3 Hair Anatomy and Histology for the Hair Transplant Surgeon

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3.1 Summary

The hair transplant surgeon should know in detail the macro- and microscopic anatomy of the human follicular unit as well as the anatomy and histology of the terminal hair follicle. In this chapter, we provide a new anatomical view of the follicular unit, beginning with its classic anatomical description but incorporating the newest information. The description of the macroscopic images of hair follicles at different stages of the cycle may be very useful for surgeons and technicians performing microscopic follicular dissections. Finally, the location of the different stem cell niches in human follicles is shown, discussing current understanding of how these cells operate during the process of hair regeneration.[15]

Key Points

- Follicular units contain terminal and vellus/miniaturized hair follicles, sebaceous glands, arrector pili muscles, eccrine coils, perifollicular dermis, adipose tissue, and a rich neural and vascular network.
- The terminal hair follicle is the prime element in hair transplantation. In order to preserve its integrity, the surgeon needs to understand this anatomy.
- Hair follicles are dynamic anatomic structures gifted with a unique capacity of self-renewal. Their anatomy changes according to the hair cycle phase.

3.2 Introduction

Historically, hair is commonly regarded as a unique mammalian feature. The first evidence of hair was recovered from a rich accumulation of fossil excrement from 55 million-year-old late Paleocene beds at Bayan Ulan in Inner Mongolia, China.[1]

Human hair varies considerably in size, shape, and density, depending on its location and stage of development. Most of the estimated 5 million hair follicles (HFs) that cover the human body are vellus hairs. They are rooted in the papillary or upper reticular dermis, and produce a barely visible hypopigmented hair shaft of less than 30 µmin diameter and often less than the thickness of the inner root sheath (▶ Fig. 3.1). In contrast, most follicles present on the scalp and used in hair restoration are terminal hairs: pigmented, long, rooted in the subcutaneous tissue, and produce thick hair shafts with a diameter bigger than 60 µm.

3.3 Updated Review of the Follicular Unit Anatomy for the Hair Transplant Surgeon

Human hair emerges from the scalp in groupings known as follicular units (FUs) (▶ Fig. 3.2a). However, strictly speaking, the FU is a histological concept first recognized by the pathologist T. Headington in 1984[2] when analyzing serial horizontal sections of scalp biopsies obtained from cylindrical punches (▶ Fig. 3.2a). Headington defined the FU as a unit structure composed of one to four terminals plus one or two vellus HFs, their associated sebaceous glands, their arrector pili (AP) muscles, the perifollicular vascular and neural plexus, and a circumferential band of fine adventitial collagen. This classical description of the microscopic anatomy of the FU has been subjected to several modifications, which can be summarized as follows.

3.3.1 The Variable Distribution of Terminal Hairs in FUs

The FUs, which in modern hair transplantation are the primary elements of transplant, can be harvested from the donor scalp either directly with a 0.8- to 1-mm cylindrical punch[16] (FUE) or by microscopic dissection after strip harvesting (▶ Fig. 3.3). It is important to note that in the donor area there is significant variation in the percentages of the different hair groupings from patient to patient and among different races. For example, more than 50% of the FUs in Caucasians are two-hair FUs, followed by three- and four-hair FUs, while only 10 to 20% of the FUs have one terminal hair (▶ Fig. 3.4). As a rule of thumb, the scalp hair density in Caucasians is about 2.5 times the FU density.[3] In contrast, most FUs in Asians have two hairs, followed by one terminal hair FUs (30–37%).[4] It is important to realize that the absolute number of FUs per square centimeter remains relatively constant (65–85 FUs/cm^2), and that it is the different proportion of natural hair groupings that determines a patient's hair density. For example, patients with high hair density have more three- and four-hair FUs than patients with low hair density, although the FU density may be the same.

3.3.2 The Anatomical Relationship of the AP Muscle with the FU

In classic anatomical textbooks, each HF appears associated with a single AP muscle. However, the current anatomical model takes into consideration the FU as a unit structure, introducing the concept of one FU served by one AP muscular unit,[5] in which the AP muscles that originate from their respective follicles join together, forming a single muscular structure that

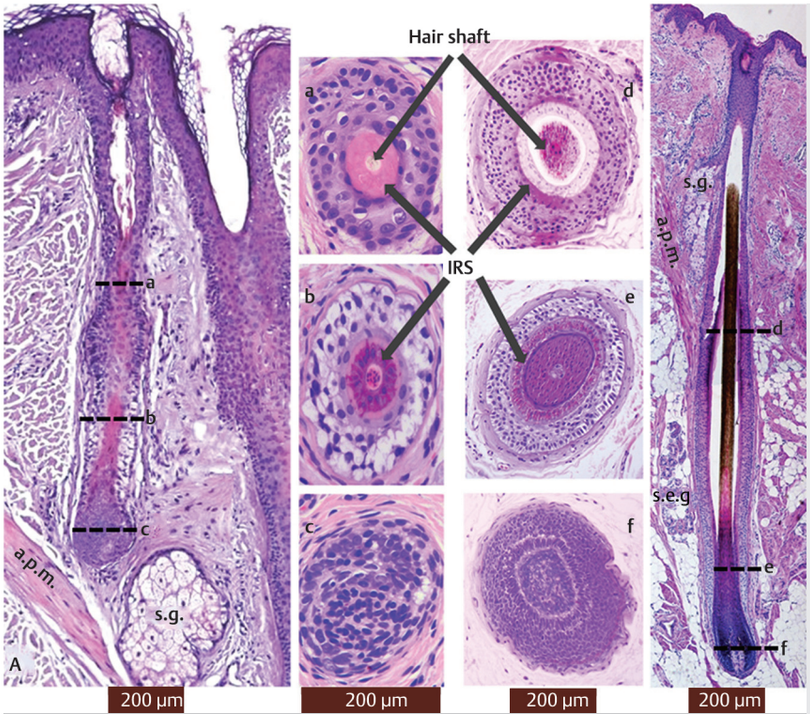

Fig. 3.1 Histological differences between vellus and terminal hair follicles. The image on the left **(a)** shows a vertical section of a vellus follicle. Note that vellus follicles do not have arrector pili muscle attachment and that the bulb is rooted in the dermis. Images **a**, **b**, and **c** are horizontal sections made at their respective level of depth. The image on the right shows a vertical section of a terminal hair follicle. Images **d**, **e**, and **f** are horizontal sections of a terminal hair follicle. The purpose of these figures is to show the differences in hair shaft diameter between vellus and terminal follicles: by definition, vellus hair shafts are thinner than the inner root sheath (IRS). Note that terminal follicles are rooted in the subcutaneous fat. a.p.m., arrector pili muscle; s.e.g., sweat eccrine gland; s.g., sebaceous gland.

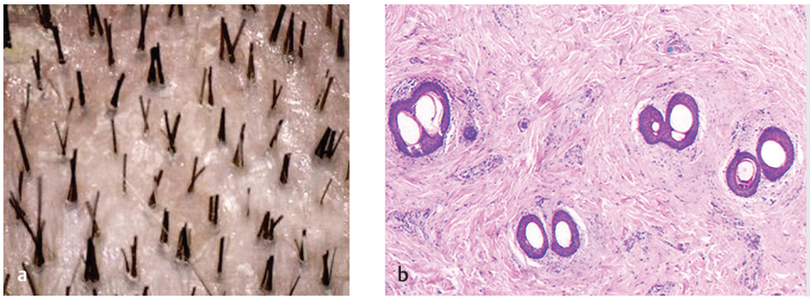

Fig. 3.2 Follicular units (FUs). **(a)** Close-up photo of the occipital scalp skin showing how hairs exit the surface, forming groups known as follicular units (FUs). **(b)** A horizontal histologic section of four 2-hair FUs. This section was made at the infundibulum level (above the sebaceous glands).

extends upward to its superior attachment zone. We could imagine the AP muscles acting as a string that ties all the HFs of each FU together, like a ribbon on a bunch of flowers (▶ Fig. 3.5). Below that area, the inferior portion of the anagen follicles tends to splay out, which is the main reason why sharp FUE punches inserted too deep (usually deeper than 3 mm) cause excessive follicular transection.

The AP muscle is attached to the follicle in a portion of the outer root sheath known as the bulge zone. It has been shown that the bulge stem cells are responsible for guiding the attachment of the AP muscle by means of the deposit of a protein called nephronectin.[6] In hair transplantation, the AP muscles are obviously transected during donor harvesting but the muscle seems to be regenerated after implantation in the recipient area[7] maintaining its contractile capacity.

3.3.3 FUs Contain Eccrine Sweat Glands

The surgeon and the hair transplant technicians cannot see eccrine glands under the stereomicroscope because they are not visible unless stained with specific dyes. Nevertheless, the majority, if not all, of FUs contain one eccrine coil (secretory portion of the eccrine sweat glands) as can be observed in many vertical histologic sections at the level of the inferior portion of the follicle (below the AP muscle; ▶ Fig. 3.5 and ▶ Fig. 3.7).[8] The functional significance, if any, of this eccrine–HF anatomic association is currently unknown.

3.4 The Terminal Hair Follicle: The Hair Surgeon's Most Precious Tissue

The terminal HF is what produces the thick and long hair shaft. It would seem to be a simple structure, but the terminal HF is in fact quite complex and can be considered a miniorgan per se, composed of many different types of cells that interact together and with the surrounding microenvironment: epithelial cells, mesenchymal cells from the dermal papilla (DP) and dermal sheath, several pools of epithelial, melanocyte, and mesenchymal stem cells involved in HF self-regeneration and pigmentation, a rich innervation and vascularization network, and

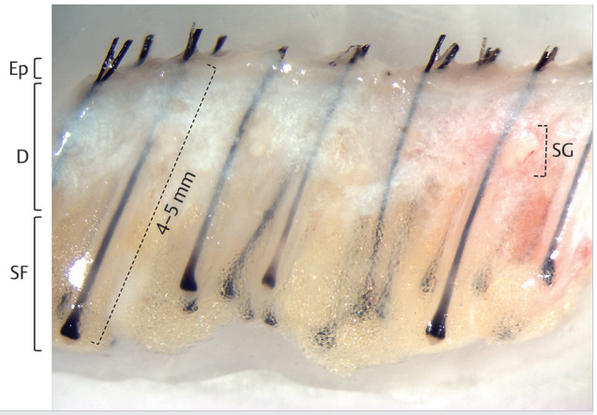

Fig. 3.3 Sliver dissected from a strip excision. This photo shows a sliver dissected under the stereomicroscope from a donor strip. It illustrates the different compartments of the scalp skin. D, dermis; E, epidermis; SF, subcutaneous fat. Note how deep the bulbs of the terminal follicles are located (normally between 4 and 5 mm). Also note the yellowish color of the sebaceous glands (SG), and the arrangement of the follicles in groupings. Other structures present in the skin including arrector pili muscles, sweat glands, small vessels, and nerves are invisible under the stereomicroscope.

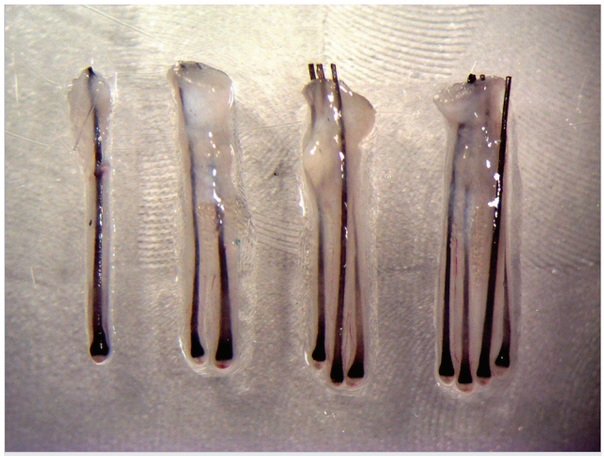

Fig. 3.4 Follicular units represent the main transplant graft. FUs of human scalp may contain one, two, three, or four terminal hairs (from left to right). These FUs were harvested with a 0.95-mm punch using the FUE technique.

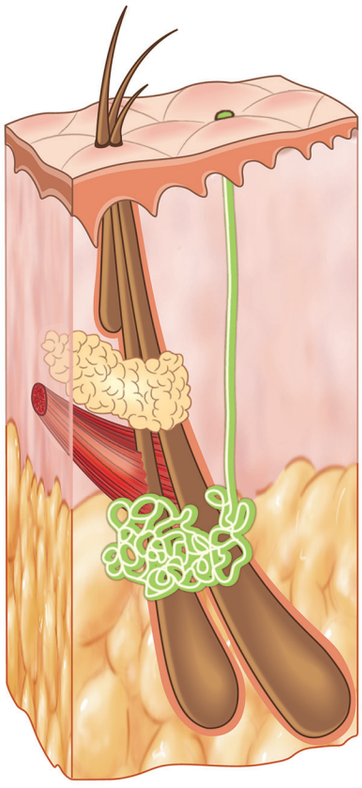

Fig. 3.5 Anatomy of the follicular unit. This is a drawing showing the spatial relationship between the different components of an FU transplant graft: hair follicles, sebaceous gland (*yellow*), eccrine gland (*green*), and arrector pili muscle (*red*).

resident immunocytes (mast cells, macrophages, T cells, and Langerhans cells). Some of these different cell types contribute to hair shaft growth and some to other very important functions (dermal remodeling, re-epithelialization after wounding, cutaneous stem cell homeostasis, etc.), which are beyond the scope of this chapter.

3.4.1 Changes in the Anatomy of the Terminal HF According to the Hair Cycle

HFs follow a continuous cycle of growth (anagen phase), involution (catagen phase), and rest (telogen phase) until a new cycle develops. In humans, these events are asynchronous, which means that each follicle contained in an FU is at a point in its cycle, which is independent of its neighbors (▶ Fig. 3.6a). In normal circumstances, approximately 90% of human scalp follicles are in anagen, and the remaining 10% in either catagen or telogen.

The anagen terminal follicles are the most common and the easiest to identify under the stereomicroscope due to their well-defined inferior segment. On the scalp, they have an average length of 4 to 5 mm, although they can be as short as 3 mm in patients with thin hair and up to 6 mm in thick hair scalps. Depending on the duration of the anagen phase, HFs in different areas of the body produce hairs of different length. For example, scalp follicles stay in anagen for a long time (2–8 years), with the hair shaft length increasing each day at an average rate of 0.30 mm.

The catagen phase starts with the destruction by massive apoptosis of the inferior portion of the HF. This event leaves an epithelial strand and a significant reduction in HF size. The DP changes from cone-shape to a more condensed shape and moves upward, remaining attached to the epithelial strand like a remnant DP. The catagen phase lasts about 2 to 3 weeks and catagen follicles can be subdivided according to their morphology in several stages (early, mid, and late catagen),[9] although in reality it should be perceived as a continuous morphological change, which ends in the telogen follicle (▶ Fig. 3.6b).

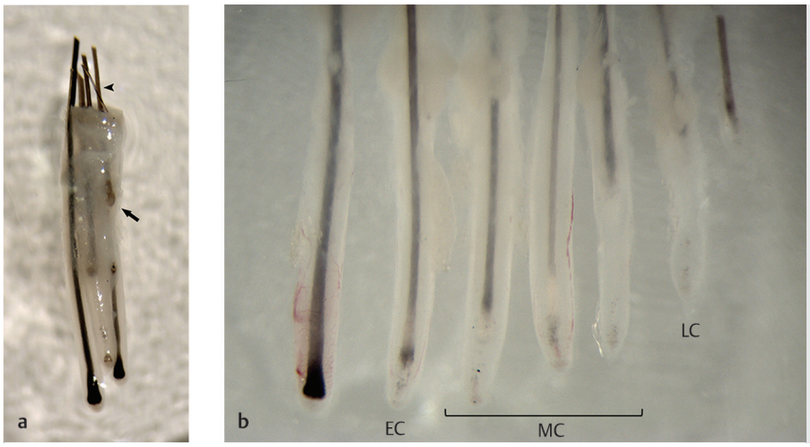

Fig. 3.6 Hair follicles within the follicular unit cycle asynchronically (or independently). **(a)** Follicular unit harvested with 1-mm punch composed of four terminal hairs and one vellus/miniaturized hair (*arrowhead*). One of the terminal hairs is in catagen, two are in anagen, and one in telogen (*arrow*). **(b)** Several hair follicles dissected under the stereomicroscope that show different stages of the hair cycle from anagen (*left*), early catagen, mid-catagen, late-catagen, and telogen (*right*).

The telogen phase represents the lack of proliferation and apparent quiescence of HFs. The hair shaft is depigmented and has a club-like base shape. The DP is very small and sits directly opposite the thin remaining epithelium in the deepest portion of the bulge known as the hair germ, which plays a very important role in the telogen-to-anagen transition. The hair germ can be identified in histologic sections of human telogen follicles with the antibody Ber-Ep4. The telogen phase of scalp follicles lasts around 2 to 3 months. The telogen-to-anagen transition (anagen re-entry) is one of the hottest topics in hair biology research, because it provides important insights into understanding the processes of HF regeneration. Finally, the shedding phase of the telogen follicle is known as exogen. The mechanisms involved in the release of the club hair are not yet fully understood, but it seems to be driven by activation and inhibition of proteases.[10] Kenogen describes an empty follicle in telogen, after the club hair shaft has been shed.

3.4.2 Histology of the Terminal Hair Follicle

It is important to know the histological division of the HF into three vertical portions (infundibulum, isthmus, and inferior portion), because it serves as a frame of reference to delineate anatomic compartments within the follicle (▶ Fig. 3.7). Briefly, the infundibulum comprises the area between the follicular ostia and the entrance of the sebaceous duct, the isthmus is the portion between the sebaceous duct and the AP muscle, and the inferior portion is the segment of the follicle below the AP muscle. The entrance of the sebaceous duct and the insertion point of the AP muscles are fixed structures, which makes this simple classification useful and immutable throughout the HF cycle. In contrast, the inferior portion of the follicle is present only in the anagen phase of the hair cycle, undergoing regression in catagen and being absent in telogen.

The HF is made of epithelial keratinocytes and mesenchymal cells. The epithelium is rather complex, disposed in eight concentric layers (▶ Fig. 3.7). The outermost layer is known as the outer root sheath (ORS). The inner root sheath (IRS) is composed of three layers: the innermost layer is the cuticle and interlocks with the cells of the hair cuticle; in the center is Huxley's layer and the outermost layer is known as Henle's layer. Between the ORS and the IRS is located the companion layer. The ORS, the companion layer, and the IRS surround the hair shaft which consists of the hair cuticle, the cortex, composed of keratinized cells which contain pigment from the melanocytes of the bulb, and the innermost medulla. The matrix is a compartment of proliferating keratinocytes that form the different follicular layers and where melanin production takes place to produce a pigmented hair shaft.

The mesenchymal component of the HF includes the dermal sheath and the DPs. The dermal sheath lines the outer surface of the HF continuously from the base of the DP to the upper bulge, and contains follicular fibroblasts, numerous capillaries, and immune resident cells. The DPs are formed also by special fibroblast-like cells in continuity with the dermal sheath. The cells of the DPs are in direct contact with the epithelium of the matrix. Both the DP and the matrix form the bulb, which represents the root of the HF (▶ Fig. 3.7). The size of the papilla correlates with the size of the follicle and the size of the hair shaft produced. The DP size is dynamically regulated during the follicle cycle: cells appear to migrate from the DP to the dermal sheath during catagen and then repopulate it in anagen.

Recently, a new fat compartment in the dermis surrounding the inferior portion of the terminal HFs as "dermal cones" has been described. This fat compartment is known as white adipose tissue. Regeneration of the HF in anagen is paralleled by expansion of this dermal adipose tissue, and adipocyte precursor cells have been implicated in the initiation of anagen. This fat dermal layer is far from being an inert spacer material, expanding in response to cold exposure and reacting to wounding and to bacterial infection.[11]

3.5 The Location of the Stem Cell Niches for Hair Follicle Regeneration

The stem cells of the follicles reside in topographically well-defined locations. These specialized tissue compartments that host the stem cells and every other component necessary for their function, including neighboring cell populations, molecular signals, and other extracellular components, are commonly referred to as "niches." There are two niches in the follicle: the

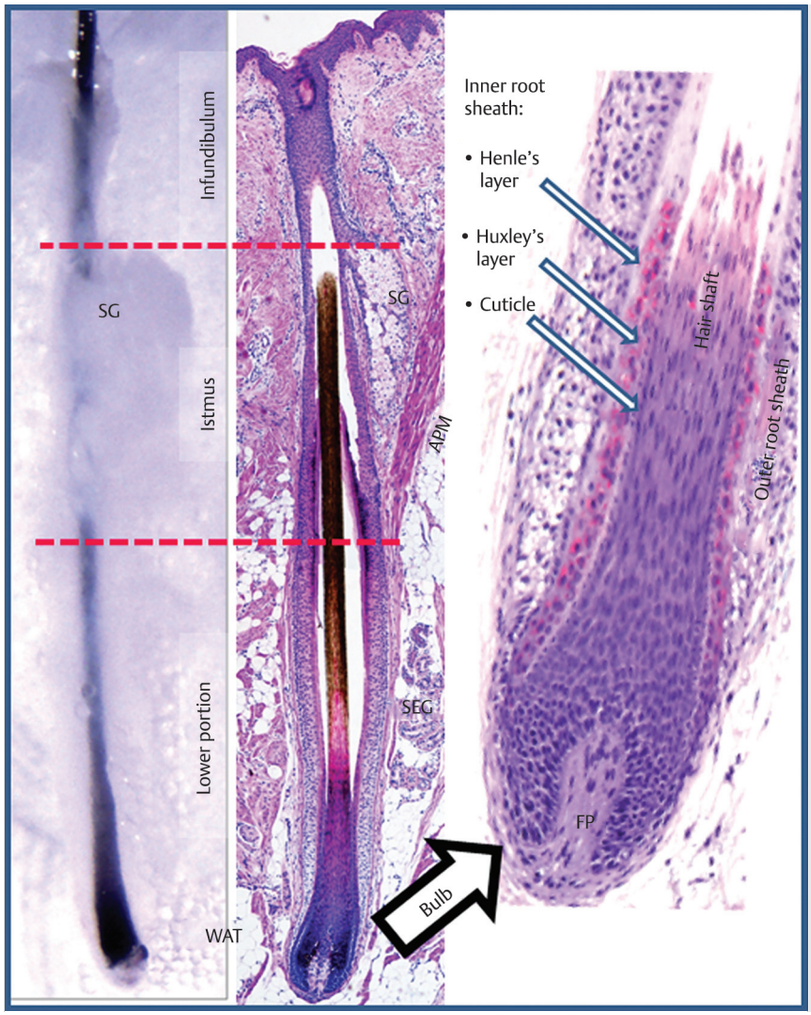

Fig. 3.7 Histology of a terminal hair follicle. Vertical section of an anagen terminal follicle stained with hematoxylin and eosin. The three compartments of the follicle (infundibulum, isthmus, and inferior portion) are delineated, as well as the different epithelial layers. Note the position of the secretory coiled portion of the eccrine gland (SEG), very close to the follicular epithelium and always below the arrector pili muscle (APM). FP, follicular (or dermal) papilla; SG, sebaceous gland; WAT, white adipose tissue.

epithelial and the mesenchymal niche. These HF niches are critical for regulating the process of hair regeneration (from telogen to re-entry in anagen), and the hair surgeon should be aware of their precise location to avoid any damage.

The epithelial follicular niche is the best studied. It is located in a region of the follicle known as the bulge. The bulge zone can be easily recognized in hematoxylin and eosin vertical sections of murine and human fetus as a prominent protuberance of the ORS. In contrast, in adult human follicles, the bulge region is barely prominent. Initially, the bulge was only known for being the attachment zone of the AP muscle, until significant interest was aroused when it was identified as the area that contained the main pool of follicular epithelial stem cells. Anatomically, the bulge in anagen follicles extends between 1 and 2 mm below the skin surface and coincides with the location of the isthmus portion of the follicle,[12] while in telogen follicles the bulge represents the deepest epithelial portion of the follicle. Specific immunohistochemical markers such as CK15 and CD200, among others, can delineate bulge stem cells in human follicles (▶ Fig. 3.8).

Recent studies made in mice follicles have uncovered very interesting aspects of the hair stem cell dynamics. It seems that the bulge zone has several compartments of organization: a more activated and a more quiescent one, and the location of the stem cells within the niche is important in predicting their fate and contribution to hair growth. Specifically, cells located in the lower bulge generate ORS lineages, while those situated further down, in the hair germ, contribute to the IRS and hair shaft layers. Cells in the mid and upper bulge do not contribute directly to the regeneration of the follicle and remain quiescent. We now know that in telogen follicles the first stem cells that become activated and proliferate to enter a new regeneration cycle are cells of the hair germ.[13] The reason why hair germ cells are the first to proliferate is thought to be due to their close proximity to the DP cells, which emanate activating signals for hair regeneration.

The mesenchymal niche is primarily composed of a group of fibroblast-like cells that form the DP and the lower portion of the dermal sheath that surrounds the bulb, which is known as the dermal cup. It is thought that in the dermal cup there is a population of self-renewing dermal stem cells, which, at the onset of each anagen growth stage, are mobilized to regenerate a new dermal sheath and supply new cells to the DP.[14] The DP cell number seems to be related to the follicle's capacity to initiate new hair growth. As DP cell numbers decline below a specific threshold, HFs are unable to initiate a new hair cycle,

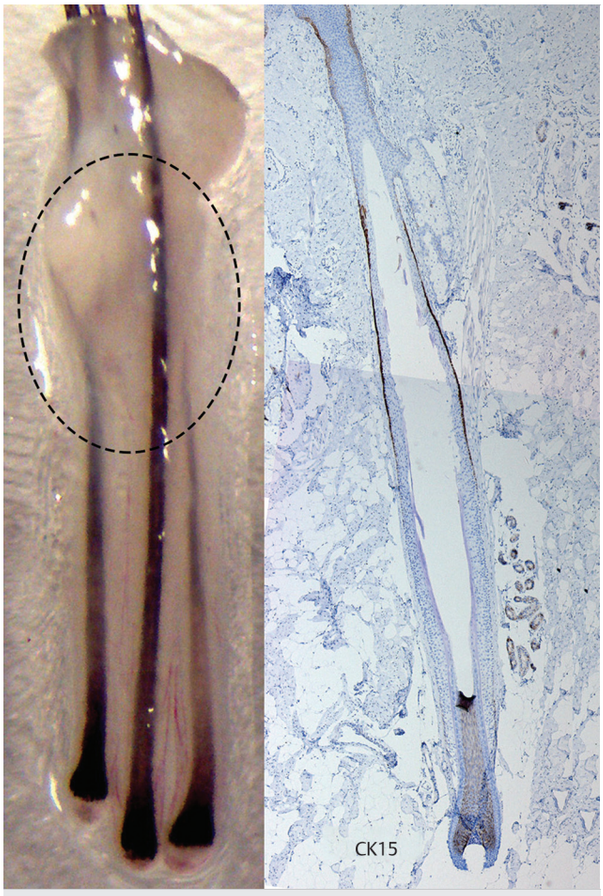

Fig. 3.8 Location of the bulge (epithelial stem cell zone). The figure on the left shows a typical FU transplant graft in which a circle has been drawn delineating the location of the bulge stem cell zone. This region, located approximately 1–2 mm below the skin, coincides with the region stained with the antibody anti-cytokeratin 15, which is a marker of human follicular epithelial stem cells (see positive brown staining of the outer root sheath cells in the bulge zone).

whereas follicles retaining a sufficient number of DP remain able to re-enter the growth phase.[15] Destruction of the DP in telogen follicles renders the HF incapable of initiating anagen growth.[13] The ability to isolate and culture DP cells in 3D cultures to maintain their inductive capacity for hair neoformation has been demonstrated by a variety of transplantation experiments; however, its translation into clinical practice in an efficient manner has not yet been possible.

Other important players in HF regeneration are molecules involved in signaling pathways as well as growth factors that regulate stem cell quiescence, proliferation, and differentiation. These molecules emanate from the DP or from the bulge or from neighboring cells. The most relevant ones in follicles include the Wnt pathway, BMP, TGFbeta, and FGF.

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Comments

15 Reminder: AU: The term “vellous” has been changed to “vellus” throughout the chapter. Please check whether this has been done correctly.

16 AU: Please define FUE if deemed necessary.