e-biomed
Volume 1, 2000
March 7, 2000

Review

Regenerative Biology and Medicine in the 21st Century

DAVID L. STOCUM

INTRODUCTION

The human body has considerable capacity for regeneration. Tissues with high rates of cell turnover, such as blood and epithelia, are regenerated continually throughout life. Other tissues, such as liver, bone, muscle, blood vessels, and adrenal cortex regenerate in response to injury. Fingertips will regenerate if amputated distal to the terminal phalangeal joint. The liver regenerates by compensatory hyperplasia, whereas other tissues regenerate by the activation of reserve stem or progenitor cells residing in the bone marrow (in the case of blood and perhaps augmenting the regeneration of mesenchymally-derived tissues) or within the regenerating tissue. However, neither bone nor muscle will regenerate across a gap, and other vital tissues such as pancreas, heart, and spinal cord respond to injury by the formation of scar tissue.

Two clinical approaches currently available to replace failing organs and tissues are organ transplantation and implantation of bionic devices. Donor shortages and immunosuppressive side effects limit use of the former, while drawbacks to the latter involve our inability to manufacture artificial materials that duplicate the durability, strength, form, function, and biocompatibility of natural tissues. In the last two decades of the 20th century, a new approach to tissue restoration, regenerative biology, has been developed, which in the 21st century will be developed clinically into regenerative medicine. Research in regenerative biology involves cell and molecular biology, developmental biology, immunology, and polymer chemistry. Regenerative medicine will use three strategies: transplantation of cells to form new tissue in the transplant site, implantation of bioartificial tissues constructed *in vitro*, and induction of regeneration *in vivo* from healthy tissues adjacent to an injury.

CELL TRANSPLANTATION AND BIOARTIFICIAL TISSUES

Reserve cell populations are proving to be much more widespread than previously thought. Multipotent stem cells have been discovered recently in the liver, pancreas, and central nervous system. In addition, mesenchymal stem cells have been isolated from the bone marrow, and there is some evidence that similar cells may even reside in the connective tissue compartments of tissues throughout the body. Pluripotent human embryonic stem cell lines have been cultured recently. The idea is to transplant stem/progenitor cells, or their differentiated products, into a lesion site where they will form new tissue, or use them to construct a bioartificial tissue *in vitro* to replace the original tissue or organ. Bioartificial tissues are made by seeding stem or differentiated cells into a natural or artificial biomaterial scaffold shaped in the appropriate form, then implanting the construct in place of the damaged tissue or organ. The use of stem cells is preferable to the use of differentiated cells harvested directly from a donor because stem cells have the potential for unlimited growth and thus supply.

Research conducted so far indicates that the use of cell transplants and bioartificial tissues to correct tissue damage is feasible. Mouse neuronal and glial cells derived from neural stem cells *in vitro*, and cardiomyocytes derived from embryonic stem cells *in vitro*, integrate into the surrounding tissue when injected

into an adult brain and heart, respectively. Multipotent human neural stem cells injected into the developing brain of mouse embryos migrate throughout the brain and differentiate site-specifically. Many bioartificial tissues are currently under development, and bioartificial skin is already being manufactured for clinical use on chronic wounds and burns. Autogeneic or allogeneic dermal fibroblasts are seeded into biodegradable scaffolds of collagen, gelatin or polyester mesh to which various other extracellular matrix components have been added. A split-thickness skin graft or a layer of cultured keratinocytes is added on top of the artificial dermis and the construct applied to the wound. The scaffold degrades and the fibroblasts synthesize a matrix similar to the matrix of normal skin. The cosmetic appearance of the skin, however, is not normal, and hair follicles, sebaceous glands, and sweat glands are not reconstituted.

Four major obstacles lie in the way of the routine use of stem cells for regeneration. The first is working out the cell signal/receptor biology required to grow the cells *in vitro* and direct their differentiation into site-specific phenotypes, as well as the physical properties and architecture of biodegradable scaffolds to support bioartificial tissue construction. Multipotent human mesenchymal and mouse neural stem cells, and mouse embryonic stem cells can be easily grown *in vitro* through the addition of leukemia inhibitory factor (LIF) to the culture medium, but mouse embryonic stem cells differentiate randomly *in vitro* and *in vivo*. However, advances in controlling stem cell differentiation *in vitro* are being made. For example, the molecular signals that regulate the differentiation of human mesenchymal and mouse neural stem cells to terminal phenotypes, as well as mouse embryonic stem cells to multipotent glial cells, have been successfully identified. Mouse embryonic stem cells differentiated to multipotent glial cell precursors *in vitro* and transplanted to the brain of myelin-deficient fetal rats, differentiate into astrocytes and oligodendrocytes which myelinate axons. We do not know, however, whether multipotential stem cells harvested from specific tissues or differentiated from ESCs *in vitro*, will make site-specific tissue when transplanted to injured adult tissues. Furthermore, the growth of human ESCs requires the labor-intensive use of irradiated feeder cells, which makes it difficult to scale-up cell numbers to quantities sufficient for clinical use.

The second obstacle is synthesizing scaffolding materials for bioartificial tissues that have the requisite topography, surface properties, and growth and differentiative signals to facilitate cell migration, adhesion, proliferation, and differentiation, as well as being moldable into the shapes of various tissues and organs. Artificial biomaterials currently in use or being tested include various ceramics, polyurethane elastomers, polyesters, polyanhydrides, and polyphosphazenes. These materials provide mainly mechanical support, migration channels, and adhesive surfaces for cells, and rely on the cells themselves for molecular signals and cues that regulate growth and differentiation. The future will reside at the interface between biology and materials science, where scaffold materials incorporating biological molecules that regulate cell adhesion, proliferation, and differentiation will be designed.

The third obstacle is immunorejection. While autogeneic cells can be used in some instances (for example, mesenchymal stem cells from bone marrow), most transplanted cells will be allogeneic. A number of genetic modification and cell biological strategies to promote host tolerance of allogeneic or xenogeneic transplants are under investigation. The simplest approach is to clone personal human embryonic stem cell lines by fusing a diploid somatic cell to an enucleated human or other mammalian egg and using the resultant blastocyst to make the stem cells. This strategy, however, generates the fourth obstacle, which is the bioethical concerns associated with the use of human embryos and human-animal hybrid "monster" experiments. These issues may be more difficult to resolve than the problem of cell growth and directed differentiation or biomaterial design.

Regeneration in vivo

Regeneration *in vivo* from remaining healthy tissues is clearly the most desirable way to restore tissue structure and function, because it bypasses the immunorejection problem and the bioethical questions surrounding human embryonic cell transplants. Stimulating regeneration from residual tissues *in vivo* is the wave of the future, but is currently the least developed approach to tissue restoration. One strategy to do this is to bridge lesions with biomaterial scaffolds that encourage tissue ingrowth from both sides of the lesion. The scaffolds ideally would contain molecular agents known to stimulate regeneration or neutralize regeneration-inhibiting factors. Skin, bone, peripheral nerve, spinal cord, tendon, and blood vessels have all

been stimulated to regenerate by various biomaterials, but regeneration is neither perfect nor complete in any case. For example, spinal cord regeneration in adult rats has been induced by bridging gaps in the cord with intercostal nerve sheaths embedded in a fibrin matrix impregnated with FGF-1. Although axons regrew across the lesion and the rats recovered their ability to support weight on their hind legs, they did not recover coordinated locomotor function.

Several major research issues must be resolved before clinical stimulation of regeneration *in vivo* becomes a reality. First, we do not know how many tissues of the body harbor stem or progenitor (more broadly, regeneration-competent) cells that can engage in regeneration. For example, there is some evidence that, in addition to the mesenchymal stem cells of the bone marrow, the connective tissue compartments of virtually every organ system in the body contain populations of mesechymal stem and progenitor cells. If so, it might be possible to induce them to regenerate the same cell types that can be derived from bone marrow mesenchymal stem cells, instead of participating in scar tissue formation. Second, if stem and/or progenitor cells do not exist in every tissue, might we be able to induce differentiated cells to become regeneration-competent by dedifferentiation? Urodele amphibians use this mechanism to regenerate a wide variety of tissues and complex structures, including limbs, tails, upper and lower jaws, intestine, lens, and neural retina. They are also able to regenerate cardiac muscle through a process that resembles the compensatory hyperplasia of liver regeneration and to regenerate spinal cord by the epithelial-mesenchymal transformation of ependymal cells.

Third, just as with cell transplantation approaches, we do not yet know enough about the biological signals and cues required to stimulate the adhesion, migration, proliferation, and differentiation of regeneration-competent cells or about the factors that inhibit regeneration in tissues that scar. Nor do we know much about how amphibian cells are able to dedifferentiate in response to injury, although it has been shown recently that myotubes derived from dedifferentiated newt limb cells are intrinsically different from mammalian myotubes in their ability to enter the S phase of the cell cycle in response to a serum factor activated by thrombin. We need to know the specific combinations of molecular signals and receptors that distinguish regeneration from scar tissue formation, including those which lead to the formation of progenitor cells by dedifferentiation. One strategy to identify these molecules is to focus on animals that regenerate well, such as amphibians. Frogs represent such a model system, being able to regenerate well at pre-metamorphic stages, but poorly at post-metamorphic stages. Our research group has opted for this approach using the frog *Xenopus laevis*. We are comparing and contrasting the temporal and spatial patterns of gene activity in a variety of tissues injured at pre-metamorphic and post-metamorphioc stages through the use of probes for genes currently known to be active in limb regeneration and through comprehensive subtractive screens to identify the whole range of molecules that stimulate and inhibit regeneration. Our goal is to then perform gain of function and inhibition of function assays using transgenic *Xenopus*, and to clone and express human homologues of the frog genes. The proteins encoded by these genes would then be used to induce regeneration-competent cells that might otherwise participate in scar tissue formation to engage in regeneration, or to induce differentiated cells to dedifferentiate and become regeneration-competent.

We have entered into an extraordinary revolution in biomedical science which has its origins in the remarkable unification of biology at the molecular level nearly fifty years ago, beginning with the discovery of the double helical structure of DNA. Regenerative biology is one of the latest, most multidisciplinary, and most exciting fields to emerge from discoveries in developmental biology over the past four decades. The basic research we are doing now will evolve into the regenerative medicine of the future and eventually into the prevention of the diseases (but not the injuries!) that regenerative medicine is designed to treat. Much remains to be done, but regenerative biology and medicine is a science whose time has come.

REFERENCES

Brockes, Jeremy P. (1997) Amphibian Limb Regeneration—Rebuilding a Complex Structure. *Science* 276:81–87.

Carlson, Bruce M. (1998) Stimulation of Regeneration in Mammals: Pipe Dream or Realistic Goal? *Wound Rep Reg* 6:425–433.

Chernoff, Ellen A.G. (1996) Spinal Cord Regeneration: A Phenomenon Unique to Urodeles? *Int J Dev Biol* 40:823–831.

Hubbell, Jeffrey A. (1995) Biomaterials in Tissue Engineering. *Biotechnology* 13:565–576.

Langer, Robert and Vacanti, Joseph (1993) Tissue Engineering. *Science* 260:920–926.

McKay, Ronald (1997) Natural and Engineered Stem Cells in the Central Nervous System. *Science* 276:66–71.

Michalopoulos, George K. and DeFrances, Marie C. (1997) Liver Regeneration. *Science* 276:60–65.

Prockop, Darwin J. (1997) Marrow Stromal Cells as Stem Cells for Non-Hematopoetic Tissues. *Science* 276:71–74.

Rao, Mahendra S. (1999) Multipotent and Restricted Precursors in the Central Nervous System. *Anat Rec* (New Anat) 257:137–148.

Stocum, David L. (1998) Bridging the Gap: Restoration of Structure and Function in Humans. In: *Cellular and Molecular Basis of Regeneration: From Invertebrates to Humans*. Patricia Ferretti and Jacqueline Geraudie, eds. John Wiley, Cambridge, pp. 411–450.

Stocum, David L. (1998) Regenerative Biology and Engineering: Strategies for Tissue Restoration. *Wound Rep Reg* 6:276–290.

Stocum, David L. (1999) Regenerative Biology: A Millenial Revolution. *Sem in Cell and Dev Biol* 10:433–440.

Address reprint requests to:
David L. Stocum
Department of Biology
Indiana University-Purdue University Indianapolis
402 N. Blackford St.
Indianapolis, IN 46202