IMAGING AND ADVANCED TECHNOLOGY

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Regenerative Medicine and the Gut

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Regenerative medicine refers to the process of creating functional tissues to augment or replace organs lost to age, disease, damage, or congenital defects.[1] Several technologies brought to bear on this challenge have had notable results. In gastroenterology and hepatology in particular, recent publications have demonstrated preclinical success in the transplantation of engineered liver tissue, augmentation of enteric sphincter function by transplantation of smooth muscle, and transplantation of enteric neurons. We begin by reviewing the basic techniques that underlie these advances, specifically stem cell biology, gene therapy, and engineered biomaterials. We describe some promising applications of regenerative medicine in dermatology, pulmonology, cardiology, neurology, and urology. Finally, we describe the state of basic scientific and preclinical research in regenerative gastroenterology.

The Tools

Stem Cells

Although the concept of regenerative medicine is as old as the myth of Prometheus, its modern era began with seminal discoveries in stem cell biology over the last couple of decades. Stem cells are defined by the capacity for unlimited self-renewal and the ability to differentiate into mature end-organ cells. They are conveniently categorized by provenance (adult, embryonic, fetal, or induced) and according to their developmental potential (totipotent, pluripotent, multipotent). Unipotent cells, or adult progenitor cells, retain the capacity for self-renewal or differentiation into a single cell type (eg, hepatocytes, skeletal myocytes). In general, in vitro propagation, expansion, and differentiation of these cells remain difficult.

Initial attempts at tissue regeneration focused on naturally occurring stem cells. Although embryonic stem cells (ESC) received great popular attention and are technically attractive owing to their pluripotency, legal and ethical objections have diminished the enthusiasm for their use. Other difficulties include the immunogenicity of transplanted ESC or ESC-derived tissues[2–4] and the potential for teratoma formation in vivo.[5] Although there has been recent progress in defining the molecular basis for this tumor risk,[6] it is clear that there are significant technical hurdles before human ESC will be ready for clinical use.

Among the promising candidates for regenerative therapy are mesenchymal stem cells, a class of multipotent cell found in several mature and immature organs, including adult adipose tissue and bone marrow, Wharton's jelly, and tooth bud. Their relative developmental flexibility, together with their availability in postnatal (even adult) mammals, lends them unique clinical promise. Yet at present we lack standardized protocols for differentiating these cells into target tissues.[7,8]

Safety and technical issues with naturally occurring stem cells have prompted the examination of other approaches to regeneration. Somatic cell nuclear transfer (SCNT) and induced pluripotent stem cells are two of the most exciting and revolutionary developments in this field. SCNT entails removing the nucleus from a recipient oocyte and fusing the enucleated oocyte with a mature donor cell, typically a fibroblast. The product is a pluripotent cell containing cytoplasm and mitochondrial DNA from the recipient oocyte and nuclear DNA from the mature donor cell.[9] SCNT has been used in reproductive cloning of several species (eg, Dolly the sheep), and as such has been subject to significant controversy and legal debate, particularly with respect to humans. Therapeutic, or "research," cloning, intended to yield cells or tissues but not whole organisms, has not been free of controversy, but research continues. Nuclear transfer remains a technically challenging procedure with a very low yield (<1%).[9] Some have also raised concerns about the possibility of exploitative sourcing of oocytes should therapeutic cloning find clinical applications.[10]

The reprogramming of mature somatic cells to assume the behavior of ESC is a major scientific breakthrough, suggesting that pluripotent cells might be derived from a patient's own mature tissue, even an easily accessible biopsy site such as the skin. With such an origin, these cells, termed induced-pluripotent stem cells (iPSC), offer a way to bypass most ethical objections to regular ESC. The

Abbreviations used in this paper: CNS, central nervous system; ECM, extracellular matrix; ESC, embryonic stem cells; GI, gastrointestinal; IAC, internal anal sphincter; ICC, interstitial cells of Cajal; iPSC, induced-pluripotent stem cells; MDCs, muscle-derived cells; nNOS, neuronal isoform of nitric oxide synthase; NSCs, neuronal stem cells; SCNT, somatic cell nuclear transfer.

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original reprogramming approach by Takahashi and Yamanaka in 2006 used retrovirus-induced expression of transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) first in mouse,[11] and later in human fibroblasts.[12] Similarly, Yu et al[13] showed that retroviral expression of OCT4, SOX2, NANOG, and LIN28 induces pluripotency in human fibroblasts. Concern over the oncogenic potential of retroviruses[14–16] has led to a refinement in techniques. It is now possible to produce iPSC without any stable genomic modification to the target cells in both mouse[17,18] and human[19,20] models. Further exciting developments in the last year include the discovery of induced pluripotency. The original approach to producing iPSC attempted to return a differentiated cell, such as a fibroblast, to an undifferentiated, pluripotent state ("de-differentiation") and then "re-differentiate" it into the desired phenotype. However, in 2010 Vierbuchen et al[21] succeeded in bypassing the de-differentiation step and converting mouse fibroblasts directly into neurons having excitable membranes and functional synapses.

Although iPSC developmental reprogramming undoubtedly has immense scientific and clinical potential, there are major challenges to translating current research into therapies. It has become clear that there are differences between the iPSCs produced by retroviral induction and ESCs. Further, the nature of the reprogramming process remains obscure, and the developmental potential of iPSCs derived by different methods, from different tissues, is unknown.[22]

Materials Engineering

Although stem cells can assume a desired cellular phenotype under the appropriate conditions, their organization into functional tissues also requires the proper spatial architecture and integration with their environment. Mammalian cells depend on biological and mechanical interaction with the extracellular matrix (ECM). For tissue regeneration, various biomaterials can replicate the effects of native ECM and form a 3-dimensional scaffold to maintain proper functional shape and cell–cell orientation, and can be loaded with bioactive factors, for example, adhesion peptides and growth factors. To be suitable for tissue engineering applications a scaffolding material should provide an appropriate 3-dimensional structure for the deposition and growth of cells, mimic normal cell–cell interactions, have limited immunogenicity, and allow for diffusion of oxygen and other nutrients. Collagen[23] and alginate[24–26] are commonly used, although, as with other materials derived from biologic sources, there have been concerns about infectious risk and immunogenicity of these products.[27] Synthetic materials such as poly(ethylene glycol), poly(lactic acid), poly (glycolic acid), and poly(lactic-*co*-glycolic acid) have the advantages of industrial production, lack of infectious risk, and decreased immunogenicity. They can also be molded or shaped by advanced fabrication techniques such as electrospinning to allow greater control over the small-scale structure of biomaterials,[28] which may enhance mechanical properties and mimicry of normal ECM.[29] Synthetic modifications to the basic polymer structure can include cross-linking peptides degradable by proteases from migrating cells and protein ligands for cell-surface receptors.[30,31]

Another source of scaffolding for engineered or regenerative tissue is decellularized natural tissue. A natural animal or cadaveric explant is washed with detergent or otherwise treated to remove cells, DNA, and other antigenic material,[32,33] and then seeded with cells capable of migrating into the residual matrix. In animal models the technique has been applied to liver,[34] lung,[35,36] heart,[37] and intestinal submucosa.[38] Perhaps the most mature clinical area of tissue engineering is skin grafting, with decellularized skin commercially available (eg, AlloDerm, LifeCell Corporation, Branchburg, NJ).

An exciting alternative to scaffold-based tissue engineering has emerged in "bioprinting," a collection of processes for depositing living cells into a defined pattern using computer-controlled machines analogous to 3-dimensional rapid-prototyping technology. Boland et al[39] describe using thermal ink jet printing to deposit neurons in a 2-dimensional pattern. The Forgacs group has demonstrated bioprinted structures composed of human endothelial cells with chicken cardiomyocytes,[40] as well as complex, branched tubular structures having concentric layers of fibroblasts and smooth muscle cells.[41]

Progress in Other Specialties

Cellular transplantation is becoming a clinically important technology. For example, autologous cultures of keratinocyte stem cells (holoclones) have now been used for >2 decades to restore defects in the skin, mucosa, and cornea,[42–44] Most recently, epidermal stem cells from an adult patient with junctional epidermolysis bullosa were transduced with a functional copy of the laminin 5-b3 gene, mutation of which is responsible for the disease phenotype. Epidermal grafts prepared from these cells were then transplanted onto the patient's legs and resulted in a local cure.[45] South Korean regulators have recently approved the first stem cell–based therapy for clinical use, injecting bone marrow–derived mesenchymal stem cells into the coronary arteries of patients with coronary ischemia.[46] A trial of a similar treatment has been reported in the United States.[46] And there seems to be some promise regarding transplantation of neurons into the central nervous system (CNS). In 1995, Kordower et al[47] reported on a 59-year-old patient with Parkinson's disease in whose brain they implanted fetal brain tissue from several donors. After 18 months they showed significant survival of neurons expressing tyrosine hydroxylase within the engrafted areas. Wernig et al[48] induced iPSC to differentiate into neurons in vitro, and implanted them into the cerebral ventricles of fetal mice, where they were found to have migrated into widespread areas of the developing brain. Subsequently, the same group injected dopaminergic neurons derived from iPSC into the brains of Parkinsonian mice and showed survival of the en-