6 Tissue Engineering

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INTRODUCTION

Tissue engineering represents an interdisciplinary field, a forum where the principles of biomaterial engineering, the molecular biology of cells and genes, and the clinical sciences interact intensively through the combined efforts of scientists, engineers, and clinicians. Tissue engineering attempts to exploit the cells' reproductive potential and to harness the body's intrinsic capacity for healing and regeneration.[1] These cells produce growth factors and cytokines, which function as architects of the repair processes. As defined by Robert Langer in 1993, tissue engineering comprises[2]:

- The isolation and manipulation of individual cells or cell substitutes, used for therapeutic infusion
- The identification of tissue-inducing substances, such as growth factors, and their appropriate delivery to their target
- Cells placed on or within matrices that permit the delivery of nutrients but protect cells from immunological destruction

The possibilities of cell engineering, matrix development, and growth-factor therapies are extensive. Imagine how application of proangiogenic proteins may reverse hypoxia in flaps, or stimulate tissue integration; how peptides may attack stubborn infections in wounds; how autologous cells from biopsies may generate an autologous dermal and epidermal cover on extensive burn wounds. Based on CT-imaging, rapid prototyping may generate a pre-shaped 3-D scaffold in which these cells can be seeded (Fig. 6.1). These scaffolds may come in the shape of an ear, a nose, a mandible, or a breast. The paucity of optimal donor tissues may be treated with off-the-shelf tissues using tissue-engineering strategies.[3–6]

However, the speed of translation of tissue-engineering science into clinical reality is slow due to the intrinsic complexity of tissues.

In this chapter, we seek to provide a perspective on the current strategies used in translational tissue engineering. Additionally, describe five clinical surgical domains, in which the integration of tissue-engineering protocols is a fact.

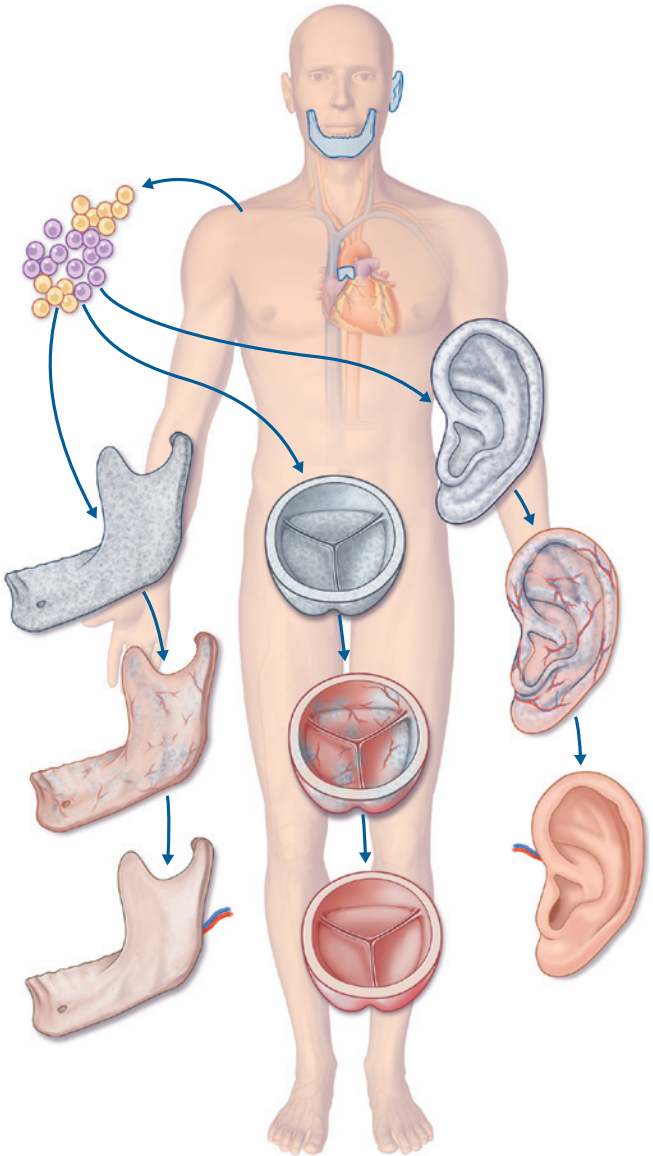

Figure 6.1 The tissue engineering concept. Data from CT-scans are used to create a 3-D-matrix by rapid prototyping technology. The porous, shaped matrices serve as a scaffold for cell seeding. Cultivated autologous cells proliferate and migrate in the matrix. A vascular network develops, stimulated and guided by growth factors, cytokines, and adhesion molecules. A macroscopical vascular supply represents the hurdle stone in this tissue-engineering strategy and is the current focus of research and translational studies.

MECHANISMS OF ACTION

CURRENT STRATEGIES IN TISSUE ENGINEERING

Three major approaches have been established toward engineering implants for regeneration: (1) implantation of engineered scaffolds and matrices; (2) implantation of neo-tissue derived from cells; (3) implantation of cells seeded within matrices.[7]

COMPLEX SCAFFOLDS AND MATRICES

The optimal bioengineered substitute must have a structure similar to the authentic tissues it must replace. It should be autologous to optimally integrate without rejection, 3-dimensional to bridge deep defects, porous to allow cell migration, bio-inductive for cells to proliferate and to topically produce extracellular matrix (ECM) components and growth factors, bio-inductive for vascular sprouts to develop within the construct and chemotactic for cells from the wound surroundings to infiltrate, stimulate vasculogenesis and optimize tissue integration.[3,6] When selective absorption of the matrix occurs, its composition and structure should be maintained. The absorption process should not result in deposition of toxic side products. The accompanying inflammatory response should be limited not to hamper cellular migration and repopulation stimulated by site-specific cues for cellular adhesion. This extensive list of requirements is self-explicatory as to why the ideal off-the-shelf scaffold has not been developed yet, despite the detailed knowledge of many biocompatible materials. It is the collaboration between material scientists, chemists, clinicians, and molecular biologists that forms the multidisciplinary environment required to design a veritable biomimetic smart scaffold.[5]

FUNDAMENTAL ROLE OF VASCULARIZATION

The absence of a vascular network capable of distributing oxygen and nutrients within the matrix is the key limiting factor in the overall success of tissue engineering. Nutrient supply and waste removal of limited-thickness engineered tissues such as skin, cartilage, or bone can initially be overcome by diffusion, until neovascularization takes over. In 1973, Folkman demonstrated that cells only survive within a 3-mm distance from a nutrient source.[8] From clinical evidence using artificial dermis in burn patients, we estimate the critical thickness to be even less, between 1 and 2 mm. The ultimate purpose of tissue engineering is to incorporate an inherent vascular bed, ready to be connected to the host vascular system. A viable vascular network within the tissue construct represents the missing link between the host and the engineered implant.

The adult vascular network remodels itself by the enlargement of existing collaterals induced by shear forces, as well as by angiogenesis; the coordinated migration and proliferation of endothelial progenitor cells and pericytes from the existing vascular bed and their subsequent maturation into endothelial cells, stabilized by these enveloping smooth muscle cells. The primary stimulus causative for capillary sprouting is hypoxia. The extracellular matrix functions as a reservoir of guidance molecules, i.e. growth factors that induce incoming blood vessels (angio-induction) and as a scaffold to guide outgrowth of blood vessels. To further enhance vascularization, proangiogenic endothelial progenitor cells can be seeded into this framework.[4] These phenomena can be induced by an in-situ or ex-vivo approach.

With in-situ cultivation of cells and tissues, the patient's own body is used as incubator. Such a strategy sounds logical and efficient. However, infiltration of undesired tissues and the obligatory use of shaped biocompatible implants as scaffold make this approach far from evident.

On the other hand, an ex-vivo approach to overcome the invasion of undesired tissues requires an immediate reperfusion once the regenerated tissue is "transplanted" in situ. Moreover, the ex-vivo revascularization step should occur in an incubator that mimics the real-time wound environment. Such strategy is also not evident.

Both approaches are intensively investigated.

PROANGIOGENIC STRATEGIES

The most obvious strategy focuses on adding proangiogenic molecules to the recipient tissues or the scaffold: growth factors such as VEGF, bFGF, PDGF, IGF-1 function as coordinators of the wound healing process, turning a temporary scaffold into a vascularized scaffold by attracting vascular progenitor cells, which will interact with local cells to grow a vascular network in situ.[4,9] These proangiogenic proteins are secreted by the cells in a specific sequence. In an *in vivo* model, these processes occur spontaneously once the cells are added to the wound. In a hostile milieu, missing cells and growth factors could be added to induce healing and integration. However, the half-life of externally added proteins to the healing wound is very short. *In-vivo* and ex-vivo gene transfer protocols may overcome the short-acting effect of proteins by turning local cells into production units of those proangiogenic growth factors. Nevertheless, to avoid tumor growth, it is essential to control this proangiogenic stimulus.[4,9–11]

In a clinical setting, prefabrication and prelamination techniques can be used to induce the formation of a macroscopical, transplantable vascular supply to tissues or tissue constructs. Similarly, hypoxia and inflammation in response to wounding are triggers to induce angiogenesis.[12]

Recent proof-of-principle strategies combine engineering with biomolecular approaches. Miller and colleagues described 3-dimensional printed networks of carbohydrate glass-molds coated with PLGA-copolymer. Constructs were encapsulated in a range of hydrogels along with living cells. Following ECM cross-linking, glass particles were dissolved to reveal patent fluidic PLGA-channels.[13]

An alternative approach is the use of photo-cross-linkable gelatin methacrylate using projection stereolithography based on computer-aided design.[14] The advantage of CAD/CAM-design is the large-scale reproducibility and the patient-specific scaffold production through the combination with CT-imaging.

Another option is to use stacking strategies, coined "cell-sheet engineering." A temperature-responsive surface is used to culture cells. Upon reaching confluency, the surface can be cooled to 20°C to reduce its hydrophobicity, and the intact cell sheet can be removed, preserving cell-to-cell junctions, ECM and cell-surface proteins. These cell sheets can then be stacked to generate multi-layered, cell-dense tissues. Stacked layers of cardiomyocytes that beat simultaneously