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NEW TRENDS IN BIOTEXTILES – THE CHALLENGE OF TISSUE ENGINEERING

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ABSTRACT

So you think that the Bionic Woman and the Six Million Dollar Man were simply science fiction stories? Think again! Rapid advances in the biological sciences and nanotechnology are becoming fused together to create the field of tissue engineering which is developing biological substitutes for the repair and regeneration of tissues and organs. This paper presents an overview of the latest concepts used in the field of tissue engineering and highlights those aspects of this multidisciplinary endeavor where polymer chemistry, fiber science and textile technology and engineering can make a significant contribution in the future design and development of novel biotextile scaffolds.

Keywords: biotextiles, biological sciences, nanotechnology, tissue engineering, scaffolds, resorbable polymers, cell culture, bioreactor, in vivo, in vitro, cell signalling, gene therapy, nanofabrication

Introduction

Recent and rapid advances in both the biological sciences and in material science, especially in molecular biology, genetic engineering, genomics, bioinformatics, biomimetics, surface science and nanotechnology are propelling us into novel collaborative research ventures so as to create new knowledge and understanding at the boundaries of previously disparate disciplines. This has been particularly true in the field of biotextiles, where we study the performance and properties of implantable textile products in a biological environment.

The objective of this paper is to show how the disciplines of molecular biology, immunology, embryology, gene therapy, biopolymers, biomimetics and nanotechnology are having an immediate impact on the study of biotextiles, implantable medical textiles and tissue engineering. Research work in this area demands an understanding of the latest technologies for polymer synthesis, fiber spinning, surface modification and nanotechnology, an appreciation of the new concepts in biological science, as well as a creative imagination to integrate these disciplines.

The Original Biotextiles Paradigm

It was back in the 1980's that Professor D.F. Williams of the University of Liverpool, UK, first defined the term "biomaterial" as a "nonviable material used in the fabrication of a medical device and intended to react with biological systems" (1). Following the same line of thinking the term "biotextiles" was defined as a "structure composed of textile fibers and designed for use in a specific biological environment (e.g. surgical implant), where its performance depends on its interactions with cells and biological fluids as measured in terms of its "biocompatibility" and "biostability" (2).

The term "biocompatibility " referred to "the ability of a biomaterial or biotextile to provoke an acceptable cellular and biological response from the host environment ", and our traditional view has been to minimize as much as possible this inflammatory or foreign body response (3). It has been generally accepted that healing occurs more rapidly if an inert biomaterial is used which the body can tolerate more readily. In fact many major developments have been made in modifying the surface properties of implants by adding surface coatings of collagen, gelatin (Figure 1), heparin, albumin or pyrolytic carbon (Figure 2) so as to make them more biocompatible.

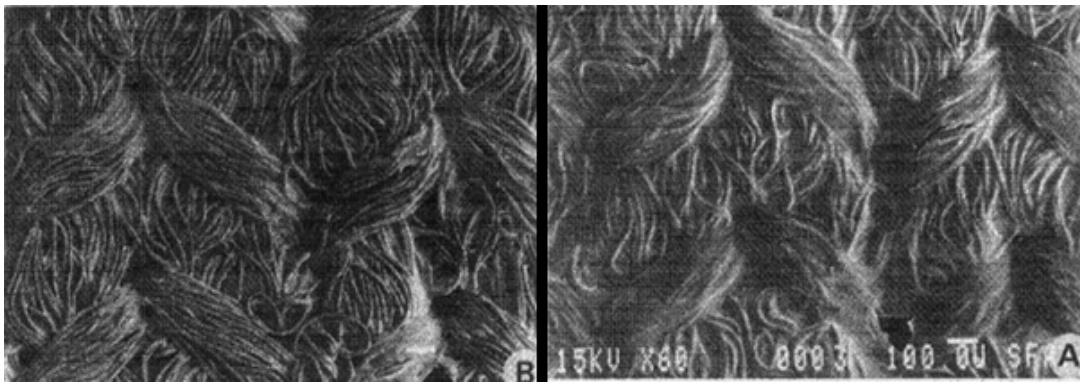

Figure 1: Knitted polyester vascular prosthesis with gelatin coating for improved

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Figure 2: Polyester vascular prosthesis with coating of pyrolytic carbon to reduce thrombogenicity.

Biocompatibility

The term "biostability" was also defined by Professor Williams to mean the "ability of a biomaterial (biotextile) to maintain its original dimensions, and its mechanical and chemical properties during long-term implantation or exposure to a hostile biological environment". This was an obvious engineering requirement for long-term implants; namely that the functional life of the implant should exceed that of the patient! In certain cases, such as the use of polyester vascular prostheses as aortic arterial substitutes in elderly patients suffering from atherosclerosis, this has been true for many years. However, for older patients requiring abdominal aortic aneurysm repair with an endovascular stent graft (Figure 3) and for younger patients requiring heart valve replacement, this has not been the case (Figure 4) (4). So more research effort is required to find new and more biostable biomaterials for such applications.

Figure 3: Scanning electron micrograph of a woven polyester endovascular stent graft retrieved from the patient after 29 months *in vivo* showing evidence of surface abrasion.

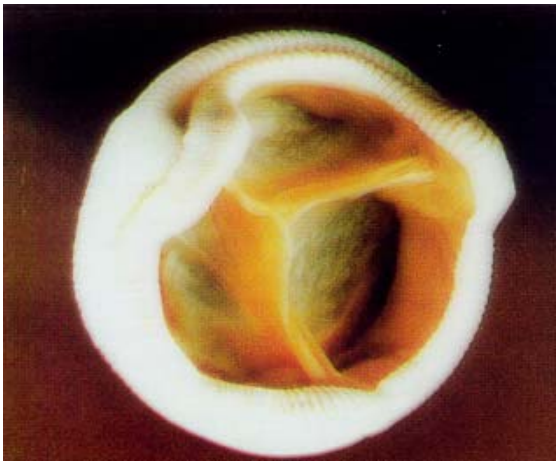

Figure 4: Porcine bioprosthetic valve with weft knitted polyester sewing ring used for heart valve replacement. Courtesy of St. Jude Medical Inc.

Biotextiles and Tissue Engineering

In North America and Europe there is a severe and growing shortage of viable and compatible human tissues and organs for transplantation into diseased and injured patients. Those who receive transplants must follow a lifelong regime of immunosupressive drugs with the concomitant risks of infection, tumor development and other side effects. So surgical reconstruction using autologous tissue (i.e. from the same patient) is an alternative strategy but not always possible due to the lack of viable donor tissue. This is particularly true for coronary artery bypass surgery where the availability of saphenous veins or mammary arteries may be limited. For other transplantations, e.g. kidneys and corneas, the availability of compatible donor tissue remains the limiting factor. Replacement with synthetic mechanical devices therefore continues to be a third option, and much research continues to be focused on reducing the risk of infection and thromboembolism and improving the biostability of devices such as bioprosthetic heart valves and endovascular stent grafts.

In the USA there are currently about 75,000 patients waiting for organ transplantation, with around 48,000 needing kidneys and 17,000 looking for liver transplants. The annual healthcare costs for the treatment of these patients exceeds US$400 billion (6). This situation is driving the increasing interest in tissue engineering research, which is defined as the "application of the principles and methods of engineering and life sciences towards fundamental understanding of the structure function relationship in normal and pathological mammalian tissues, and the development of biological substitutes for the repair and regeneration of tissue and organ function" (7). This work was pioneered by Langer and Vacanti in the early 1990's. There are two main thrusts for tissue engineering research. They are i) the *in vivo* route, and ii) the *in vitro* approach.

Tissue Engineering by the *In Vivo* Route

The objective of this route is to initiate tissue engineering therapies inside the body for the repair and regeneration of damaged or diseased tissue. Some examples from animal and clinical studies have already shown that this approach can be successful for blood cell and nerve regeneration (both peripheral and spinal cord), skin repair, remodeling of defective bone, cornea and retina, and for repairing damaged myocardium (heart muscle) following a myocardial infarction (heart attack).

Figure 5 illustrates various examples of *in vivo* therapies for treating leukemia (cancer of the blood), and repairing injured

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myocardium by the injection of stem cells that will differentiate and proliferate into the desired cell line. This approach relies on the pre-existence of extracellular matrix (ECM) proteins (7). For the repair of large defects ECM may be absent, and the implantation of a scaffold either with or without growth factors is necessary to serve the role of the ECM. As discussed later this porous scaffold may serve a number of roles, but its main purpose is to promote and regulate cell attachment and proliferation.

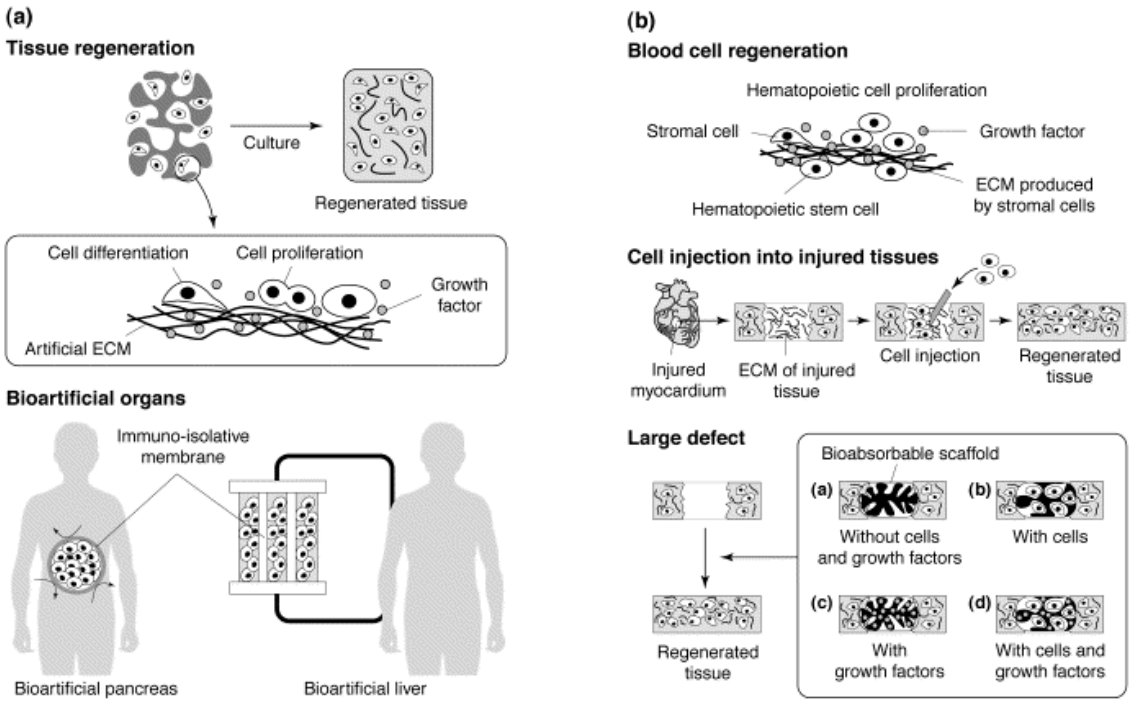

Figure 5: Basic principles in tissue engineering : (a) *in vitro* and (b) *in vivo* tissue engineering.

In certain applications such as the repair of severed peripheral nerves, the generation of fibroblastic connective tissue can be disruptive and prevent the proximal neuron from growing towards and reattaching to the distal stump. To prevent this from happening a narrow tubular shaped nerve guide woven from resorbable polyglycolic acid (PGA) yarns can serve as a barrier membrane. By using the biotextile filled with appropriate growth factors to join the two neural stumps, the proximal neuron regenerates and can bridge gaps as wide as 8 mm, so as to re-establish distal neural activity (Figure 6).

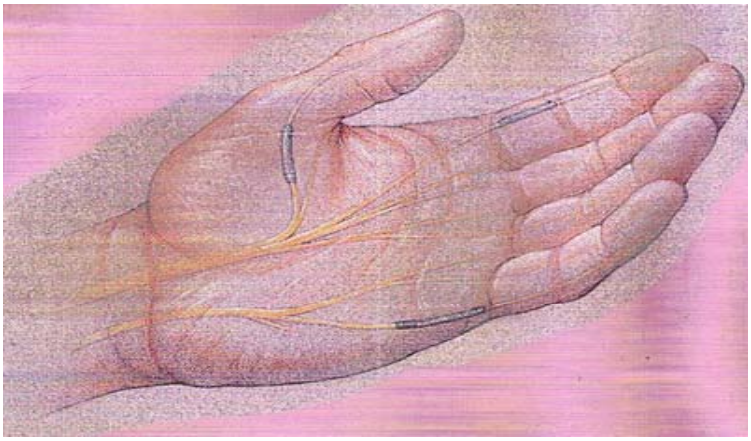

Figure 6: Tubular nerve guide woven from polyglycolic acid (PGA) yarns used for the regeneration of injured peripheral nerves. Courtesy of Neuroregen LLC.

Another example of using biotextiles for *in vivo* tissue engineering is the knitted external cardiac support. The hearts of patients with congestive heart failure have impaired pumping capacity. As their hearts attempt to compensate by pumping faster, the size of the heart becomes enlarged (dilated) and, because the valve leaflets do not grow an equivalent amount, the valves become increasingly less efficient, exacerbating the disease state. In an attempt to limit the growth of such dilated hearts, the company Acorn Cardiovascular Inc., has developed a warp knitted polyester cardiac support device, which is installed tightly around the diseased heart (Figure 7). Studies on animals have confirmed that not only does it halt progressive dilation and myocardial stretching, but it also improves cardiac function and pumping efficiency by allowing the damaged myocardial cells to repair, remodel and regenerate. In other words, the implantation of the device reverses the disease state, which makes this an alternative innovative therapy for patients who have side effects from traditional drug regimes (8).

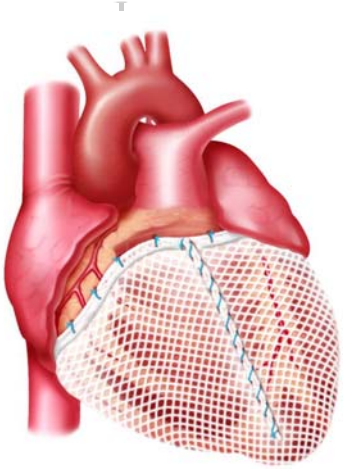

Figure 7: Implantable cardiac support device which improves cardiac function of patients with dilated cardiomyopathy, and allows remodeling of the heart muscle. Courtesy of Acorn Cardiovascular Inc.

Tissue Engineering by the *In Vitro* Approach

But not all diseases and injuries can be reversed or controlled by *in vivo* therapies. Much research is therefore directed towards developing tissue engineering constructs which can be manufactured in controlled cleanroom settings so as to provide an unlimited supply of functional tissue products. It is anticipated that these would be a class of generic off-the-shelf devices for

- implantation into patients to restore normal function of, for example, blood vessels, heart valves, livers, pancreas, bladders, tendons and cartilage,
- the manufacture of extracorporeal devices, such as kidney dialysis and artificial liver machines, and
- use in complex tissue cultures for the production of enzymes, drugs and growth factors, and for toxicological and pharmacological assays.

Figure 5 shows schematically how such approaches can work through *in vitro* tissue regeneration followed by implantation or by use in an extracorporeal device.

Elements of Tissue Engineering Strategies

In addition to controlling the cell culture conditions in a laboratory or production bioreactor (Figure 8) with respect to the supply of nutrients, the pH, temperature and CO_2 / O_2 atmosphere, the success of creating a tissue engineered construct depends on planning and controlling the following four elements of the process :

- viable cell line from a known and reliable source
- resorbable polymer material
- porous scaffold
- growth factors for signalling

Figure 8: Laboratory bioreactor for *in vitro* tissue engineering. Courtesy of Enduratec Inc.

Cell Sources for Tissue Engineering

A critical component of tissue regeneration is the need to select a viable type of cell that is clinically relevant, will not cause a negative immunological response, and has the capacity for rapid expansion to produce the volume of tissue required. One obvious approach is to harvest autologous cells by direct biopsy from the patient, which is a

technique used for skin and most organs, including liver, heart, blood vessels, bone, bone marrow and cartilage. However, for certain tissues, e.g. heart valve, direct biopsy is not feasible, and the disease state of some patients precludes the use of their material. So alternative strategies of harvesting human (allogeneic) or non-human (xenogeneic) cells is also being pursued. Technologies employing cell encapsulation, immune protection, extracorporeal systems and genetic manipulation are being developed to permit the use of these types of xenogeneic tissues and organs. Evenso, serious concerns remain over ethical issues and the transmission of infectious agents, such as viruses.

Biologists have recently recognized the unique biological properties of stem cells which are defined as "undifferentiated cells that can proliferate and have the capacity for both self-renewal and differentiation to one or more types of specialized cells" (9). Human stem cells come from two sources. Embryonic stem cells are derived from the inner cell mass of early aborted fetuses, and are capable of differentiating into almost any cell line. As adult humans we also carry stem cells in our bone marrow, brain, liver, skin and blood stream. These adult stem cells have a limited range of differentiated cell lineages or phenotypes depending on their location, and so are less versatile for tissue engineering purposes. In theory, appropriate adult stem cells can be harvested from a patient, incorporated into a tissue engineered construct and then returned to the individual without the need for immunosuppression (9)

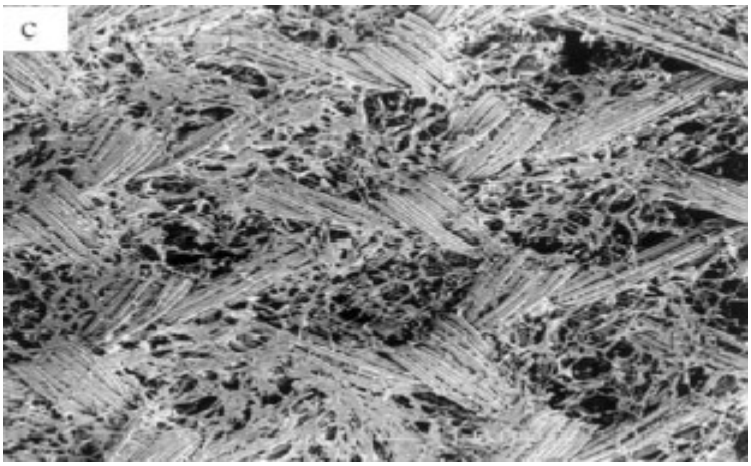

Figure 9: Tissue engineering with a collagen scaffold.

Selection of Polymers for Tissue Engineering

Collagen is the most abundant structural protein in the body, and is known to exhibit minimal inflammatory and antigenic responses. It has high strength and flexibility, and is already approved by regulatory authorities for medical applications related to wound dressings and artificial skin (Figure 9). As a fiber forming polymer it is particularly suited to tissue engineering applications since it contains cell adhesion domains, that is arginine-glycine-aspartic acid or RGD sequences, which elicit specific cellular interactions that assist in retaining the cell phenotype and activity, particularly for fibroblasts and chondrocytes (10).

Other naturally occurring polymers and biopolymers include silk proteins, alginates,

polysaccharides isolated from seaweed, and chitosan, derived from shell fish protein. These have been spun into fibrous meshes and nonwoven structures that have shown promise as tissue engineered scaffolds for the seeding of chondrocytes and osteoblasts (11, 12). Polymers such as poly-α-hydroxy acids (polyesters) of glycolic acid, lactic acid and copolymers and ethylene oxide block copolymers are widely used in tissue engineering because of their inherent ability to resorb (degrade) by hydrolytic or enzymatic mechanisms and return the polymer to natural metabolites and lactic acid which are readily excreted from the body (Figure 10). Several studies have focused on the seeding of chondrocytes in animal models for cartilage repair; while others have evaluated the culture of urethelial and smooth muscle cells for the regeneration of bladder tissue and blood vessels in dogs (13, 14). Other polymers of interest include polyhyaluronic ester which has supported cartilage tissue developments on novel nonwoven structures (15), and polyethylene terephthalate and polyurethanes which likewise can be manipulated into porous nonwoven structures (16).

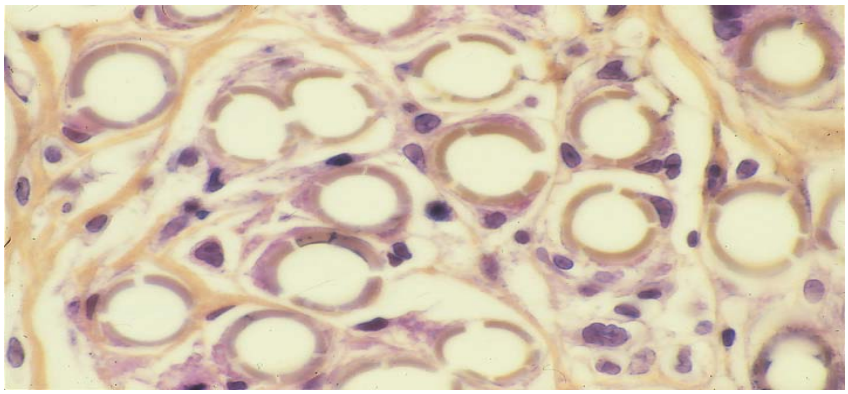

Figure 10: Histological cross-section of polyglycolic acid/polypropylene bicomponent fibers showing dynamic cellular infiltration after 28 days in an rat subcutaneous model, HPS stain.

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Porous Scaffolds for Tissue Engineering

Any scaffold material for tissue engineering must have the right composition, biocompatibility and 3-dimensional architecture. For example, it may be expected to function as artificial extracellular matrix (ECM), and elicit the biological and mechanical functions of native ECM. The following seven criteria are currently considered necessary for designing the ideal scaffold structure.

i) The surface should allow cell adhesion, promote cell growth and permit the retention of differentiated cell functions.

ii) It should be biocompatible and not produce cytotoxic degradation products.

iii) It should be bioresorbable so that eventually it is eliminated from the body.

iv) The porosity should be sufficient to provide space for cell adhesion, ECM generation, and minimize diffusional constraints during cell culture.

v) The pore structure should be open and uniform enough to permit homogeneous cell distribution throughout the entire scaffold.

vi) The material should possess sufficient physical integrity and mechanical stability to enable the construct to be handled and relocated.

vii) The material should be processable into a 3-dimensional structure and sterilizable.

Clearly biotextiles created with resorbable fibers can meet all these criteria, and for this reason we believe biotextile structures, whether woven, knitted or nonwoven (Figure 11), are uniquely suited to serve as tissue engineering scaffolds and compare favorably with other fabrication techniques such as phase separation, gas forming methods, emulsion freeze drying, porogen leaching and 3-dimensional prototyping.

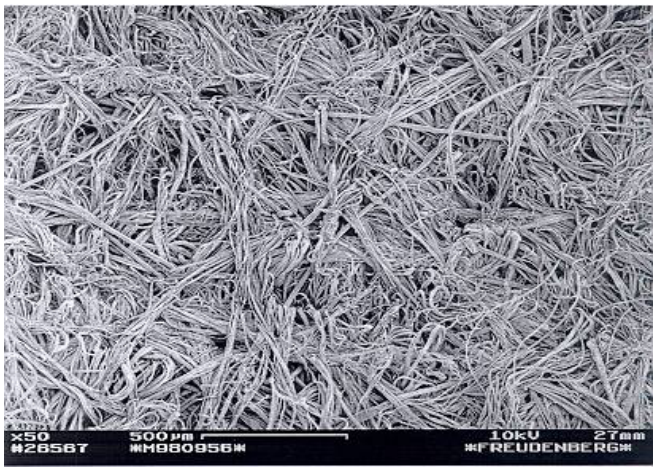

Figure 11: Porous nonwoven structure fabricated from microdenier fibers.
Courtesy of the Nonwovens Co-operative Research Center.

But there are many challenges that need addressing, including such simple questions as which fiber diameter and linear density is required? What is the optimal overall porosity and pore size distribution? Does the fiber orientation distribution influence the direction and spacing of cellular proliferation? The cellular response to biotextile has been shown to depend directly on fiber diameter, with finer fibers generating thinner surrounding tissue capsules in vivo (17). Also recent reports on the preferred scaffold porosity suggest that fibroblast cells spread and proliferate more rapidly and uniformly on scaffolds containing pores in the 10-22 αm range (16).

Signals for Cell Proliferation and Growth

Cellular activity and function are influenced by different types of signals associated with the cellular environment. They include:

i) Extracellular matrix signals. The extracellular matrix in which most cells grow consists of a mixture of collagens, proteoglycans, hyaluronic acid and various glycoproteins such as fibronectin, vitronectin and laminin. Cells must interact with these macromolecules in order to function in terms of adhesion, migration and differentiation of phenotype expression. Adhesion is achieved through cells binding to the arginine-glycine-aspartic acid-serine (RGDS) peptide sequence on fibronectin and by other mechanisms. When such adhesion proteins have been added to the surface of a TE scaffold, they have been found to enhance cell adhesion, migration and proliferation. For example, neurons exposed to adhesion proteins have been observed to grow more rapidly and for longer so as to bridge and repair sciatic nerve defects.

ii) Signals from cytokines and growth factors. Growth factors also effect cell function by binding to cell surface receptors and triggering signal transduction cascades which result in either the promotion or inhibition of cell division, differentiation, mobility and gene expression . Each growth

factor as its name implies has a specific function, e.g. fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-□) and platelet derived growth factor (PDGF). The challenge is to find an appropriate delivery mechanism for these signaling proteins and to know when and at what concentration to deliver them to the TE construct within the bioreactor.

iii) Spatial organization. The correct spatial orientation of cells in either a 2 or 3-dimensional array is essential for their successful culture. Nanofabrication techniques, micro-stamping and micro-patterning, and ink-jet printing techniques have been shown to provide the surface contour or topography or surface chemistry for controlling the cellular array (18). This has also been applied to hippocampal cells and other nerve cell constructs to ensure that they develop an active neural network and exhibit polarized neural function.

iv) Mechanical modulation. Mechanical modulation of engineered tissue is essential in order to develop the appropriate orientation, cell morphology and mechanical properties in the tissues with respect to the extracellular matrix molecules (Figure 12). This is particularly important when creating tissue constructs from cardiac, venous and arterial smooth muscle cell populations so that they can develop resistance to the mechanical strains experienced *in vivo*.

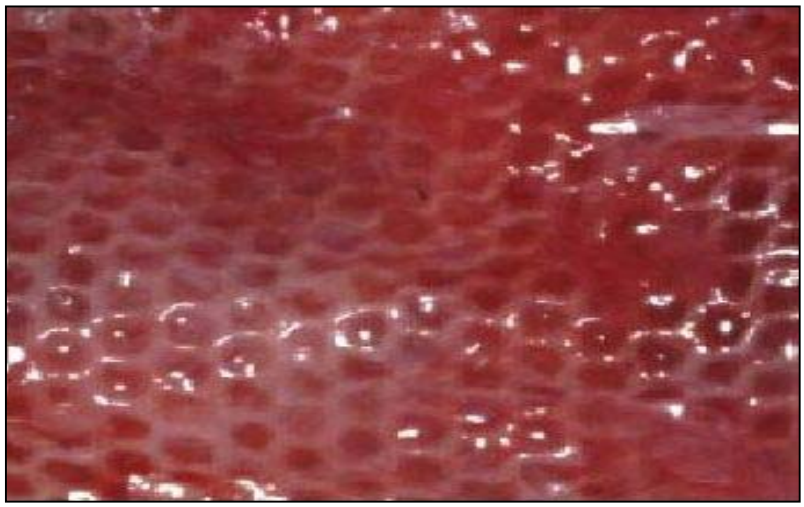

Figure 12 : Porous warp knitted structure with stretch and recovery properties engineered to support the growth and proliferation of heart muscle cells seen after 6 months in a canine model.
Courtesy of Acorn Cardiovascular Inc.

v) Electrical signals. We have demonstrated in our laboratory that electrical stimulation can be used effectively in controlling cellular functions when growing endothelial cells on semiconductive polypyrrole fabrics (19). Other *in vitro* and animal studies have shown that the applied voltage influences the direction and rate of migration of corneal epithelial cells or sciatic nerve cells.

vi) Intercellular interactions. Cell-cell interactions have proven to play crucial signalling roles in regenerating complex

tissue structures such as those for liver, blood vessels and nerve constructs. This can occur either through direct cell-cell contact or via secreted molecules such as ECM components. Such interactions provide physical support from one cell type to another and induce favorable phenotype expression. Co-culturing with other cells can enhance the maintenance of the phenotype of a primary cell culture *in vitro*.

vii) Gene delivery. Recent developments in genetic engineering have provided an additional powerful tool for those in the tissue engineering field to provide signalling cues for cell proliferation and growth. For example, my colleagues have recently synthesized an elastin-mimetic peptide polymer through genetic engineering and electrospun elastin nanofibers into a nonwoven scaffold (20). A 81 kDa recombinant protein based on the elastomeric peptide sequence of elastin was expressed in E.coli using a pGTA plasmid vector. Other ways of incorporating plasmid DNA into tissue engineered constructs have involved the release of plasmid encoded PDGF from a 3-dimensional resorbable polyglycolide-co-lactide polymer matrix which, when implanted subcutaneously in rats, increased the time for granulation tissue production to over 4 weeks. This technology is still in its infancy, and potential barriers, such as plasmid vector inactivation and gene transfer inefficiency, need to be overcome.

Conclusions

From this discussion it is evident that textile researchers have much to contribute to the development of scaffold materials for tissue engineering. Depending on the application and cell line under investigation, there is a need to study bioresorbable polymers, whether naturally occurring, synthetic or genetically engineered, surface modification chemistries, fiber size and cross-section and type of fabric structure, so as to ensure that they will provide appropriate strength, compliance, resorption rate, electrical conductivity, microstructure, 3-dimensional architecture, porosity, permeability and cell recognition properties that can be used to regulate cell proliferation and growth.

We have traveled a long way from our 1980's definition of biomaterial as a nonviable material. Indeed this previous concept that preferred biocompatibility means an inert material with minimal inflammatory response, and that biostability means no change in mechanical and chemical properties *in vivo*, have been completely reversed. For today's tissue engineering needs a biotextile scaffold requires its biocompatibility to be expressed in terms of its "ability to interact with cells and extracellular matrix components so as to optimize cell function and generate functional tissue". In the past we may have thought of the Bionic Woman and the Six Million Dollar Man simply as science fiction characters, but with the current rapid advances in the biological and genetic sciences, in nanotechnology, biotextile engineering and the delivery of targeted healthcare therapies, they will soon become part of today's reality in the twenty first century.

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