Special feature: Cellular engineering

Tissue engineering in the USA

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Abstract—*Tissue engineering is the application of the principles and methods of engineering and the life sciences towards the development of biological substitutes to restore, maintain or improve functions. It is an area which is emerging in importance worldwide. In the USA it has been actively fostered by the National Science Foundation, both through research grants and the sponsorship of a series of workshops starting in 1988. This brief review of activities in the USA focuses on cell culture technology as a foundation for tissue engineering and then discusses examples of applications. These include artificial skin and the use of encapsulated cells in the development of bioartificial organs. Also discussed is the reconstitution of a blood vessel in culture, both for use in basic research and for implantation as an artificial blood vessel in bypass surgery. In conclusion, other potential applications are mentioned as well as generic areas of technology for future development.*

Keywords—*Artificial skin, Bioartificial organs, Biological substitutes, Blood vessels, Cell culture technology, Encapsulated cells*

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1 Introduction

TISSUE ENGINEERING is an activity within the field of medical and biological engineering which predates its name. Although still in its infancy and still evolving, a working definition is as follows:

> 'Tissue engineering' is the application of the principles and methods of engineering and the life sciences toward the fundamental understanding of structure/function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve functions.

Implied in the above is the essence of tissue engineering, i.e. the use of living cells, together with extracellular components, either natural or synthetic, in the development of implantable parts or devices for the restoration or replacement of function.

The term 'tissue engineering', certainly in regard to its use in the USA, originated at a bioengineering panel meeting held at the US National Science Foundation (NSF) in Washington, DC during spring 1987. This led to a special meeting the following October focusing on the subject of tissue engineering, which also was held at NSF, and subsequently tissue engineering was identified by NSF as an emerging technology and a priority for funding.

In early 1988 an NSF-sponsored workshop on tissue engineering was held at Lake Tahoe, California (see SKALAK and FOX (1988) for the proceedings of this meeting). It was at this meeting that the definition stated in the first paragraph was developed. In 1989 a special US-Japan symposium on tissue engineering was held at the Winter Annual Meeting of the American Society of Mechanical Engineers (ASME),* and in April 1990 a tissue engineering workshop was held in Keystone, Colorado. The abstracts from this meeting are published in a supplement to the *Journal of Cellular Biochemistry* (FOX and BURGER, 1990), and a number of the presentations from this workshop are published by ASME as full-length manuscripts in a special issue of the *Journal of Biomechanical Engineering* (SKALAK, 1991). Out of this workshop came recommendations for a number of areas of generic technology which are in need of further development, and these will be discussed at the end of this paper.

There will be other workshops and meetings in the area; however, each of the three meetings noted above has been important in its own right, coming at the beginning of this emerging technology. A key to this technology is the use of living cells, and this as a basis of tissue engineering will be expanded upon in the next section.

2 Cell culture technology

If the living cell is the key to the tissue engineering of implantable parts and devices, then the advent of mammalian cell culture technology, i.e. the growing of mammalian cells out of the body, represents an event which opened the door for this field. Modern cell culture dates back to the early part of this century when a French scientist, Alexis Carrel, working at the Rockefeller Research Institute in New York, started a culture from a small slice of heart muscle taken from a chick embryo (LEFF, 1983). This culture continued for several decades, although along the way the heart muscle cells died out and only fibroblast cells continued to proliferate. Carrel's historic chick-cell culture finally was allowed to expire 34 years after it was started—and two years after his own death.

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Cell culture has led to research which has paved the way for a number of important breakthroughs in the life sciences. This includes the study of cellular processes, molecular biology and the ability to genetically manipulate cells, and the resulting development of new drugs, with much of recent drug-related research and product development being based on recombinant DNA technology.

Although engineers have been slow to move into the area of cell culture, there are real engineering needs associated with the evolution of this technology. These range from the basic process of cell culture, particularly the scale-up of these processes to something larger than a laboratory bench-top system, to the design and manufacturing of pumps, seals, filtering systems, and the like for use in cell culture. An example is the continuous culture of mammalian cells, whether the cells may be anchorage-dependent or ones grown in suspension. Mammalian cells are known to be fragile, and one of the problems is to provide the necessary nutrient transport while at the same time not damaging the cells. In the process waste products must be removed, and temperature and pH controlled.

Furthermore, cell growth is influenced by a variety of factors including media composition, the surface to which the cells are adherent (if anchorange-dependent cells) and the mechanical environment in which the cells reside. It is the influence of a cell's mechanical environment which has been the focus of the research in our laboratory at Georgia Tech. Fig. 1 illustrates the influence of one type of mechanical stress, the frictional shear stress imposed by a steady laminar flow, on cultured vascular endothelial cells (ECs).

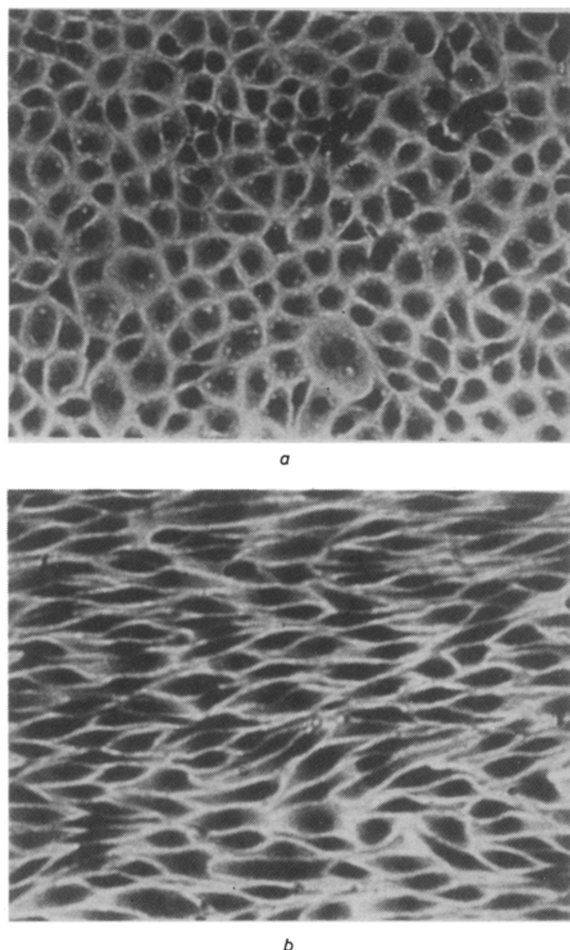

Fig. 1 *Photomicrographs of cultured bovine aortic endothelial cells grown on Thermanox under static culture. (a) Control conditions; (b) under a steady laminar shear stress (8·5 Nm^{-2}) for 24 h; flow from left to right. Reproduced from* LEVESQUE *and* NEREM *(1985); used with permission of ASME*

As may be seen, in response to such a flow, vascular ECs elongate in shape and align their major axis with the direction of flow. As part of this response there are major alterations in cell function, and further discussion of this will be left to a later section.

A few years ago Invitron, a company in the USA based in St Louis, Missouri, had an advertisement with the headline, 'The cultural revolution has begun'. This is undoubtedly true, and there is no evidence for this stronger than the emergence of tissue engineering. A key to tissue engineering is the ability to reconstitute tissue and organ equivalents in culture from specialised cells and matrix molecules (BELL *et al.*, 1989). In the next few sections cell culture, as a foundation of this emerging field, will be examined in terms of specified product applications.

3 Artificial skin

An excellent example of tissue engineering and the importance of cell culture is the development of artificial skin (VAN BRUNT, 1991). The use of the term 'artificial' here must be qualified because many of the approaches, in using living cells and matrix molecules, are quite natural. One company which is a pioneer in the effort to develop a tissue-engineered artificial skin is Organogenesis, Inc., a company located in Cambridge, Massachusetts (BELL *et al.*, 1981; 1983). Here cells, together with extracellular matrix and nutrients, are grown to produce a living skin equivalent. The cells are from human neonatal foreskin, and dermal fibroblasts are 'mixed' with extracellular matrix bovine type I collagen, this being necessary so as to surround the cells so that they can form tissue. On top of this human epidermal cells are added.

Organogenesis started human clinical trials on the use of its skin equivalent for grafting a little over two years ago. These trials, although in an early stage, are going well. In addition, they have developed an *in vitro* skin model for use in dermatological research and for assaying cell viability. Marketed under the trademark TestSkin, two versions are available. One is a living dermal equivalent, i.e. without epidermal cells, and the other a living skin equivalent like that described in the previous paragraph. One of the applications of TestSkin is in testing the toxicity of cosmetic products.

Another type of artificial skin graft is that developed by Dr Ioannis Yannas and his co-workers (YANNAS *et al.*, 1982; TOMPKINS *et al.*, 1989). This involves a highly porous collagen matrix which serves as a template for the graft. When the graft is attached to a wound, fibroblasts migrate to it from surrounding tissue and permeate the collagen 'sponge'. These fibroblast cells produce new collagen, the original matrix is slowly degraded, and epidermal cells from the edges of the wound grow inwards and cover the graft area. During this entire process, a silicone rubber sheet covers the wound area; this is to provide a barrier to fluid loss and infection and for mechanical strength during the healing process. In a more advanced version, the matrix is covered by cells from the recipient isolated from a small biopsy.

Still another entry in the artificial skin market is from Marrow-Tech located in La Jolla, California. They have developed a skin equivalent which consists of both dermal and epidermal cells. The difference, compared with the Organogenesis product, is that in this case cells are cultured on a biodegradable mesh of either polyglycolic or polygalactic acid. The goal is to provide an organised dermis with these meshed grafts which will provide a foundation for the patient's own epidermal cells. Marrow-Tech has applied this technology in the development of a

product sold under the trademark 'skin^2' ('skin squared'). This is a line of toxicity screening kits which contain cultures of actively growing human skin tissue. In addition, the company has received government approval to begin clinical trials of a product for use with burn patients. This three-dimensional dermal replacement is marketed under the trademark 'DermaGraft'.

Finally, a different approach is that of the company, BioSurface Technology, in Cambridge, Massachusetts. This in fact was the first skin substitute developed and came from the laboratory of Dr Howard Green at Harvard Medical School, who devised a system for growing sheets of human keratinocytes, the outer epidermal cells, from small samples of a burn victim's own skin.

There thus are a number of companies actively working to market commercially a tissue engineered artificial skin. As important as this is as the first type of product being developed with the technology of tissue engineering, there are a number of other applications of this emerging technology.

4 Encapsulated cells

One of the important areas of tissue engineering is the development of biological substitutes based on the encapsulation of cultured cells. Important engineering issues in the design of these encapsulated cell devices are

(i) shape
(ii) porosity and macrostructure
(iii) surface microstructure and chemistry
(iv) the choice of implantation site.

Shape is important because it may be necessary for proper function. Porosity and macrostructure affect the transport of nutrients to the implanted cells. Microstructure and surface chemistry will influence how cells attach and grow and also whether or not they function normally. Finally, the choice of implantation site will affect not only the size and dimensions of the device but also its overall effectiveness.

One of the important areas for use of encapsulated cell technology is in the development of bioartificial organs. Of particular interest has been the pancreas, the liver and the kidney. In regard to the pancreas, pancreas transplants have failed to be useful because of immunological rejection. An alternative approach is the development of implantable insulin pumps; however, these are not without problems either. This thus has led to the interest in developing a bioartificial pancreas.

Although there are several possible designs for a bioartificial pancreas, one involves the use of microencapsulated islet cells (Colton and Augoustiniatos, 1991; Lim and Sum, 1980; Friedman, 1989). In such a device the islet cells, which secrete insulin, are surrounded by a semipermeable membrane. This membrane must be permeable to the transport of insulin so that it can be passed into the bloodstream. The cells also will need nutrients, oxygen, and other molecules necessary for the maintenance of metabolic function. However, the membrane must in addition protect the islet cells from bacteria, lymphocytes and other proteins responsible for immune rejection. Important in the design of a bioartificial pancreas is its ability to respond rapidly to changes in glucose level. Equally necessary is the long-term survival of the islet cells and the secretion of the insulin. As part of this it is also important that islet cell function does not change with a buildup of hormones. In all of these areas microencapsulated islet cells function fairly well. However, one problem is that of insulin production rate, which is on the low side.

There are other applications of encapsulated cell technology in tissue engineering. Cima *et al.* (1991) have shown that both liver and cartilage cells can be transplanted successfully, at least in small animals, using cells which are encapsulated in a degradable polymer substrate. Neurological deficits also can be treated by transplantation within the brain of polymer-encapsulated cells which release the missing neurotransmitter, and Aebischer *et al.* (1991) have investigated the ability of encapsulated dopamine secreting cells to reverse experimental Parkinson's disease.

Finally, a major tissue-engineering market where encapsulated cell technology may have application is the development of blood substitutes, i.e. an artificial blood (Pool, 1990). Current efforts are based on the chemical cross-linking of haemoglobin, in many cases bovine derived; but there are potential problems associated with both incomplete cross-linking and the introduction of the foreign, bovine proteins into the body. In future the use of microencapsulation, together with stem-cell culture and controlled haematopoiesis, should prove important. In fact, Marrow-Tech hopes to be the first company to grow a bone marrow in culture, which will proliferate once transplanted into a human and provide the various types of blood cells required by the body.

5 Reconstituting blood vessels in culture

Another application of tissue engineering is in the development of an artificial blood vessel for use in the bypass and replacement of diseased arteries. A number of groups have been interested in using cell culture technology for the development of such tissue-engineered vascular prostheses. Much of this effort has been focused on hybrid vascular grafts, i.e. a graft constructed out of synthetic material such as Dacron or polytetrafluoroethylene (PTFE), but seeded with cultured endothelial cells (ECs) prior to implantation to provide a natural interface with flowing blood (Zilla *et al.*, 1987). Although initial results in terms of increased graft patency are promising, it is clear that this only partially simulates an actual, living blood vessel (Stanley *et al.*, 1982).

Others have attempted to use the co-culture of ECs and smooth muscle cells (SMCs) in the construction of an artificial blood vessel. Jones (1982) attempted to do this by using cloned bovine pulmonary artery ECs grown on top of rat SMCs in tissue culture dishes. He found that both cell lines behaved differently when grown together, ECs attached more quickly to plastic than to the smooth muscle layer; however, the mixed cultures were stable and could be maintained for up to six months without detaching from the dish. Weinberg and Bell (1986) constructed an artificial blood vessel using bovine aortic ECs, bovine aortic SMCs and advential fibroblasts. This was composed of three layers, one of ECs, one of SMCs together with collagen, and one of fibroblasts and collagen. The ECs were shown to form a flattened monolayer covering more than 90 per cent of the surface and acting as a permeability barrier for large molecules, e.g. albumin. ECs were also shown to release prostacyclin (PGI_2) and produce von Willibrand factor. Finally, van Buul-Wortelboer *et al.* (1986) cultured human umbilical vein ECs on top of human umbilical artery SMCs in calf-skin collagen. They found that the ECs elongated when grown with SMCs, compared with a cobblestone shape when cultured on plastic. The stability of this artificial vessel was up to ten days, after which the collagen was degraded by SMC collagenases. Furthermore, the growth of SMCs was lower when co-cultured with ECs, with this inhibitory

effect being observed for both subconfluent and confluent ECs.

The development of an artificial vessel is an example of both parts of the definition given in the introduction of this paper. Before one can achieve the goal of a biological substitute, in this case an artificial blood vessel made at least in part from living cells, one must develop a fundamental understanding of the structure/function relationships intrinsic to vascular biology. This involves the study of the structure and function of both vascular ECs and vascular SMCs, their interaction with each other, the role of the extracellular matrix and the influence of the environment in which ECs and SMCs reside, including the mechanical stresses to which they are exposed. Our laboratory at Georgia Tech is investigating the influence of mechanical stresses on vascular cells. The major focus has been on the effect of flow on both subconfluent and confluent vascular EC monolayers. As shown in Fig. 1, in the presence of a steady-state laminar flow, vascular ECs elongate in shape and align their major axis with the direction of flow. As part of this adaptation to flow there are a variety of other alterations in structure and function which take place (LEVESQUE *et al.*, 1989; NEREM and GIRARD, 1990), and these are summarised in Table 1. Furthermore, in the presence of a pulsatile flow, although qualitatively the changes are similar, quantitatively there are differences. Thus vascular ECs can discriminate between different types of flow environments.

In vivo ECs reside in a flow environment. Thus, to study vascular endothelial biology in static culture is at best a simulation of a region of flow stasis and at worst artefactual. Furthermore, ECs respond differently to differing flow environments, and one should never collectively talk of flow as a single stimulus. Just as there are different chemical agonists, each with its own separate effect, there also are different types of flow 'agonists', a variety of types of flow environments, each of which will have its own 'agonist' effect. Thus, as important as cell culture studies have been to the study of vascular biology, it is clear that there is much more which needs to be done if we are to engineer the cell culture environment so as to make it truly simulate physiological conditions. Factors which need to be included, in addition to a realistic flow environment, are the medium, extracellular matrix components and neighbouring cells.

Table 1 Laminar flow effects on cultured vascular endothelial cell structure and function

Cell shape and alignment	Endocytosis
F-actin localisation	Protein secretion
Cell mechanical stiffness	Proto-oncogene expression
Cell proliferation	Intracellular signalling

An illustration of this is as follows. One of the major differences between ECs *in vivo* and *in vitro* is that *in vivo* vascular endothelium has a very low turnover rate, whereas *in vitro*, for a confluent EC monolayer in static culture, the cell turnover rate is several orders of magnitude higher. It is possible to reduce the cell turnover rate observed *in vitro*. For example, by placing a confluent EC monolayer in a laminar flow where the shear stress is $3\ N\ m^{-2}$ or higher, our studies show that the rate of cell turnover can be significantly reduced (LEVESQUE *et al.*, 1990). It has also been shown that ECs in contact with SMCs, i.e. an EC-SMC co-culture, results in a 50 per cent reduction in EC turnover. These two together suggest that, to achieve a low cell turnover rate, comparable to that observed in *in vivo*, it may be necessary to have cultured ECs both in contact with neighbouring SMCs and in a flow environment. Thus, the cell culture environment would have to be engineered to include both co-culture and flow.

These results also have implications for the development of a tissue-engineered vascular graft. If a low EC turnover rate is a requisite, one comparable to that found *in vivo* in vascular endothelium, this will not be realised simply with EC pre-seeded on a synthetic material; it will take an EC-SMC co-culture, i.e. the reconstitution of a complete blood vessel, and there are several groups working on this, with Weinberg and his co-workers at Organogenesis being the furthest along in their efforts.

An alternative approach to the treatment of cardiovascular disease is gene therapy (NABEL and NABEL, 1991). Here cells genetically modified so as to enhance the secretion of a desired substance, e.g. the thrombolytic agent tissue plasminogen activator (tPA), will be implanted into the body. Although the use of recombinant DNA technology may appear to be primarily a molecular biology effort, an important aspect is the development of vehicles for introducing the cells into the cardiovascular system. One approach would be through the use of a vascular graft lined with genetically modified ECs (WILSON *et al.*, 1989). This thus brings us back to the need to develop tissue-engineered vascular prostheses.

6 Concluding discussion

There are many other areas of tissue engineering which are currently receiving attention. These include both orthopaedic implants and neural prostheses. Each of these is important in its own right; however, it is the neural area which perhaps poses the greatest challenge.

Under the right conditions, there appear to be considerable regenerative capacities for both the central and peripheral nervous system. AEBISCHER *et al.* (1991) has shown for the latter that bridging the gap at a lesion site with a synthetic material in effect provides a guidance channel which facilitates the regrowth of injured peripheral axons. KHAN *et al.* (1991) have investigated the ability of carbon filaments to serve as a scaffold for the regrowth of injured axons, and they conclude that such filaments may have clinical utility by providing a surface which both facilitates adhesion and serves as a guide. In fact, an extremely important area in general is the interaction of cells with surfaces and how to modify such surfaces so as to promote a certain type of cellular behaviour.

Another area of importance to tissue engineering is that of drug delivery (LANGER, 1990). New approaches are of a variety of types. One is to chemically modify the drug so as to selectively alter properties important to its distribution within the body. An example of this is the design of a drug so as to allow it to cross a normally impermeable barrier, with one important application being the blood/brain barrier. Another approach is the entrapment of the drug in small vesicles which can be injected into the bloodstream. These small vesicles are microparticulates or colloidal carriers and in general are composed of such substances as proteins, lipids, carbohydrates and synthetic polymers. Controlled release systems are yet another new approach to drug delivery. Here the delivery of the drug at a predetermined rate can be facilitated through the placement of the drug in a polymeric material. It should be noted that another important application of controlled release

polymer technology is in the development of sensors for the continuous measurement of chemical concentrations within the body. One example of this is a fluorescence energy transfer assay where the reactants are provided by a controlled-release system (BARNARD and WALT, 1991).

At the conclusion of the Keystone, Colorado, workshop noted in the introduction to this paper, a final discussion session was held. The purpose of this was to address questions relating to priorities for future work. These are presented in Table 2, and this was provided as a set of recommendations to the National Science Foundation. As may be seen, the topics listed are generic to many of the tissue-engineering applications discussed here.

From this discussion, it hopefully is clear that there are challenges ahead. Many of these relate to the basic science.

Table 2 Generic areas for technology development

Antigens/monoclonal antibodies
Biosensors for determining tissue biologic concentrations
Cell growth in three dimensions and under active conditions
Cultivating recalcitrant cells
Expression of proteins in implanted cells
Polymeric systems for delivering biologics
Vehicles for cell introduction

But tissue engineering involves the transition from basic science to engineered products, i.e. to the commercialisation of this science and technology. It is this which has been emphasised here, and to realise the potential of this field in terms of products and devices will require the participation of engineers, albeit a unique type of engineer, one who can bridge the two worlds of engineering and molecular and cell biology.

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