those of their actual counterparts in vivo. The three-dimensional systems, if readily available and standardized, would therefore have relevance as defined basic research models. In addition to their use for better understanding metabolic cell processes, cell differentiation, and cell-cell and cell-matrix interactions, those tissue systems

that undergo predictable differentiation are likely to be of value for the propagation of difficult to cultivate viruses that depend on a differentiation cycle for growth signals. Similarly, the propagation of tumor cells, or genetically deficient cells, often difficult or impossible to subculture, for purposes of selecting a strategic therapy, may be enhanced by providing engineered tissue substrates in or on which to grow them (see Quesenberry et al., this issue).

Model systems constituted with human cells are of special value in testing the safety of substances encountered in the work place or home or used deliberately to achieve a remedial or cosmetic result. They are also of value in screening substances and formulations for their safety and efficacy. The closer the resemblance of a model system to its actual tissue or organ counterpart, the greater its predictive value and the better it can be used to understand its responses to applied chemicals or physical stimuli. Mechanistic similarity is a valuable feature for a model system for guiding drug design and the development of drug delivery and mode of action strategies.

There is a remarkable confluence of interest as groups engaged in gene insertion and those concerned with tissue engineering begin to deal with the issue of returning genetically altered cells to the body (see Chen et al., Geller et al., this issue. What is being sought are vehicles that incorporate the remedial cells, that provide for their delivery to the recipient, and that insure vascularization and their persistence. All are issues that are approachable experimentally. A facet of the gene insertion technology of great usefulness in transplantation studies is that of providing innocuous markers to permit tracking and persistence studies of transplanted cells.

While in its early incarnation tissue engineering will depend heavily on progress in the realm of basic and applied research, its technological success will ultimately be determined by how it is supported by the public sector and embraced by the private sector. The products of tissue engineering will be manufactured products needing both old and new machinery for their fabrication. Since many products of tissue engineering are living materials or materials that would soon be occupied by living cells, they need special handling. Manufacturing will call for sterile operations, quality assurance and control, observance of regulatory requirements, and innovative packaging, storing, and shipping.

In this overview of tissue engineering, I have tried to define the new field in part by pointing to the subdisciplines on which it depends. Progress will be governed perhaps by the recognition given by the subdisciplines to problems central to tissue engineering and on funding and collaborations driven by interest in it for the ends of basic and applied research and in the social usefulness of the engineered products. I expect also that some tissue engineers will become the renaissance scientists needed to bridge the disciplines that compose the field.

Eugene Bell
Organogenesis Inc.
83 Rogers Street
Cambridge, Massachusetts 02142
and
Department of Biology
Massachusetts Institute of Technology