Muscling in on Tissue Engineering

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The establishment of a Tissue Engineering Research Centre (TERC) and other activities in Australia, reflects the major developments in bioengineering research to construct ‘living’ tissue replacements that are attracting considerable world-wide clinical and commercial interest. Construction of ‘artificial’ skeletal muscle illustrates some of the key issues to be considered.

Tissue Engineering is an emerging interdisciplinary field that applies the principles of biology and engineering to the development of substitutes to restore, maintain or improve the function of body tissues [1, 2]. Examples of ‘non-living’ engineered body parts such as artificial joints, contact lenses and the bionic ear are already in wide clinical use and others such as artificial corneas are now realities. The extension of this approach to the engineering of ‘living’ tissue constructs that become integrated within the patient is of enormous clinical interest in many areas and is attracting much commercial attention. Although cells have been cultured outside the body for many years, it has only recently become possible to grow complex three dimensional tissue
constructs *ex vivo* to meet clinical needs. A sophisticated understanding of cell biology and developments in stem cell biology, combined with new biomaterials have opened up many challenging possibilities to greatly extend existing approaches. The growth of sheets of skin *ex vivo* for transplantation after extensive burns is already widely used clinically and construction of living three-dimensional tissues for blood vessels, heart valves, tendons, bladders, bone and cartilage replacement is well advanced. Reconstruction of nerves to repair spinal defects and other central nervous system damage is a major challenge (see article in next issue) and construction of neo-organs e.g. liver, kidney, pancreas, heart, could overcome the problem of limited availability of donor organs. Tissue engineering combined with the availability of human stem cells presents the possibility for construction of a wide range of ‘spare body parts’ from the patient’s own cells (for further information on applications see http://www.cs.cmu.edu/~webwatch/#Organizations).

In order to bring together researchers in these diverse fields to share expertise and stimulate discussion for future collaborations, a local Tissue Engineering Research Centre (TERC) has been established in Western Australia (http://www.terc.uwa.edu.au). TERC recently organised a highly successful visit by Professor Joseph Vacanti from Boston to Perth to participate in a Workshop on “Frontiers in Tissue Engineering” (held on October 5th 2000). Joseph Vacanti is a surgeon who pioneered Tissue Engineering and is internationally recognised as a world leader in this new area (http://web1.tch.harvard.edu/faculty/vacanti.html). Diverse collaborations and creative thinking are central to such bioengineering and a collaborative Studio for Art and Science, called SymbioticA, was recently established in the Department of Anatomy and Human Biology to facilitate innovative interactions.
between artists and scientists. Such cross-disciplinary activities have already led to two WA artists involved in “Tissue Culture & Art” currently working in Professor Vacanti’s laboratories in Boston on tissue engineering related projects (http://www.tca.uwa.edu.au).

**Bioengineering skeletal muscle masses**

The longstanding research interests of our laboratory relate to the repair of mature skeletal muscle. The research falls into two broad areas: understanding the wide range of factors that control the activation, proliferation and fusion of myoblasts (the muscle precursor cells) in regenerating mature skeletal muscle *in vivo* [3]; and myoblast transplantation strategies for therapeutic gene replacement [4] in muscle diseases (see http://www.anhb.uwa.edu.au/staff/grounds/default.htm). It is a challenge to extend this broad based cell biology knowledge to the design and pre-assembly *ex vivo* of fully vascularised skeletal muscle tissue for potential clinical transplantation for anatomical reconstruction and treatment of muscle diseases [5, 6, 7]. Three broad aspects can be considered: (A) design of the ‘artificial’ vascular network, (B) the extracellular matrix scaffold for the muscle, and (C) formation of the skeletal muscle fibres and subsequent appropriate innervation. These are briefly outlined below.

**(A) The vascular network.**

This is an area of intensive research, as it is fundamental to the construction of most bioengineered organs e.g. liver. The idea is to construct hollow vascular tubes using
some biocompatible material as a scaffold, to line this structure with endothelial cells, and provide smooth muscle cells and fibroblasts to populate the outside of the vessels: ideally these cells will become appropriately organised and the artificial scaffold will be replaced over time with endogenous matrix. This artificial vascular system will be filled with fluid (initially tissue culture media) and used to feed the cells of the bioengineered organ as it grows in a bioreactor, i.e. the nutrients will be delivered, and waste products removed via these vessels. Superior vessels result when such artificial vascular constructs are grown under pulsatile conditions (similar to what happens when the heart beats) [2]. At the time of transplantation the main input (artery) and output (vein) vessels will be attached by microsurgery so that the ‘vascular supply’ to the bioengineered organ/tissue will be maintained. In the case of skeletal muscle which is a highly vascular tissue, when the vascular supply is disrupted by transplantation (or extensive injury) this normally results in necrosis and regeneration. However, new muscle formation is impaired in central areas of large muscles (greater than 5gm) due to prolonged ischaemia, thus it is highly desirable for clinical purposes to maintain continuity of vascular supply. It is envisaged that this vascular network will adapt after transplantation to the biological needs imposed upon it.

There are many problems associated with the simplistic scenario outlined above. These include the technical construction and design of the scaffold and dispute over how detailed the initial network need be (see Figure 1).
Figure 1. **Vascular scaffold showing two extreme designs.** Note: these should be considered with respect to an overall three-dimensional structure. A complex initial network with some capillary beds is indicated at the top - this presents many more potential problems for occlusion of the small vessels. An alternative (shown below), is simple ‘plumbing’ with perhaps some slight constriction to slow the circulating fluid. Both designs assume subsequent vascular sprouting to form fine capillaries (indicated in Figure 2), and also replacement of the simple scaffold (supporting the endothelial cells) by layers of basement membrane, collagen, elastin and other ECM components, plus smooth muscle cells.

For example, are capillaries required from the onset or will they subsequently develop *de novo* by sprouting in response to physiological demand? What is the most basic structure that would suffice? The issue of endothelial cell behaviour and problems of hyperplasia and occlusion of the vessels is a key issue that builds upon much research into cardiovascular disease. These challenges test to the utmost our understanding of all aspects of these biological situations. (Such issues are the topic of a workshop on ‘Tissue Engineering, Remodelling and Angiogenesis’ to be held at the 7th World Congress on Microcirculation, in Sydney in August 2001.)
(B) The extracellular matrix (ECM)

The connective tissue lying outside the muscle fibres (especially collagen) is of tremendous importance structurally for transmission of the contractile forces, that occur along the length of the myofibres as well as at tendons [8]. See Figure 2.

Figure 2. The complex ECM scaffold. The scaffold of interstitial collagen fibrils (that surrounds individual skeletal muscle fibres) is shown (at the top). These are connected to ECM molecules in the basement membrane that is in intimate contact with the sarcolemma of the myofibres (see Figure 3). The location of a myofibre within the ECM scaffold and associated blood vessels (capillaries) are also shown.

It is also well recognised that molecular connections from the internal environment of muscle fibres, through the sarcolemma, to the specialised ECM that forms the basement membrane around myofibres, are critical for normal muscle function: defects in key molecules here (e.g. dystrophin, sarcoglycans, laminin) lead to severe myopathies [9]. Furthermore, new muscle formation is far more effective within the basement membrane, due in part to exclusion of fibroblasts but also because of laminins in the basement membrane that are known to facilitate myogenesis, compared with components of the interstitial ECM such as collagen 1. In tissue culture, basement membrane components clearly enhance myoblast migration and fusion, but seem to be
less critical for cell proliferation. The question arises of what (if any) structural and molecular composition of ECM scaffold (both interstitial and basement membrane) is required to optimise new muscle formation in a bioreactor? Alternatively, to what extent might new muscle cells self-assemble along lines of tension, and subsequently produce (along with fibroblasts) the basement membrane and other ECM components as required? Various scaffolds have been used to grow myoblasts (of the C2C12 cell line) in vitro, including synthetic biodegradable non-woven meshes of polyglycolic acid [6], patterned organosilane grids [7] and collagen gels in different shapes [10]. Beyond this, the important role of the ECM has barely been addressed.

(C) New muscle formation

Skeletal muscle has a terrific capacity for regeneration. The myoblasts are classically considered to arise from satellite cells that lie beneath the basement membrane on the outside of mature muscle fibres (Figure 3).

![Formation of skeletal myofibres](image)

**Figure 3. Formation of skeletal myofibres.** The myoblasts (mononucleated precursor cells) proliferate and fuse to form multinucleated myotubes. The myotubes fuse together and mature into innervated myofibres. Note: innervation (usually by a single synapse in the mid-region of each myofibre) is essential for muscle survival and function. The ECM scaffold consisting of interstitial connective tissue (mainly collagen I) and the basement membrane (including laminin-2, proteoglycans and collagen IV) is indicated.

These cells can be extracted from muscle tissue and readily expanded in culture. A range of growth factors and ECM molecules that enhance myoblast proliferation and
fusion have been identified from both \textit{in vitro} and \textit{in vivo} studies [4]. Thus the ingredients are available for generation of new skeletal muscle \textit{in vitro} within appropriate three-dimensional scaffolds. See Figure 3. Ideally, to minimise immunological problems of rejection after transplantation, the myoblasts would be autologous and be derived initially from a muscle biopsy taken from the potential patient. The rapid death of transplanted histocompatible cells \textit{in vivo} after exposure to tissue culture conditions as used in myoblast transfer therapy [3] emphasises the need to consider a potentially adverse host immune response to such tissues constructed \textit{ex vivo}.

Beyond the satellite cells, other sources of myoblasts from putative stem cells are now attracting much interest as it has been demonstrated that skeletal muscle can be derived from dermal fibroblasts, from interstitial/mesenchymal stem cells and from bone-marrow derived stem cells [4]. The opportunity to harvest such human stem cells and direct them into the myogenic lineage is the subject of intensive research in many laboratories [4,11]. In patients with Duchenne muscular dystrophy where the skeletal muscles are severely compromised, autologous myoblasts might be generated from such alternative sources and genetically corrected prior to myoblast transfer therapy. Genetic manipulation of autologous myoblasts, either to replace a missing gene such as dystrophin, or to allow transient controlled expression of key growth factors to enhance some aspect of myogenesis (e.g. IGF-I), offers many powerful possibilities.

It is a fascinating challenge to undertake such bioengineering. While each tissue presents its own unique problems, many fundamental issues are in common. It is
anticipated that many innovative approaches and rapid advances in Tissue Engineering will occur in the next few years.

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References


