92e Tissue Engineering

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Tissue engineering is a field that applies principles of regenerative medicine to restore the function of various organs by combining cells with biomaterials. It is multidisciplinary, often combining the skills of physicians, cell biologists, bioengineers, and material scientists, to recapitulate the native three-dimensional architecture of an organ, the appropriate cell types, and the supportive nutrients and growth factors that allow normal cell growth, differentiation, and function. Tissue engineering is a relatively new field, originating in the late 1970s. Early studies focused on efforts to create skin substitutes using biomaterials and epithelial skin cells with a goal of providing barrier protection for patients with burns. The early strategies employed a tissue biopsy, followed by ex vivo expansion of cells seeded on scaffolds. The cell–scaffold composite was later implanted back into the same patient, where the new tissue would mature. However, there were many hurdles to overcome. The three major challenges in the field of tissue engineering involved: (1) the ability to grow and expand normal primary human cells in large quantities; (2) the identification of appropriate biomaterials; and (3) the requirement for adequate vascularization and innervation of the engineered constructs.

ISOLATION AND GROWTH OF CELLS

The original model for tissue engineering focused largely on the isolation of tissue from the organ of interest, the growth and expansion of the tissue-specific cells, and the seeding of these cells onto three-dimensional scaffolds. Just a few decades ago, most primary cultures of human cells could not be grown and expanded in large quantities, representing a major impediment to the engineering of human tissues. However, the identification of specific tissue progenitor cells in the 1990s allowed expansion of multiple cell types, and progress has occurred steadily since then. Some cell types are more amenable to expansion than others, reflecting in part their native regenerative capacity but also varying requirements for nutrients, growth factors, and cell–cell contacts. As an example of progress, after years of effort, protocols for the growth and expansion of human cardiomyocytes are now available. However, there are still many tissue-specific cell types that cannot be expanded from tissue sources, including the pancreas, liver, and nerves. The discovery of pluripotent or highly multipotent stem cells (Chap. 88) may ultimately allow most human cell types to be used for tissue engineering. The stem cell characteristics depend on their origin and their degree of plasticity, with cells from the earliest developmental stages, such as embryonic stem cells, having the greatest plasticity. Induced pluripotent stem cells have the advantage that they can be derived from individual patients, allowing autologous transplants. They can also be differentiated, in vitro, along cell-specific lineages, although these protocols are still at an early stage of development. Human embryonic and induced pluripotent stem cells have a very high replicative potential, but they also have the potential for rejection and tumor formation (e.g., teratomas). The more recently described amniotic fluid and placental stem cells have a high replicative potential but without an apparent propensity for tumor formation. Moreover, they have the potential to be used in an autologous manner without rejection. Adult stem cells, such as those derived from bone marrow, also have less propensity for tumor formation and, if used in an autologous manner, will not be rejected, but their replicative potential is limited, especially for endoderm and ectoderm cells.

Stem cells can be derived from autologous or heterologous sources. Heterologous cells can be used when only temporary coverage is needed, such as replacing skin after a burn or wound. However, if a more permanent construct is required, autologous cells are preferred to avoid rejection. There are also practical issues related to tissue sources. For example, if a patient presents with end-stage heart disease, obtaining a cardiac tissue biopsy for cell expansion is unlikely to be feasible, and bone marrow–derived mesenchymal cells may provide an alternative.

BIOMATERIALS AS SCAFFOLDS FOR TISSUE ENGINEERING

The biomaterials used to create the scaffolds for tissue engineering require specific properties to enhance the long-term success of the implanted constructs. Ideally, the biomaterials should be biocompatible; elicit minimal inflammatory responses; have appropriate biomechanical properties; and promote cell attachment, viability, proliferation, and differentiated function. Ideally, the scaffolds should replicate the biomechanical and structural properties of the tissue being replaced. In addition, biodegradation should be controlled such that the scaffold retains its structural integrity until the cells deposit their own matrix. If the scaffolds degrade too quickly, the constructs may collapse. If the scaffolds degrade too slowly, fibrotic tissue may form. Also, the degradation of the scaffolds should not alter the local environment unfavorably, because this can impair the function of cells or newly formed tissue.

The first scaffolds designed for tissue regeneration were naturally derived materials, such as collagen. The first artificially derived material for tissue engineering used a biodegradable scaffold made of polyglycolic acid. Naturally derived scaffolds have properties very similar to the native matrix, but there is an inherent batch-to-batch variability, whereas the production of artificially derived biomaterials can be better controlled, allowing for more uniform results. More recently, combination scaffolds, made of both naturally and artificially derived biomaterials, have been used for tissue engineering.

An emerging area is the use of peptide nanostructures to facilitate tissue engineering. Some of these are self-assembling peptide amphiphiles that allow scaffolds to form in vivo, for example at sites of spinal cord injury where they have been used experimentally to prevent scar formation and facilitate nerve and blood vessel regeneration. Peptide nanostructures can be combined with other biomaterials, and they can be linked to growth factors, antibodies, and various signaling molecules that can modulate cell behavior during organ regeneration.

VASCULARIZATION AND INNERVATION

Implanted tissue-engineered constructs require adequate vascularity and innervation. Judah Folkman, a pioneer in the field of angiogenesis, made the observation that cells could survive in volumes up to 3 mm^3 via nutrient diffusion alone, but larger cell volumes required vascularization for survival. Adequate vascularity was also essential for normal innervation to occur. This was a major challenge in the field of tissue engineering, which largely depended on the patient's native angiogenesis and innervation. Even if sufficient cell quantities are available, there is a theoretical limit on the types of tissue constructs that could be created. In response to this challenge, material scientists designed scaffolds with much greater porosity and architecture. Scaffold designs included the creation of thin, porous sponges comprised of 95% air, markedly increasing the surface area for the resident cells. These properties promoted increased vascularity and innervation. The addition of growth factors, such as vascular endothelial growth factor and nerve growth factor, has been used to enhance angiogenesis and innervation.

LEVEL OF COMPLEXITY FOR THE ENGINEERING OF TISSUES AND ORGANS

All human tissues are complex. However, from an architectural aspect, tissues can be categorized under four levels. Flat tissue structures, such as skin, are the least complex (level 1), comprised predominantly of a single epithelial cell type. Tubular structures, such as blood vessels and the trachea, are more complex architecturally (level 2) and must be constructed to ensure that the structure does not collapse over time. These tissues typically have two major cell types. They are designed to act as a conduit for air or fluid at a steady state within a defined physiologic range. Hollow nontubular organs, such as the stomach, bladder, or uterus, are more complex architecturally (level 3). The cells are functionally more complex, and these cell types often have a functional interdependence. By far, the most complex are the solid organs (level 4), because the amount of cells per cm^2 are exponentially greater than any of the other tissue types.

For the first three tissue levels (1–3), when the constructs are initially implanted, the cell layering on the scaffolds is thin, not unlike that seen in tissue culture matrices. The cell layering continues to