Chapter 3
Tissue homeostasis

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Chapter contents

Chapter objectives:

- To know the definition of the terms regeneration and homeostasis
- To recognize that different tissues have different regeneration capacity
- To acknowledge that the brain and heart are regenerating organs
- To understand how tissue regeneration can be analyzed by labeling experiments
- To recognize a stem cell niche
- To understand that stem cell niches share common regulatory systems
- To understand that tissue regeneration has consequences and offer opportunities in the development of tissue engineering products

"I'd give my right arm to know the secret of regeneration"

Oscar E Schotte, quoted in Goss (1991).

3.1 Introduction

The ability to regenerate larger parts of an organism is connected to the complexity of that organism. The lower developed the animal, the better the regeneration ability. In most vertebrates, the regeneration potential is limited to the musculoskeletal system and liver. In the hydras (a 0.5 cm long fresh-water cnidarian) the regeneration is made through morphollaxis, a process that does not require any cell division. If a part of the organism is lost through traumatic injury, other cells adapt to the new situation and the result is a smaller but fully functional hydra.

In higher organisms like Salamanders, belonging to the urodele amphibians (a family of adult vertebrates that can regenerate their limbs after amputation), the regeneration is initiated by epimorphosis, a process characterized by dedifferentiation and high proliferation of the local cells. The epimorphosis process starts immediately after the amputation of the limb when a wound epidermis is formed through the migration and proliferation of epithelial cells. In a zone underlying the epidermis, mesenchymal cells (muscle, cartilage and bone) lose their phenotype and start to dedifferentiate into blastemal cells which are the progenitor cells of the regenerating limb. Within the blastema, the cells undergo proliferation and redifferentiation into the various cell types needed to regenerate the limb. During this process the initial muscle cells show plasticity by being able to redifferentiate into muscle, cartilage and bone (Figure 3.1).

This ability of limb regeneration has obviously been lost in humans although we have the capacity for regeneration after injuries in a few tissues such as the liver, where we are able to regenerate a substantial part if it is resected surgically, bone and connective tissue. Furthermore, young children and

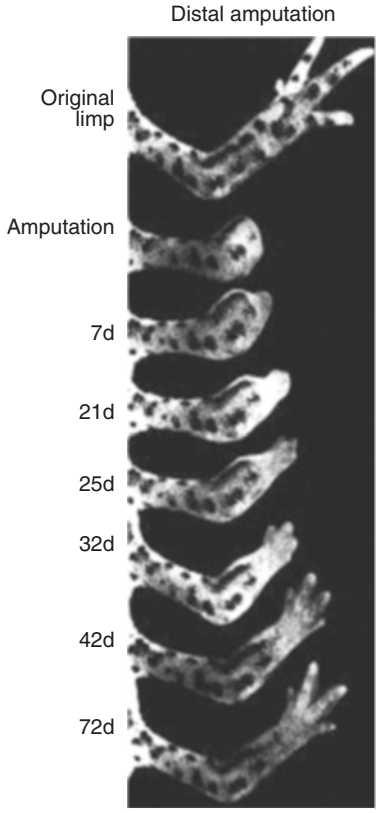


Figure 3.1 Tissue regeneration in salamander. (From Goss, R.J. *Principles of Regeneration*, NY: Academic Press, 1969.)

even adults have the ability to regenerate finger tips. Regeneration of these tissues mirrors embryonic control mechanisms of differentiation from stem cells. In the skin, brain, bone and muscle of mammalians, a subset of the cell population are selected during embryonic and fetal life to be used during childhood growth and for tissue regeneration throughout life.

Box 1 Regeneration potential of tissues during lifetime

- 3 tons of blood
- 500 kg intestinal epithelium corresponding to 40 km of intestine

This concept has also been demonstrated in the heart tissue (Laflamme *et al.*, 2002).

Embryonic development results in a determined cellular structure where each cell has its function in a specific place. During subsequent growth, where the organism becomes larger cells proliferate but within their determined fate. Some animals like the fish, continue to grow throughout life although mammals stop growing in size; although the cell proliferation continues resulting in a constant renewal of cells in the body.

All organs are developed by the end of the first trimester, and the process continues during the rest of the gestation of the fetus growth, and after birth until the growth spurt in adolescence is over. Most people consider this to be the end stage of human growth but nothing could be more wrong. We stop growing, and throughout life cells and tissues in the human body are exposed to internal and external stress-factors which lead to injury and loss by apoptosis or necrosis. However, to sustain the function of the tissues, the affected cells need to be replaced through a process called regeneration. During our lifetime, we are growing at a constant rate with a cell turnover of about 1% of the body weight each day (Box 1).

In some sense the organs of the body can be characterized as a cellular homeostasis where cells are constantly produced in order to balance the continuous cell death (Figure 3.2). The balance between cell production and cell death must be properly maintained since even small differences will give dramatic effects; if the liver cells produced exceeds the cell death by 1%, the liver weight would be equal to the initial body weight within a time period of 6–8 years.

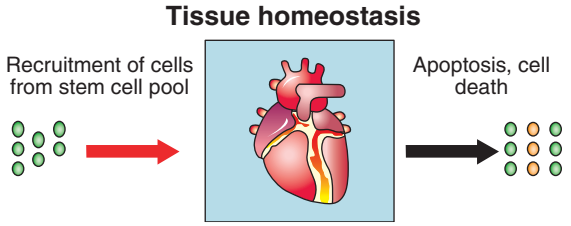


Figure 3.2 In the tissue regeneration process there is a balance between cell proliferation and cell death.

The tissue maintenance also has to control the supporting tissues for a specific organ: organs need mechanical strength which is provided by the extracellular matrix produced by the connective tissue cells (fibroblast, chondrocytes, bone cells). Most tissue also need a blood supply provided by the capillaries and lining endothelial cells as well as innervation provided by the nerve cells. Cellular debris cleaning and tissue defense against bacteria and viruses are provided by macrophages and lymphocytes. All of these cells are necessary to support the specialized cells of each organ; the cleaning process of the liver cells, the muscle cell contraction in the heart and the specialized endocrine cells.

The internal structure of each organ must maintain the intricate mixture of cells although the specialized cells are renewed. This is orchestrated by the internal determination memory of each specialized cell and its stem cells that pass the information on to its progeny. However, the specialized cell is highly adaptive to the surrounding environment and can change its phenotype. Articular cartilage chondrocytes can be isolated and propagated in monolayer cultures where the typical type II collagen production is changed

to a type I connective tissue type. However, when reimplanted into a cartilage environment, these cells turn on their type II collagen synthesis again. A second example of adaptation properties are the epidermal skin cells where mechanical load gives a different thickness of the skin. Just compare the back of your hand with the front of your palm or your foot soil. Bone tissue is also constantly adapting structurally to the changing load of the tissue. Not all organs regenerate in the same way – instead different organs have different rates of regeneration.

3.2 Tissues with no potential of regeneration

Although our body has the capacity to regenerate to a large extent, there are tissues with no or limited capacity of regeneration. For example, the central part of our lens consists of lens lamellas that are embryonic fossils that will not have changed since they were developed during embryonal life. The cell populations of the photo receptor of the retina and the auditory organ of Corti are not regenerated. In mammals, all cells in the auditory organ of Corti becomes terminally mitotic by embryonic day 14 and a similar phenomenon occurs in avian basal papilla by embryonic day 9 and thus one would expect that neither avian nor mammals would be able to regenerate auditory cells (Rubel *et al.*, 1995). However, in contrast to mammals, cells in the avian cochlear epithelium re-enter the cell cycle, and divide and differentiate into new hair cells after experimental loss of hair cells. A challenge for the future is to decipher the differences between the species and hopefully in the future auditory cells can be induced to regenerate also in humans.

3.3 Tissues with slow regeneration time

Our bones are constantly being renewed by an active process which involves the breakdown and rebuilding of new bone matrix by the osteoblasts. This constant renewal, governed by the continuous optimization of the load-bearing role of the bone by a functionally adaptive remodeling activity (which is more active in growing bone), is dominated by high-magnitude, high-rate strains presented in an unusual distribution. Adaptation occurs at an organ level, involving changes in the entire bone architecture and bone mass. This process is continuously ongoing and results in a total turnover time of 3 years for the whole bone structure.

Cartilage has a similar turnover rate over time, although the individual matrix components are renewed at various rates. The two major extracellular components in articular cartilage, collagen type II and aggrecan, are relatively longlived in the tissue and as a consequence undergoes non-enzymatic modifications by reducing sugars, thus ending in accumulation of advanced glycation endproducts. These accumulated endproducts reflect the half life of the components that for collagen is estimated to be 100 years and for aggrecan to be 3,5 years. The cellular turnover in cartilage is probably limited and the localization of stem cells in articular cartilage is so far undetermined. However, the common dogma of cartilage as homogenous tissue with only one cell type, the chondrocyte, producing the extracellular matrix consisting of mainly collagen type II fibres, and the high molecular weight aggregating proteoglycan aggrecan is changing. The tissue is instead heterogeneous with distinct cellular characteristics in different zones from the surface zone with flattened discoid cells secreting surface proteoglycans, through the middle zone. Rounded cells produce not only collagen type II and aggrecan but also cartilage intermediate layer protein and the deep and calcified zones with larger cells produce type X collagen and alkaline phosphatase. Furthermore, cells isolated from different compartments of the articular cartilage demonstrate a phenotypic difference between the cells located at the surface and cells in the deeper layer when subjected to agarose suspension cultures (Archer *et al.*, 1990; Aydelotte *et al.*, 1988). Within the mesenchymal tissue hyaline cartilage chondrocytes are usually considered tissue restricted and without the broader differentiation potential seen in bone marrow derived mesenchymal stem cells (Caplan, 1991).

This concept has been challenged by the demonstration that isolated articular chondrocytes are able to take on several phenotypic identities within the mesenchymal lineage; cartilage, adipose cells, osteoblast-like cells and muscle cells (Barbero *et al.*, 2003). In contrast to mesenchymal stem cells, chondrocytes form only cartilage, and not bone, in an *in vivo* osteochondrogenic ceramic implantation assay (Tallheden *et al.*, 2003) indicating a different *in vivo* default pathway.

Within articular cartilage there are subgroups of clonogenic cells that have heterogenous the capacity for hyaline cartilage formation (Barbero *et al.*, 2003) which could explain the plasticity seen in primary isolated articular chondrocytes. However, for articular chondrocytes, multipotency can be demonstrated even on the clonal level (Brittberg *et al.*, 2005).

Recent studies have demonstrated that the surface layer is involved in regulation of joint development and growth as well as responsible for the post-foetal and adult appositional growth of the joint (Archer *et al.*, 2003). The surface cells harbor a progenitor cell population with high growth potential that participate actively in the repair of cartilage injury by migration into the defect.

3.4 Tissues with a high capacity of regeneration

If the liver is resected, cells undergo limited dedifferentiation allowing them to reenter the cell cycle while maintaining all critical differentiated functions. Cells subsequently start to divide and the liver is regenerated in a short time – usually within weeks. This regeneration works through a homogenous process of simultaneous proliferation of the hepatocytes. The liver does not overgrow its original size; instead it adapts itself to the organism. This can be demonstrated by the fact that adult resected liver lobes implanted into children adjust to the smaller host. This process is potentially controlled by a circulating homeostatic factor – the hepatocyte growth factor.

Human regeneration in intestine and epidermis of the skin is characterized by a high turnover rate where cells constantly proliferate and differentiate; a process that is finalized by a discarding of the cells into the intestine, or off the body. The regeneration is maintained by a special resident cell type, the stem cell situated at the basal lamina. These cells persist throughout life their function is to maintain homeostasis, and effect tissue regeneration and repair (Figure 3.3).

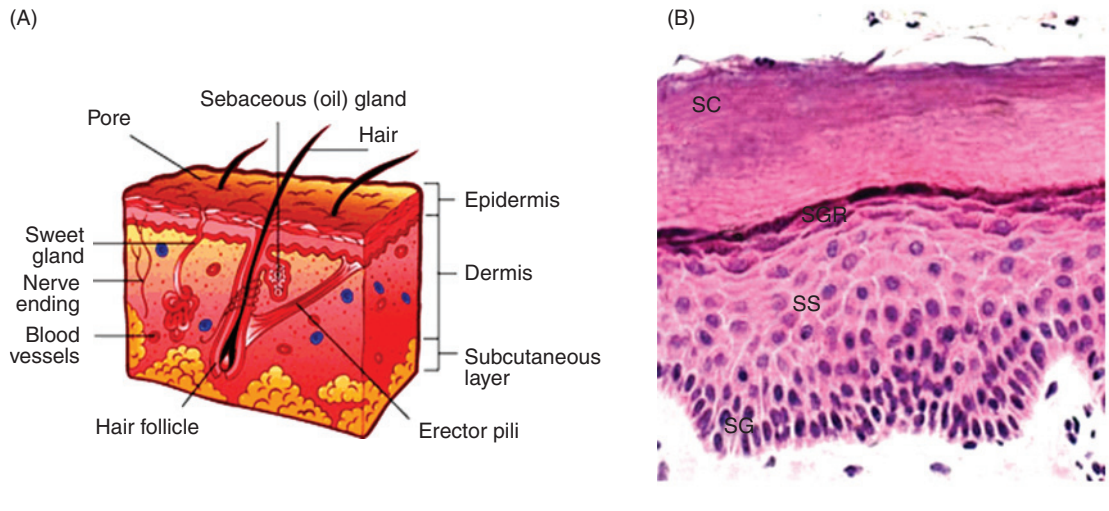
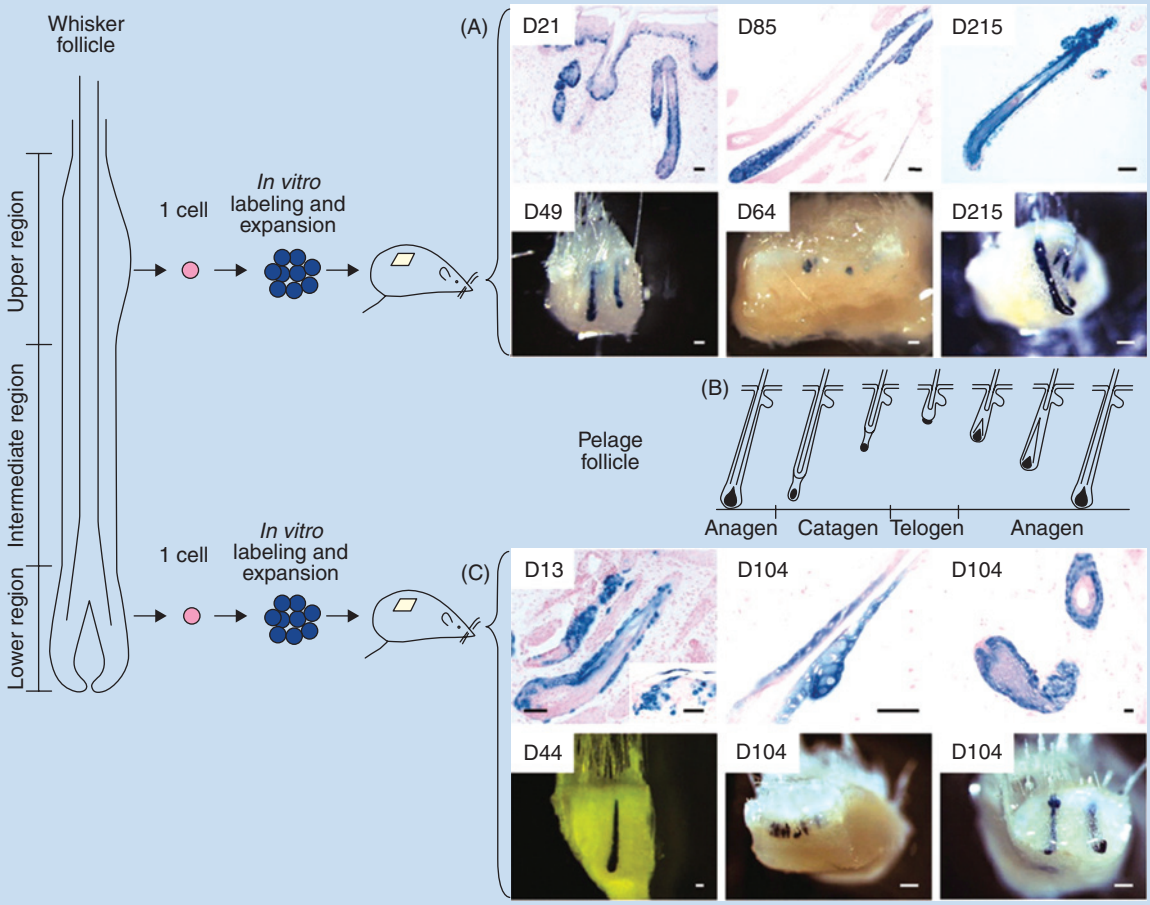


Figure 3.3 Schematic drawing of skin with hair follicle (left) and microscopic picture of epidermis (right) Abbreviations: SC = stratum corneum, SGR = stratum granulosum, SS = stratum spinosum, SG = stratum germinative.

Classical Experiment

Clonogenic keratinocytes are long-term multipotent stem cells (Claudinot *et al.*, 2005) In order to demonstrate that cells in the hair root sheet are multipotent stem cells, single keratinocytes were isolated from the upper or lower regions of whisker follicles of adult rats. Clones were labeled by using a defective retrovirus bearing a β-gal gene and subcloned, and labeled cells (passages 11–12) were injected into newborn mouse skin. Transplanted clonogenic keratinocytes, whether from the upper or lower regions, contributed to all epithelial lineages of hairy skin, including sebaceous glands, and participated in the hair cycle of pelage follicles for months. Note the variable degree of chimerism and that the epidermis is not labeled in long-term grafts (Lower in A and C). Insets show duration of transplantation in days in A and C; (B). Schematic representation of the hair cycle phases of a pelage follicle.



Multipotency of clonogenic keratinocyte. Scale bars: biopsies stained for β-gal in toto, 100 μm; microscopic sections, 50 μm. (From Claudinot, S., Nicolas, M., Oshima, H. et al. *(2005). Long-term renewal of hair follicles from clonogenic multipotent stem cells.* Proc Natl Acad Sci USA, *102(41): 14677–14682.)*

In the mid-1980s, researchers developed a method for growing a type of human skin cells called keratinocytes (which populates the skin's upper, or epidermal, layer) outside the body (Rheinwald and Green, 1975). The secret behind the culture method was that the keratinocyte stem cells could be propagated and that the contaminating fibroblasts of the dermis were growth inhibited. The technique is based on a culture system of mouse-derived fibroblast cells that inhibit contaminating fibroblast growth as well as the differentiation of the keratinocytes. The medium was further enriched with a growth factor Epidermal Growth Factor (EGF) that stimulates keratinocyte migration and proliferation. After several days in such an environment, few starting keratinocytes grew into a sheet of epidermal-like tissue.

Further investigation of the potential of the skin has revealed that the hair follicle contains stem cells with a pluripotency to produce skin, sebaceous glands and hair follicles (see Classical Experiment).

3.5 Tissues where regeneration was not considered – the paradigm shift in tissue regeneration

3.5.1 The brain as a regenerative organ

Until recently, the common understanding has been that nerve cells of the human brain no longer have the capacity for renewal. The only period where cells could be renewed was through the embryogenesis or during the prenatal period. In the adult brain plasticity existed, but only through increased complexity of new synapses, dendrites and neuritis. This belief could seemingly be unfounded with the knowledge of the existence of stem cells in most tissues of the body. However, the inability of the brain to self-repair is clinically obvious and the lack of appropriate techniques has hindered the discovery of adult brain regeneration. With the emerging radioactive techniques in the 1960s, researchers used ^{3}H-thymidine that could be incorporated into the DNA of dividing cells and visualized by autoradiography to demonstrate nerve cell renewal in rats, guinea pigs and cats. The scientific attitude towards these experiments was skeptic and little attention was attributed to the studies. The data was revisited in the mid 1970s, and neurogenesis was demonstrated in the dentate gyrus and olfactory bulbs of adult rats by alternative methods using electron microscopy. The work was followed by other researchers demonstrating regeneration in the adult brain of mammals and birds. In 1998 researchers were able to demonstrate cell division in human post mortem tissues using the thymidine marker 5-bromo2′deoxy-uridine (BrdU) (Box 2) (Eriksson *et al.*, 1998), discoveries that were later confirmed by others (see State of the Art Experiment).

3.5.2 The heart as a regenerative organ

Scientific data published in recent years have radically changed the view of the regenerative potential of the heart, and thus opened the possibility of cell therapy as well as new pharmacological concepts for treatment of cardiac insufficiency. According to the dominating dogma, the heart tissue is regarded as postmitotic without regenerative capacity and it has from birth a finite number of cells that is gradually

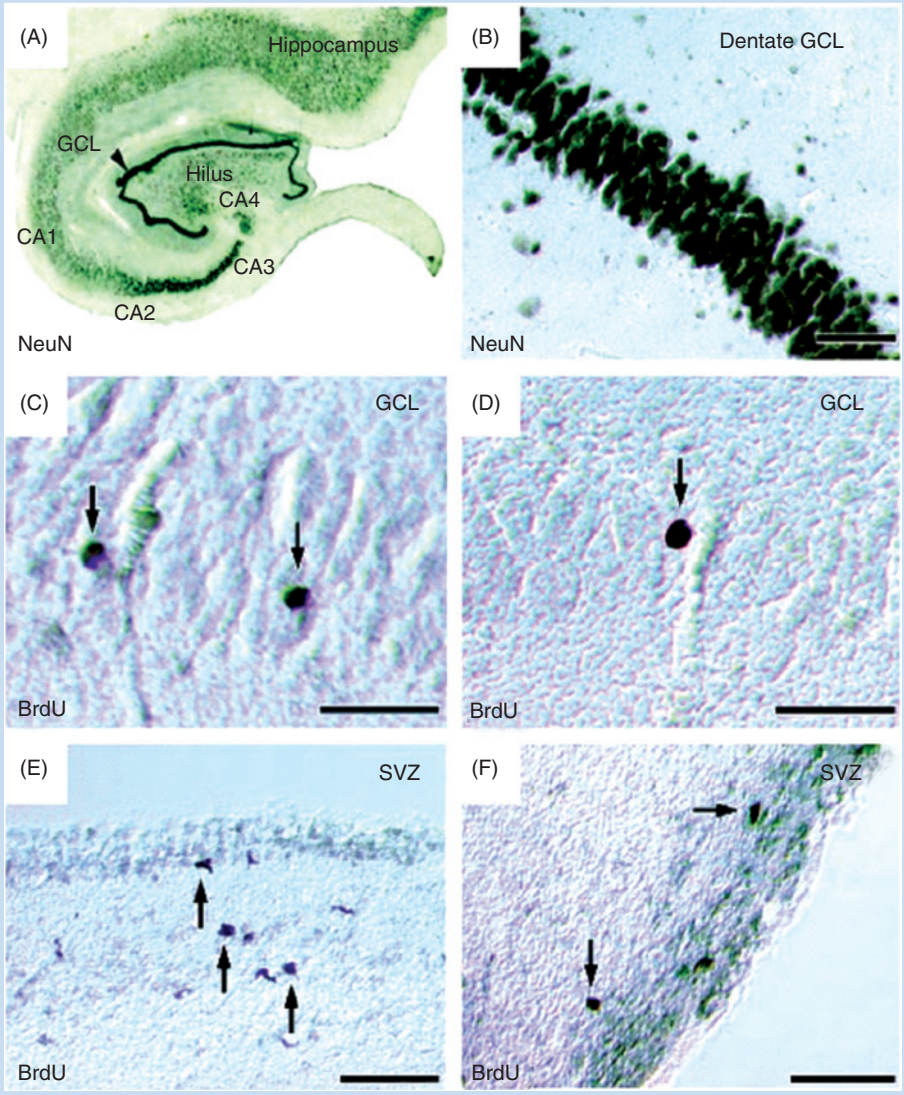
Box 2

To measure DNA synthesis or cell proliferation, 5-bromo2′-deoxy-uridine (BrdU) can be incorporated into DNA in place of thymindine. Cells, which have incorporated BrdU into DNA, can be quickly detected using a monoclonal antibody against BrdU. The binding of the antibody is achieved by denaturation of the DNA. This is usually obtained by exposing the cells to acid, or heat.

State of the Art Experiment

Newly generated cells can be detected in the adult human brain in patients previously treated with BrdU (Eriksson *et al.*, 1998).

In order to investigate the fundamental question whether neurogenesis occurs in the adult human brain tissue specimens were obtained



Detection of newly formed cells in the human brain using various techniques (see text). All scale bars represent 50 μm. (From Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, et al. *(1998). Neurogenesis in the adult human hippocampus.* Nat Med, *4(11): 1313–1317.)*

postmortem from patients who had been treated with the thymidine analog, bromodeoxyuridine (BrdU), that labels DNA during the S phase. Areas of the human brain previously identified as neurogenic in adult rodents and monkeys was used for the experiment. With immunofluorescent labeling for BrdU in combination with the neuronal markers, NeuN, calbindin or neuron specific enolase (NSE) demonstration of new neurons generated from dividing progenitor cells in the dentate gyrus of adult humans was possible. a, localization for the neuronal marker NeuN; b, the hippocampal dentate gyrus granule cell layer (GCL) visualized with immunoperoxidase staining for NeuN; c, differential interference contrast photomicrograph showing BrdU-labeled nuclei (arrows) in the dentate granule cell layer (GCL); d, differential interference contrast photomicrograph showing a BrdU-labeled nucleus (arrow) in the human dentate GCL. BrdU-positive nuclei have a rounded appearance and resemble the chromatin structure of mature granule cells and are found within the granule cell layer; e, differential interference contrast photomicrograph showing BrdU-positive cells (arrows) adjacent to the ependymal lining in the subventricular zone of the human caudate nucleus. Cells with elongated nuclei resembling migrating cells are in the rat subventricular zone (SVZ); f, differential interference contrast photomicrograph showing BrdU-positive cells (arrows) with round to elongated nuclei in the subventricular zone of the human caudate nucleus.

reduced over time. The only compensating mechanism for loss of heart tissue is thus hypertrophy and not through proliferation of individual cardiomyocytes. This view is in contrast to the biological reality in newt and zebra fish where regeneration of heart tissue is seen after injury (McDonnell and Oberpriller, 1984) (Poss *et al.*, 2001) (Figure 3.4).

The dogma of non-existing regeneration potential of cardiomyocytes has been challenged by individual researchers (McDonnell and Oberpriller, 1983; Oberpriller and Oberpriller, 1974) but the finding of y chromosome-containing cardiomyocytes, smooth muscle cells and endothelial cells in female-donated hearts in male recipient patients, gave new support to the view of the human heart as a regenerating organ (Quaini *et al.*, 2002). The demonstration of dividing myocytes in the normal and pathologic heart tissue has given further support to the hypothesis that the heart harbors a pluripotent stem cell niche supporting a normal slow regeneration (Beltrami *et al.*, 2001).

Cardiomyocyte progenitors with a specific phenotype (Lin(−) c-kit(+)) have been identified in rats. The cells are able to self renew and have multipotency with ability to form myocytes, smooth muscle cells and endothelium. When cells are injected into injured cardiac tissue the cells are able to induce regeneration of myocardium, including new vessels and endothelium (Beltrami *et al.*, 2003). Recently it was demonstrated that stem cells with the Sca-1(+) marker in mice have a homing potential to injured myocardium and the effect was due to both cell fusion and regeneration of new cells (Oh *et al.*, 2003). These results clearly demonstrate that heart tissues in mammalians that mainly consist of terminally differentiating cells also has the potential of a classic regeneration organ (Beltrami *et al.*, 2003). Interestingly, a stem cell niche has been demonstrated in the mouse heart (Figure 3.5). In these niches containing cardiac stem cells and lineage committed cells the supporting fibroblasts and myocytes are connected to the stem cells by connexins and cadherins (Urbanek *et al.*, 2006).

3.6 Consequence of regeneration potential for the tissue engineering concept

The regeneration capacity of a single organ requires careful considering when approaching a therapeutic

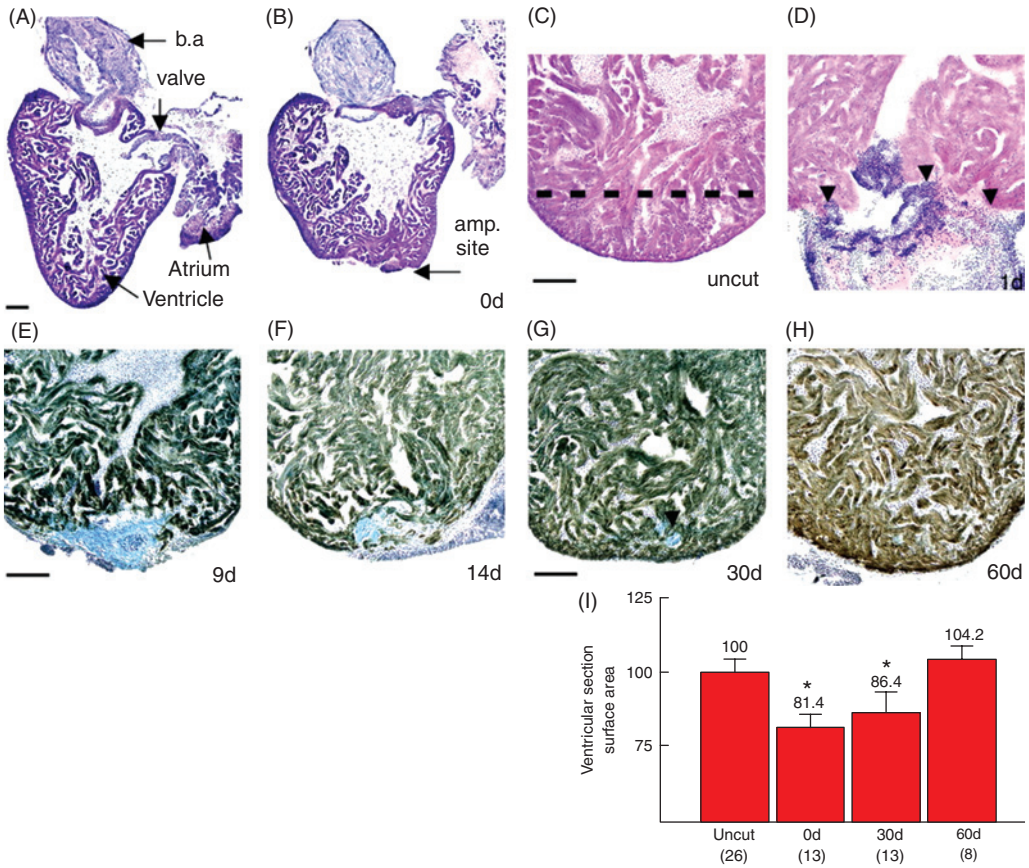


Figure 3.4 Regeneration of ventricular myocardium in the resected zebrafish heart. Hematoxylin and eosin stain of the intact zebrafish heart (A) before, and (B) after about 20% ventricular resection, b.a., bulbous arteriosus; (C) An intact ventricular apex at higher magnification, indicating the approximate amputation plane (dashed line). All images in this and subsequent figures display longitudinal ventricular sections of the amputation plane; (D) 1 dpa. The large clot is filled with nucleated erythrocytes (arrowheads); (E) 9 dpa. The heart section is stained for the presence of myosin heavy chain to identify cardiac muscle (brown) and with aniline blue to identify fibrin (blue) (accessed from http://www.sciencemag.org/cgi/content/full/298/5601/2188#R5#R5). The apex is sealed with a large amount of mature fibrin; (F) 14 dpa. The fibrin has diminished, and the heart muscle has reconstituted; (G) 30 dpa. A new cardiac wall has been created, and only a small amount of internal fibrin remains (arrowhead). (H) 60 dpa. This ventricle shows no sign of injury; (I) Quantification of healing at 0, 30, and 60 dpa. Values represent the size of the largest ventricular section (mean ± SEM; $*P < 0.05$); parentheses indicate the number of hearts examined (accessed from http://www.sciencemag.org/cgi/content/full/298/5601/2188#R5#R5). Scale bars, 100 μm. (From Poss, K.D., Wilson, L.G. and Keating, M.T. (2002). Heart regeneration in zebrafish. *Science*, 298(5601): 2188–2190.)

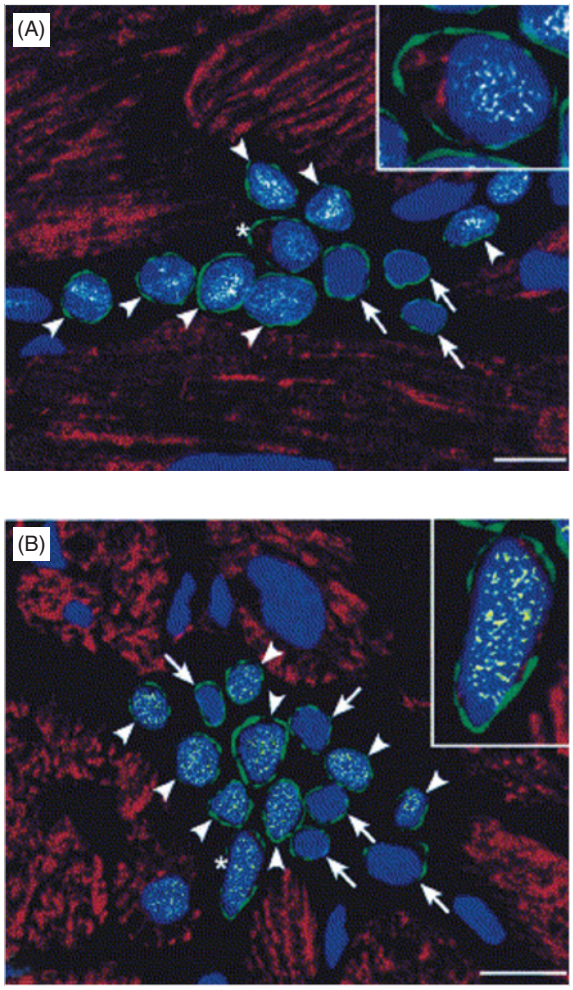


Figure 3.5 Clusters of Primitive and Early Committed Cells in the Heart, A, Cluster of 11 c-kit^{POS} cells (green) with three expressing c-kit only (arrows), seven expressing Nkx2.5 (white dots; arrowheads) in nuclei (blue, propidium iodide, PI), and 1 Nkx2.5 and α-sarcomeric actin in the cytoplasm (red; asterisk, see inset); B, Cluster of 15 c-kit^{POS} cells with five c-kit^{POS} cells only (arrows), eight expressing MEF2C (yellow dots; arrowheads), and one expressing MEF2C and α-sarcomeric actin (asterisk, see inset). Bars, 10 μm. (From Beltrami, A.P., Barlucchi, L., Torella, D. *et al.* (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, 114(6): 763–776.)

concept. If each organ is schematically described as an equilibrium between cells produced and cells discarded, the newly implanted tissue construct needs to consider the fact that this equilibrium has to be maintained by adding progenitor cells, or by not stopping or interfering with the normal process of regeneration.

The stem cell niche concept is central to most of the approaches we are discussing in tissue engineering. Stem cell niches are composed of a cellular microenvironment that supports the stem cells and enables them to maintain tissue homeostasis (Moore and Lemischka, 2006). A cellular interaction between the stem cells and the niche cells exists, with the goal to fulfill the lifetime support of differentiated cells. The niche cells shelter the stem cells from differentiation stimuli, apoptotic signaling and other stimuli that would challenge the reserve of stem cells. The stem cell niche also protects the stem cell pool from overactivity, e.g. unnecessary hypertrophy and must be activated in a timely sense to produce progenitor and transiently amplifying cells. Thus, the overruling control is to balance between cell quiescence and activity.

Today there are three identified and well-defined stem cell niches that harbor stem cells responsible for the maintenance of a continuous turnover of cells during its life time. These are the interstitial stem cell niche (ISCN) of the intestine, the hair follicle epidermal stem cell niche (HFSCN) and the hematopoietic stem cell niche in the bone marrow (HSCN).

The stem cell niches share common properties and requirements. All harbor stem cells that give rise to several different cell lineages. The transient amplifying cells that are produced must migrate into their proper location in order to fulfill the correct function. They are dependent on the surrounding cells consisting of mesenchyme and for the HSCN on the osteoblasts. The intricate signaling from the mesenchyme is one of the control mechanisms regulating the stem cell niche.

Interestingly, the three stem cell niches share interesting entities. An anatomical organization coordinates stem cell control and fate, both stimulating

and repressor signaling systems are integrated and intercellular signaling pathways are shared. Bone morphogenetic protein (BMP) functions as a negative regulator on stem cell proliferation by suppressing nuclear β-catenin accumulation. This is counteracted by Wnt signaling (Figure 3.6).

Numerous regulatory signaling pathways have been revealed by global gene expression profiles of quiescent and activated HSCN as well as more committed progenitor populations (Eckfeldt *et al.*, 2005). Furthermore, a comprehensive genome analysis of an HSC supportive cell population has been described which further enlightens these interesting structures. The future of tissue engineering and regenerative medicine lies in increased knowledge of anatomical organization and location of stem cell niches in different organs. Future efforts will be focused on constructing proper microenvironments *in vitro* to accurately mimic the *in vivo* function of the niches, including understanding of the migration behavior of the transient amplifying (TA) cell population.

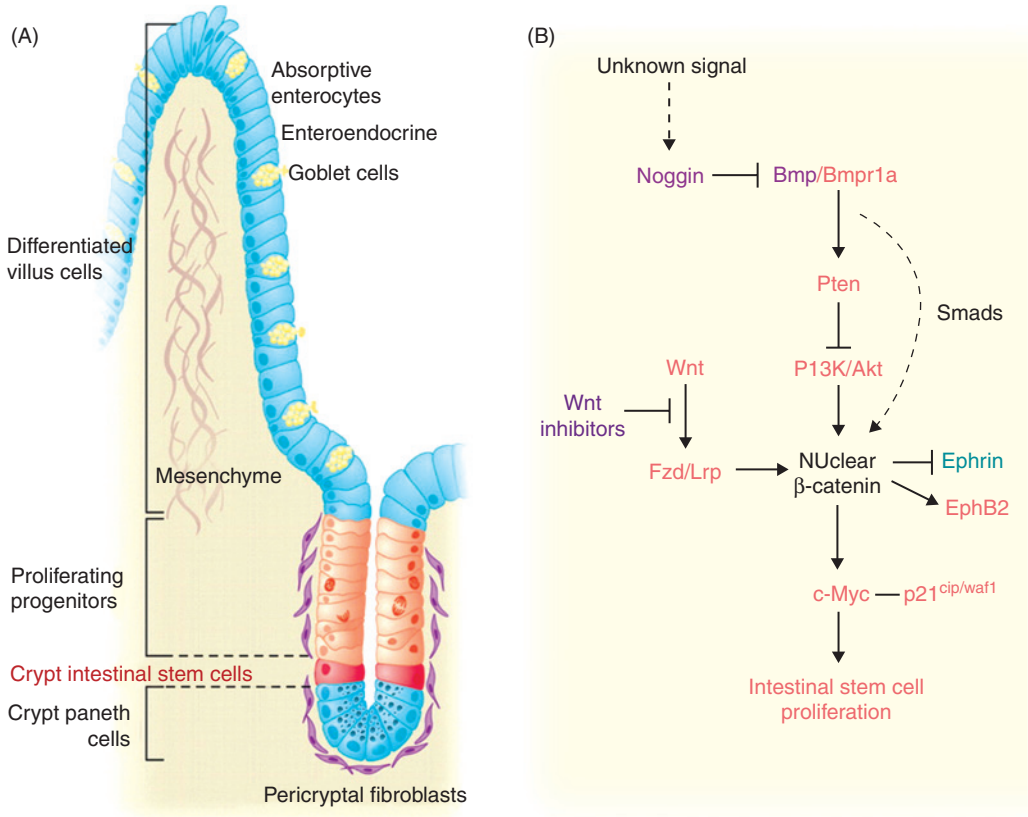


Figure 3.6 Stem cells within their niche in the small intestine. A, Schematic diagram of the major types and spatial orientations of cells found within the crypt niche and the villus; B, Interactive signaling pathways that mediate ISC proliferation. Colors represent the cell types sending and receiving the signals as displayed in (A). (From Moore, K.A. and Lemischka, I.R. (2006). Stem cells and their iches. *Science*, 311(5769): 1880–1885.)

3.7 Cell migration of TA cells

One central issue regarding stem cell niches and the maintenance of an organ is how a TA cell is moved from the stem cell niche to the proper location. Cell migration must be properly maintained in any potential scaffold produced. This is either provided by the laid down extra cellular matrix or by, e.g. nanofibers, giving the cells a proper attachment and migration potential. Examples of how nature has solved these issues are given in the hair follicles which show how cell trafficking is controlled for transient amplifying cells. Hair shafts contain a multipotent stem cell region – the bulge region – that contains three-potent epidermal cells that are able to form the epidermis (the skin), the sebaceous glands and the hair (Oshima *et al.*, 2001) (Figure 3.7).

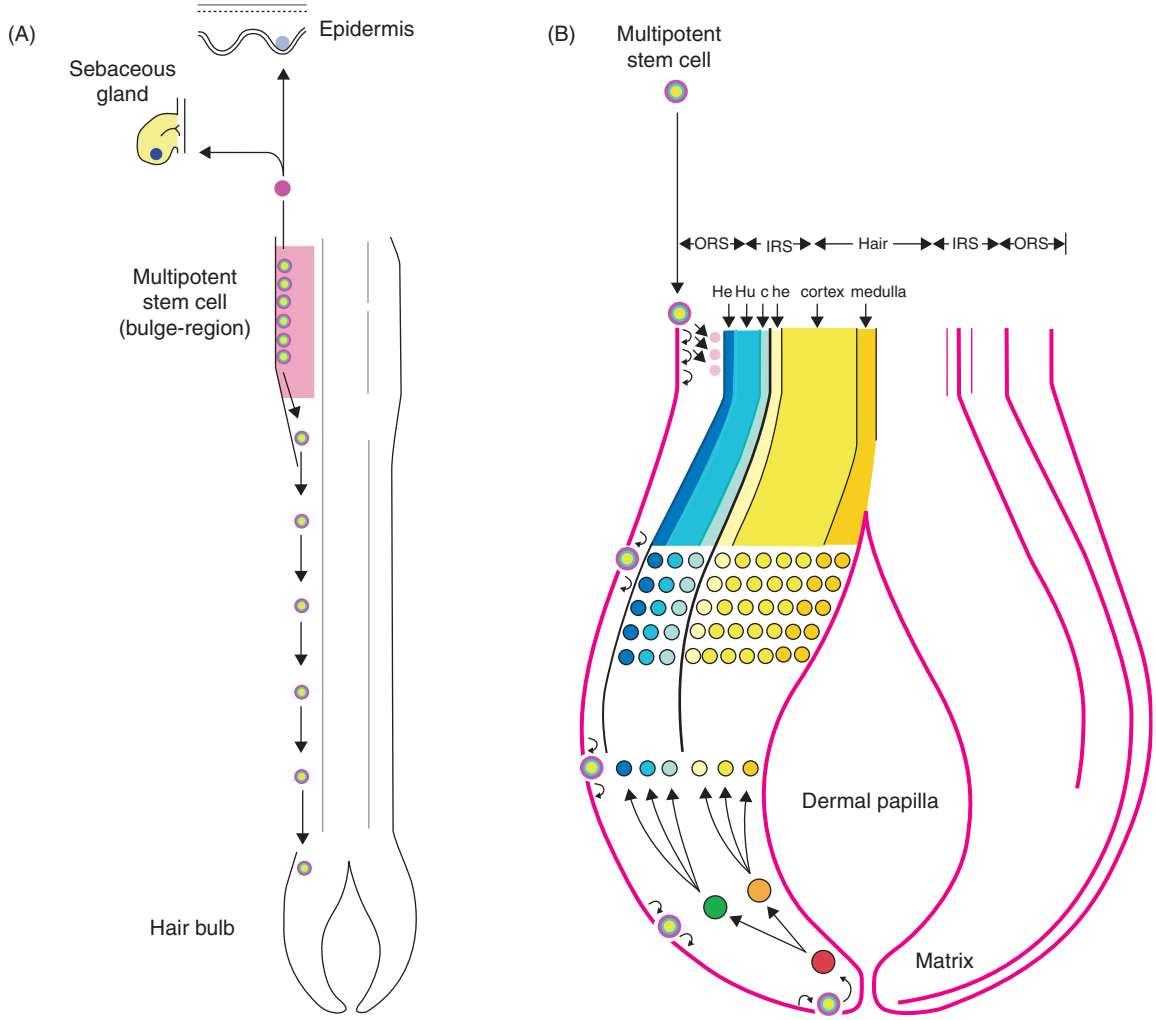


Figure 3.7 Migration behavior of TA hair root cell generated from stem cell bulge. (Oshima, H., Rochat, A., Kedzia, C. *et al.* (2001). Morphogenesis and renewal of hair folliclies from adult multipotent stem cells. *Cell*, 104(2): 233–245.)

With time, the stem cell moves by trafficking along the hair to the root. Similar trafficking can be found after implantation of mouse embryonic stem cells in the mouse brain using labeled cells traceable by magnetic resonance imaging.

3.8 Future developments

The emerging understanding of tissue regeneration will open new frontiers for tissue engineering researchers. The tissue engineering concept of engineering whole functional organs will probably change towards manipulating the enormous potential of autologous tissue regeneration. One could envision local implantation of chemical substances able to enhance stem cell recruitment. Perhaps even nanoconstructed small stem cell niches could be implanted that locally will enhance tissue regeneration. Furthermore, large fibers unable to support cell migration of TA cells and transduction of cellular biomechanics might be replaced by nanofibers produced by electrospinning or other means.

3.9 Summary

- Tissue regeneration after injury is limited to a few tissues in vertebrates including humans with the liver, blood and bone as examples of tissues with full regeneration capacity after injury.
- Tissues in the body undergo a constant renewal over time with approximately 1% of the body renewed each day. Tissues with high regeneration capacity are the intestine skin and blood. Others tissue like bone have a slow cellular turnover.
- Skin transplantation to burn victims was introduced in the 1980s. By the development of a stem cell culture system for human keratinocytes based on fibroblast feeder layers and addition of EGF a $1\,cm^2$, skin bisopsy is able to regenerate $2\,m^2$ of skin.
- Each tissue is maintained in a steady-state equilibrium where the cell loss due to apoptosis, shredding of cells or for other reasons is balanced by cell renewal from stem cells.
- The brain and heart were considered non-regenerating but recent knowledge gained from labeling experiments of human tissue revealed cell regeneration capacity in the human brain and heart.
- Most organs contain a defined stem cell niche where transient amplifying cells are constantly released. Well-defined stem cell niches are identified in the skin, intestine and bone marrow.
- Stem cell niches share common cellular signaling pathways usually well-conserved over species borders.
- When discussing the concept of tissue regeneration by artificial means, i.e. by implantation of scaffolds with or without cells, one has to acknowledge the normal regeneration mechanisms in the tissue. The stem cell niche releases cells into the organ and cells migrate to the location of function. Such migration behavior should be considered in the tissue engineering approach by the introduction of a nanoscale 3D web able to support migration.

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