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Engineered Cells and Tissue

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Introduction

Tissue engineering applies biological and engineering principles to the study of cells and tissues to develop functional cell and tissue substitutes. It has recently emerged as a new interdisciplinary science that can be used to repair injured body parts. Skin, bone, and articular cartilage are the first success stories: the FDA has already approved a living skin product, autologous chondrocyte implantation for cartilage repair, and others are flowing through the regulatory pipeline. Techniques that use scaffolds, matrices, transforming growth factors, and pluripotent mesenchymal cells have substantial treatment potential.

A symposium presented on Day 1 summarizes current developments and research directions in this field. The speakers presented the fundamental principles of tissue engineering, including the biology of mesenchymal stem cells, mechanisms of cell differentiation, preservation and maintenance of cell/tissue function, the chemistry of biocompatible and bioactive scaffolds, and the potential of regenerative medicine.

An Overview

First author and moderator Rocky S. Tuan, MD,^[1] from Thomas Jefferson University in Philadelphia, PA, presented an overview of cell and tissue engineering. Stem cell technology utilizes cells that have multiple differentiation potential and may be converted (*in vitro* or *in vivo*) to the tissue types needed for functional tissue substitution. Embryonic stem cells are derived from the blastocyst. They may be maintained as undifferentiated cells. They are responsive to differentiation signals *in vitro* and *in vivo* and can be used for generating transgenic animals and modeling gene mutations.

Adult stem cells are derived from marrow stroma and connective tissues. They may be culture expanded as undifferentiated cells. Stem cells can be used for the following applications: stably transfecting selected cell lines, introduction into blastocoel cavity, production of chimeric and transgenic animals, and potential uses in tissue engineering. Multipotential stromal mesenchymal stem cells can be used to develop articular cartilage, bone, and so forth.

Important issues with mesenchymal or differentiated cells used as tissue or organ substitutes include availability, expandability, inducibility, proliferation, programmed

maturation, response to environment, immunocompatibility, and their ability for recombinant manipulation.

Resorbable or nonresorbable matrices or scaffolds, used for tissue repair or replacement can be natural or synthetic (ie, ceramic or metallic). The important features of scaffolds include mechanical stability, cell retention, endo- and exo-biocompatibility, bioactivity, and degradability.

The future of tissue engineering will explore the application of paradigms of developmental biology to cell and tissue engineering, cells sources, their isolation, maintenance, and expansion, the development of scaffolding biomaterials, growth factors, and differentiation genes.

Biodegradable Polymer Scaffolds

Antonios Mikos, MD,^[2] from Houston, TX, focused on the use of biodegradable polymer scaffolds. One of the goals of tissue engineering technology is bone replacement. However, the complexity of bone makes it difficult to replace it with traditional biomaterials. Allogeneic or autogeneic tissue can be used, but serious concerns about safety and donor morbidity remain.

The functions of scaffolds include substrate for anchorage-dependent cells, stimulant for specific cellular response, carrier for growth factors, and retention of cells in defect. Synthetic biodegradable scaffold materials are optimal. The important properties of scaffolds are its porosity, pore size, and pore structure. Highly porous scaffolds with interconnected pores of diameter 200 to 400 μ m are ideal. Mechanical properties are also important as the scaffold must minimize stress shielding while providing support. Degradative properties, such as degradation time coupled to rate of tissue regeneration, and degradation mechanism (bulk vs surface erosion) are important. Degradation products should be noncytotoxic. And the scaffold should be sterilizable. The final product should be reproducibly manufactured, have a long shelf life, be easily handled in surgery, and should fit irregularly shaped defects.

Applications for scaffolds include tissue induction, migration and proliferation of host bone cells, cell transplantation, and delivery of bioactive molecules. Bone morphogenic proteins, angiogenic factors, and other bioactive molecules are important for the activation of scaffolds. Polyglycolic acid (PGA) and poly L-lactic acid (PLLA) are the most common (FDA-approved), most widely investigated preformed scaffolds. Scaffolds made of these materials can be used with *in vitro* culture systems to produce large pieces of tissue before implantation.

Another exciting option are injectable scaffolds. They can fill defects of all sizes and shapes, can be used in minimally invasive procedures and cross-link *in situ* to create highly porous scaffolds. Injectable composite materials are particularly suitable for cavities as they are highly unsaturated materials that can be polymerized *in vivo*.

Regeneration of Adult Stem Cells

Daniel R. Marshak, MD,^[3] from Osiris Therapeutics and Johns Hopkins University School of Medicine, Baltimore, Maryland, discussed the use of adult stem cells in regenerative tissue technology.

Stem cells can be isolated from adult bone marrow, but they are rare cells, capable of extensive expansion and multilineage differentiation. The number of mesenchymal stem cells (MSCs) found in bone marrow appears to decline with age, as they are normal diploid human cells that are not immortal. Mesenchymal stem cells are progenitors of structural and connective tissues, such as bone, cartilage, tendon, ligament, muscle, marrow stroma, and fat.

MSCs uniformly differentiate *in vitro* to multiple lineages, including bone, cartilage and fat. Colonies of MSCs derived from single cells display multilineage differentiation comparable to the parental cells. Factors that influence MSC differentiation are polypeptide growth factors and cytokines, basal nutrients, cell density, spatial organization, mechanical forces. MSCs can differentiate to osteoblastic cells *in vitro* and *in vivo* and show the molecular markers of osteogenic cells. In studies of segmental gaps in the rat femur, ceramic implants containing syngeneic MSCs form substantial bone in 8 weeks. In studies of segmental gaps in the canine femur, implantation of autologous MSCs results in callus formation at 8 weeks and a complete bridge at 16 weeks.

Allogeneic MSCs used for bone regeneration do not express large amounts of major histocompatibility complex (MHC) II or co-activators. In a mixed lymphocyte reaction, MSCs do not stimulate T-cell activation or proliferation. Ectopic implants of allogeneic MSCs do not show lymphocytic infiltration and are not rejected. In ceramic cubes, ectopic implants of allogeneic MSCs form bone, and therefore, the goal is a universal MSC product for bone repair. Also, tendon differentiation from allogeneic MSCs appears feasible. Cartilage implants with allogeneic MSCs show no rejection.

Tissue engineering is likely to revolutionize orthopaedic surgery. Engineered tissue substitutes, like bone and articular cartilage, are already available. This technology will take the field of orthopaedics beyond the reactive treatment to the replacement and regeneration of bone, osteochondral defects and ligaments.

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