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TISSUE ENGINEERING

Tissue engineering is an emerging interdisciplinary field that applies biology and engineering to the development of viable substitutes, which restore, maintain, or improve the function of tissues or organs. This type of therapy differs from the standard therapies in that the engineered tissue becomes integrated within the patient, affording a potentially permanent and specific cure of the disease. Tissue engineering crosses numerous medical and technical specialities. It involves cell biologists, molecular biologists, biomaterial engineers, computer-assisted designers, robotic engineers and developers of bioreactors, where tissues are grown and nurtured.

To engineer living tissues *in vitro*, culture cells are coaxed to grow on bioactive degradable scaffolds that provide the physical and chemical basis to guide their differentiation and assembly to 3D tissues. Coaxing cells to form tissues in a reliable manner is the quintessential engineering design problem. Food and Drug Administration (FDA) in USA, has approved 5 engineered tissues and presently several companies are spending a lot of money to develop new products.

Scaffolds are porous, degradable structures fabricated from either natural materials (collagen, fibrin) or synthetic polymers (polyglycolide, polylactide, polylactide coglycolide). They can be sponge-like sheets, gels or highly complex structures with intricate pores and channels fabricated using new materials—processing technologies. Virtually all scaffolds used in tissue engineering are intended to degrade slowly after implantation and be replaced by new tissue. Polyglycolide, unlike polylactide, does not dissolve in solvents such as chloroform. Thus, 3D polyglycolide scaffolds can be sculpted by dipping them in a solution of polylactide dissolved in chloroform and shaping the wet fabric on a mould. When the chloroform evaporates, the polylactide serves as a solid glue to hold the fabric in the desired shape. A scaffold made in this way in the shape of a bladder and seeded with urinary epithelial and smooth muscle cells and implanted into dogs, acquired near-normal function.

There are three principal therapeutic strategies for treating diseased or injured tissues in patients: (i) implantation of freshly isolated or cultured cells, (ii) implantation of tissues assembled *in vitro* from cells and scaffolds, and (iii) *in situ* tissue regeneration. For cellular implantation, individual cells or small cellular aggregates from the patients or a donor are either injected into the damaged tissue directly or are combined with a degradable scaffold *in vitro* and then implanted. For tissue implantation, a complete 3D tissue is grown *in vitro* using patient or donor cells and a scaffold and then is implanted once it has reached "maturity". For *in situ* regeneration, a scaffold implanted directly into the injured tissue stimulates the body's own cells to promote local tissue repair.

Sources for cells for implantation include autologous cells from the patient, allogeneic cells from a human donor who is not immunologically identical to the patient, and xenogeneic cells from a different species. Each category may be further delineated in terms of whether the cells are adult or embryonic stem cells, or a mixture of differentiated cells at different stages of maturation. Some approaches use cell mixtures, whereas others rely on separation or enrichment of stem cells.

Allogeneic cells have been used successfully to treat skin ulcers, diabetes and liver disease. Patients with diabetic or venous skin ulcers have been treated with two FDA-approved living skin products engineered in the laboratory. One product is made up of neonatal dermal fibroblasts obtained from human foreskins. They are expanded in culture and seeded into thin scaffold composed of the polymer, polylactide coglycolide, which breaks down gradually in the presence of water. The cells on their scaffold are cultured in custom-designed bioreactors for several weeks until they form tissue similar to the inner dermal layer of skin. This neo-dermis is then frozen for use later on. The second skin product has both dermal and epidermal layers. It is made up of dermal fibroblasts in a collagen solution that forms a gel when heated to body temperature; the gel is coated with several layers of human epidermal cells (keratinocytes). After transfer to the patient, this skin product is at least partially replaced by host skin cells as healing progresses. The dermal fibroblasts in the skin products naturally secrete extracellular matrix proteins and are able to respond to growth-regulatory molecules secreted by the host. These skin products can persist for up to 6 months after implantation.

There is an FDA-approved autologous cell product for the repair of articular cartilage. A small piece of cartilage is removed from the healthy section of a patient's injured knee. Cartilage cells are isolated, grown in culture, and then implanted at the injury site. In another approach, mesenchymal stem cells have been harvested from patient's bone marrow, grown in culture, and then induced to differentiate into cells that can help to repair damaged bone, cartilage, tendon, or ligament.

Stem cells hold good promise for treating damaged tissue where the source of cells for repair is extremely limited or not readily accessible. Embryonic stem cells are attractive because they can be expanded in any undifferentiated state *in vitro* and can be induced to form many different cell types. Adult bone marrow stem cells can be collected from the circulation (after mobilization with cytokines) and used clinically to treat a range of blood disorders. Recent reports that marrow-derived stem cells can give rise to hepatocytes, cardiac muscle cells, and lung tissue suggest that efficient recruitment of bone marrow stem cells to sites of injury or their injection into target sites may provide a source of cells for tissue repair.

One of the principal constraints on the size of tissues engineered *in vitro* that do not have their own blood supply is the short distance over which oxygen can diffuse before being

consumed. Once implanted in the patient, cells in the engineered tissue will consume available oxygen within a few hours, but will take several days for the growth of new blood vessels that will deliver oxygen and nutrients to the implants. How can this problem be overcome? Implanting cultured cells directly into the existing vascular beds of the patient's liver and spleen appears to be a promising strategy. Some diabetic patients with pancreatic islet cells implanted into their liver exhibited normal glucose tolerance for several months after the procedure. Unfortunately, cells implanted for the repair of bone or tendon, for example, cannot exploit existing vascular beds. Inducing or speeding up growth of new blood vessels by engineering a scaffold to slowly release growth factors, such as vascular endothelial growth factors or fibroblast growth factor, may be the answer. Formation of new blood vessels can also be induced using engineered skin products because the dermal friboblasts that they contain produce angiogenic growth factors. The need for preformed vascular beds or rapid angiogenesis could be avoided altogether by exploiting what may be a common property of many stem and progenitor cells—their resistance to low-oxygen conditions.

By the inclusion of endothelial cells (which form blood vessels) in cultures of the cells to be expanded, the rudimentary tube-like vessels form within the assembly tissue. Another promising method is to form fully vascularised tissues for implantation that contain blood vessels of sufficient size that they can be fused with the patient's own blood vessels during surgery. The complexities associated with organizing millions of cells into 3D structures such as blood vessels can be simplified using computer modeling, which translates the tissue's 3D structures into 2D template. Composed of a degradable polymer, the 2D template precisely guide cells to their correct positions, the engineered tissue finally being folded up to form the 3D structure.

Scaffolds can be designed to release growth factors that induce cellular differentiation and tissue growth *in vitro*, or cell migration into the wound site *in vivo*. The fragile nature of proteins has motivated design of scaffolds that release naked plasmid DNA containing genes that encode growth factors. New bioactive materials, such as those that covalently incorporate growth factors and other molecules that regulate cell behaviour, offer alternatives for enhancing scaffold performance.

A crucial mainstay of tissue engineering is the biomaterial from which scaffolds are prepared. Many biomaterials direct the growth of cells in culture. However, tissue regeneration *in vivo* involving the guided growth of nerve, bone, blood vessels or corneal epithelia across critical injury sites requires that cells receive more specific instructions. The ideal biomaterial for a scaffold would selectively interact with the specific adhesion and growth factor receptors expressed by target cells in surrounding tissues required for repair of damaged tissue. The scaffold could guide migration of these target cells into the injury site and stimulate their growth and differentiation, finally degrading in response to matrix remodeling enzymes released by the cells as tissue repair progresses.

Cell motility is an adhesion-dependent process required for cell migration, angiogenesis, and regrowth of several nerve ends, among many other physiological events. Intermediate adhesion is required for optimal cell migration. *In vivo* bone scaffolds coated with adhesion proteins containing the amino acid sequence Arg-Gly-Asp (RGD) promote maximal tissue ingrowth only at intermediate values of ligand surface density; likewise, only at an intermediate density do adhesion proteins on scaffolds induce neural progenitor cells to extend neuritis, a prerequisite for nerve regeneration. Cells are also responsive to the nanoscale spatial organization of RGD peptides- such peptides more effectively induce cell adhesion and migration when they are clustered rather than random.

The correct molecular and macroscopic architecture of cartilage, blood vessels, bone, and other tissue is essential for proper tissue function. Connective tissue cells grown on 3D scaffolds *in vitro* secrete biochemically appropriate extracellular matrix molecules of cancer metastasis that mimic the lodging of a single tumour cell in a capillary bed, which would facilitate the development of antimetastatic drugs.

Many current medical therapies may be improved upon by tissue engineering with significant financial savings. For example, in standard organ transplantation, a mismatch of tissue types necessitates life-long immunosuppression, with its attendant problems of graft rejection, drug therapy costs, and the potential for the development of certain types of cancer. There is always the potential for rejection of the tissue, and the surgery, itself, carries some risk.

The increasingly intimate combination of engineering and biology offers the prospect of sophisticated physiological *in vitro* models of many different human tissues. These physiological surrogates will ultimately allow major advances in prevention and diagnosis and molecular treatment of diseases that are currently considered potential targets for tissue engineering. However, much research work is needed to improve the tissue engineering techniques, which will improve the lives of millions of patients in times to come.

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