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Restoration of hair growth by surgical implantation of follicular dermal sheath

KENNETH A. HORNE and COLIN A. B. JAHODA*

Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland

*Author for correspondence at present address: Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, England

Summary

The capacity of lower follicle dermal sheath to restore hair growth was tested by removing the lower halves of follicles, and then immediately implanting material containing dermal sheath cells from these bases, into the remaining upper epidermal follicle cavity. Over 60% of recipient follicles produced stout emergent vibrissa fibres and some operations resulted in multiple hair production from a single follicle. Histological examination revealed new dermal papillae within large bulb structures which were sited below the level of amputation - a feature that indicated that the new dermal papilla was derived from implanted material. For many follicles, the failure to produce emergent fibres could be accounted for after histological examination. These results provide clear evidence that lower follicle dermal sheath cells are capable of replacing those of the dermal papilla and it shows that they can do so in the context of the upper follicle. However, because elements of lower follicle epidermis were present in the implant material, the interactive sequence of events cannot be established. Dermal sheath cells have immense potential for papilla cell replacement: questions remain as to whether the distinction between sheath and papilla cells is one of context, or whether the transition requires specific external influences.

Key words: vibrissa, dermal sheath, hair growth, dermal-epidermal interactions, fibroblast differentiation.

Introduction

The mammalian hair fibre is the product of a small peg of tissues known as the hair follicle which lies immediately underneath the skin's surface, with the distal part of its epidermal structures in direct continuation with the cutaneous epidermis externally. Although small, the follicle comprises a highly organised system of recognisably different layers arranged in concentric series. Vibrissa follicles are hair follicles that have become specialised for a role in tactile sensation and can be many times larger than the pelage follicles of the same species. Certain of the concentric layers within the vibrissa follicle are greatly elaborated, and in the rodent vibrissa follicle 12 major zones can be readily identified.

At the base of any active follicle lies the hair bulb, which consists of a body of dermal cells - known as the dermal papilla - contained within an inverted cup of epidermal cells. Irrespective of follicle type, the hair fibre, together with several supportive epidermal layers, is produced by the germinative epidermal cells within this follicular epidermal matrix. The basal stalk projects through a hole in the bottom of the epidermal matrix giving the dermal papilla physical continuity with the mesenchymal or dermal sheath. In the rat vibrissa follicle, this mesenchyme forms a highly distinctive sleeve surrounding the follicular epidermis.

The large size of the rat mystacial vibrissa follicle renders it amenable as a model for hair growth studies and specific microsurgical experimentation, a property that Cohen (1961) first took advantage of. Then, in an illuminating series of experiments, Oliver (1980 review) revealed the importance of follicular epidermal associations with the dermal component, that is the dermal papilla and the adjacent mesenchymal sheath. When the dermal papilla alone was removed, or the follicle end-bulb containing the epidermal matrix and dermal papilla was surgically amputated, the regeneration of a new dermal papilla was shown to be a necessary prerequisite for renewed hair production (Oliver, 1966b). In both cases, the new papillae were considered to have been formed by the dermal sheath cells. Furthermore, surgical removal of the end-bulb with more than one-third of the lower vibrissa follicle, leads to permanent cessation of hair growth due to the inability of the tissues at that level to establish a new epidermal matrix-dermal papilla organisation (Oliver, 1966a,b). The transplantation of isolated papillae into inactivated vibrissa follicles demonstrated the powers of the rodent vibrissa dermal papilla to induce hair growth (Oliver, 1967b; Ibrahim and Wright, 1977), and the human dermal papilla has recently been shown to have similar capabilities (Horne et al., 1989).

After embryonic development of the hair follicle, the dermal papilla cells remain mitotically quiescent in situ (Pierard and De La Brassine, 1975); however, papilla cells

from different follicle types can be grown in culture (Jahoda and Oliver, 1981, 1984; Messenger, 1984; Withers et al., 1986; Reynolds, 1989). Subsequent transplantation studies, again utilising rat vibrissa follicles that had been inactivated according to the methodology of Oliver (1967b), revealed that, following culture, cells of the vibrissa dermal papilla still retained the ability to assume the role of an active dermal papilla and to promote hair growth in vivo (Jahoda et al., 1984; Horne et al., 1986).

The present work extends aspects of previous regeneration and implantation experiments. We investigated the potential of mesenchymal sheath from the lower third of the follicle to adopt the role of the dermal papilla when dissected free, then reimplanted into the upper half of ablated follicles.

Materials and methods

The main steps in the implantation procedure are shown diagrammatically in Fig. 1, and basic follicle anatomy in Fig. 2.

Removal of the follicular bulbs

Five adult male inbred hooded rats were used for the experiment. Anaesthesia was induced with halothane and oxygen, and maintained by injection of hypnorm (fentanyl-fluanisone, 0.4 ml/kg subcutaneously) and diazepam (2.5 mg/kg intraperitoneally). The vibrissa follicles of the posterior dorsoventral rows (rows A and B) of the left mystical pad were plucked, and then approached via a surgical incision posterior to row A. Their bases were then exposed (Oliver, 1966a), and the bulbar portions clipped from selected follicles and placed in Eagles Minimal Essential Medium (MEM) [Gibco]. The following two steps of the operative procedure were then undertaken simultaneously.

Preparation of the mesenchyme from the amputated bulbs

Using two pairs of finely sharpened watchmaker's forceps, the collagen capsule of each follicular bulb was inverted and the dermal sheath was dissected free of the capsule and the dermal papilla.

The dermal sheath was placed in MEM containing 2 mM L-glutamine where it was manipulated under low magnification in order to remove any obvious traces of adherent epidermis. At this stage, some samples were removed for histological and immunohistochemical observation. The material was then transferred to a small drop of fresh medium and physically disrupted to provided segments of a size suitable for implantation.

Preparation of the vibrissa follicles as sites of implantation

Whilst the mesenchyme sheath was being procured from the amputated bulbs, anaesthesia was maintained and the follicles were further prepared in readiness for the implant procedures. To this end, the remainder of the lower half of each follicle was removed with a transverse cut some way above the level of nerve penetration in the capsule wall (Figs l and 2). This left behind a hollow tube lined with outer root sheath within the capsule of each follicle. The prepared follicles were then continually bathed in MEM containing antibiotics and the outer root sheath was kept free from accumulating blood and other tissue debris.

Implantation of isolated mesenchyme sheath

Pieces of cleaned, isolated mesenchyme sheath were lifted from the MEM and placed in juxtaposition to the outer root sheath, attempting to insert at least some of them inside the cut end

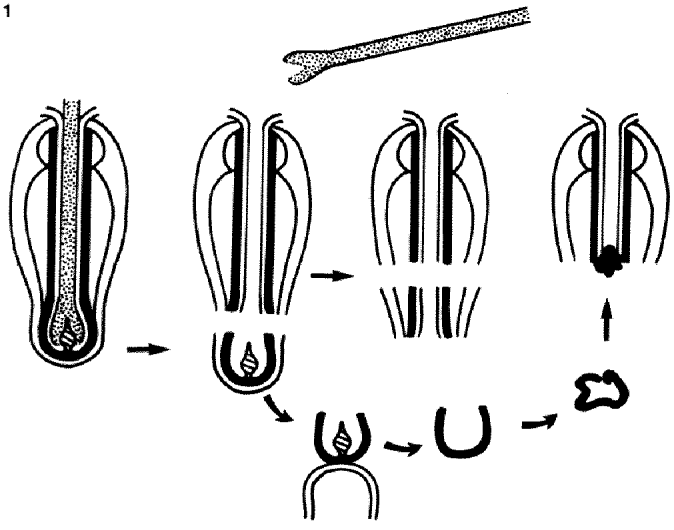


Fig. 1. Diagram of the main operational procedure. The mesenchymal sheath (shown as a solid black line somewhat exaggerated in thickness) is dissected from the follicle base and implanted into the transected follicle in contact with the outer root sheath layer of the epidermis.

Fig. 2. Low-power view of normal histological appearance of rat vibrissa follicle in the early stages of anagen, containing the root of the club vibrissa (cr), which was formed during the previous anagen phase, and the new growing shaft (g), which will eventually replace it. Within the base of the follicle is the hair bulb comprising the epidermal matrix (em) and the dermal papilla (dp). The dermal papilla is contiguous via the basal stalk (bs) with the mesenchyme sheath (ms). The line drawn at A represents the level below which transverse section of the follicle permits spontaneous regeneration of a new bulb, and above which regeneration is not possible; the line drawn at B represents the level of amputation utilised for the present experiment, therefore excluding completely the risk of follicles spontaneously regenerating new bulbs. Large arrow, shows the level at which the nerve penetrated; rs, ring sinus; cs, cavernous sinus. Alcian blue; Weigert's haematoxylin and Curtis' Ponceau S.

Fig. 3. Base of a control follicle biopsied 36 days following transection below the level indicated by A in Fig. 2. It has spontaneously regenerated a new bulb, which is typically narrow with a slender dermal papilla (dp), and has formed inside the collagenous capsule, above the level of the cut inflicted at operation (arrow). The opening in the base of the follicle is covered over with scar tissue (s). Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×144.

Fig. 4. The follicular epithelium (fe) persists as a solid peg extending to the transected end of a control follicle biopsied at 10 months. Subsequent to removal of the lower half of the follicle proximally, at the level of amputation indicated by B in Fig. 2, it has been unable to regenerate a new bulb, and has therefore remained incapable of hair fibre production. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×230.

Fig. 5. Follicle biopsied 10 months after removal of the proximal half (level B in Fig. 2) and subsequent implantation of mesenchyme sheath. A pigmented bulb is visible projecting extrafollicularly at the base - the cut edge of the collagen capsule is discernable as a faint line above the bulb. An actively growing (anagen) whisker is present (g) together with the previous, postimplantation club whisker (c). The ring sinus is visible inside the capsule as a dark band. ×12.

(Fig.1). In five experimental rats between two and five follicles received implants.

Control follicles

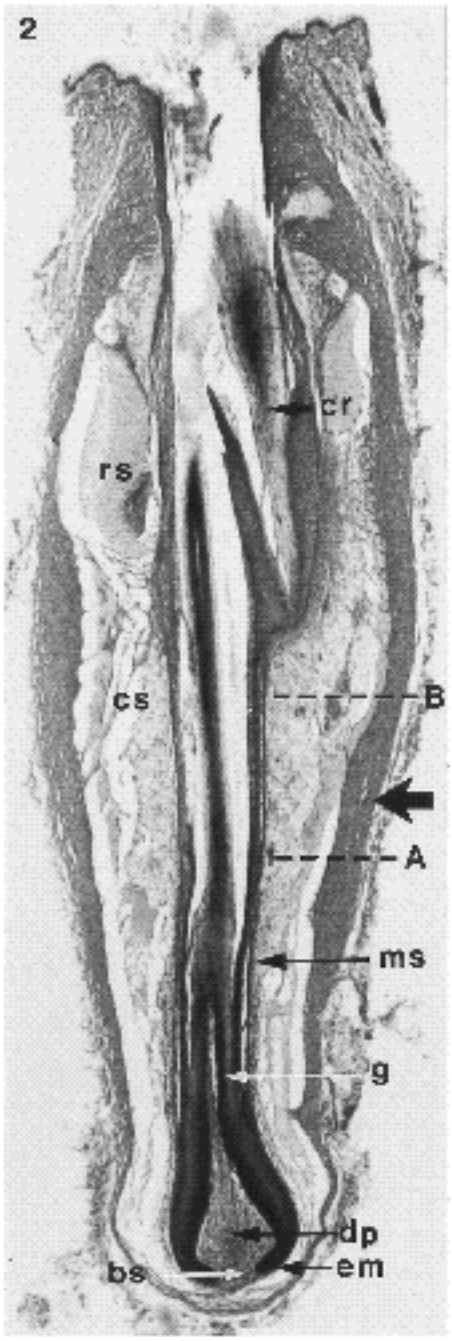
In addition to the experimental follicles thus described, two types of control follicles were prepared. Regeneration controls comprised four follicles from which only the bulbar portions were amputated prior to removal of the vibrissa shafts. In five non-regeneration controls, the rest of the lower half of the follicle was removed subsequent to amputation of the bulb and the fibres were then plucked as described above. The wounded ends of the controls were then manipulated in the same manner as those of the follicles destined to receive implants in order to prepare them with similarly open-ended tubes of epidermis; however, both control types remained non-implanted and received no further treatment.

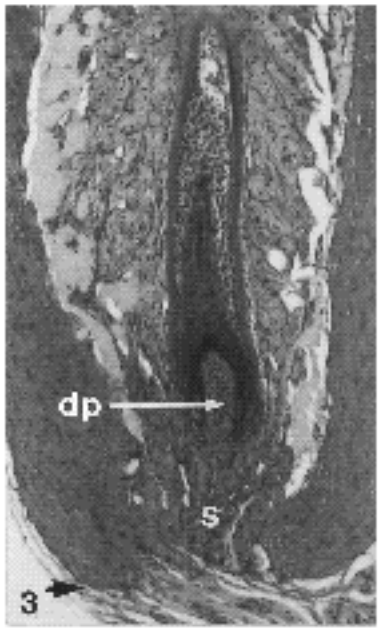
All incisions were closed by stitching with three or four, 5-0 coated vicryl sutures (Ethicon). At no time after the operations did the animals have difficulty in drinking or feeding.

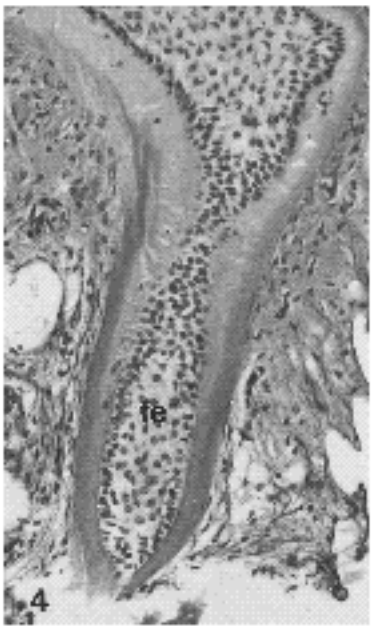
Postoperative techniques

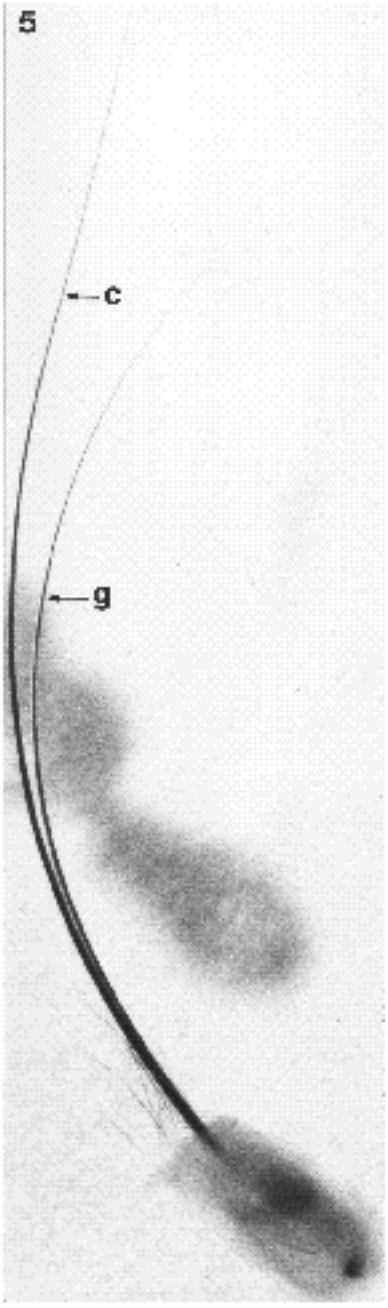
From 4 weeks postoperatively, the selected follicle sites were observed for evidence of fibre production. Two animals were killed and biopsied 36 days postoperatively, two were left for a long-term duration of 10 months, and one died at 9 months. All follicles were biopsied and examined macroscopically, and all except those from the animal that died were further processed and examined histologically.

Specimens were fixed in Mirsky's solution (National Diagnostics), embedded in paraffin wax and serially sectioned at 8 µm parallel to the longitudinal axis. Sections were stained in a com-









bination of Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S.

Sections were photographed with a Zeiss ICM 405 inverted microscope equipped with epi-illumination for fluorescence observations, using Kodak Panatomic X or Tungsten 160 colour transparency film.

Results

Control follicles

The results of the experiment are summarised in Table l, from which it can be seen that both types of control follicle behaved as anticipated, and in accordance with previous findings (Oliver, 1966a).

All four of the follicles that only had bulbs amputated were found to have undergone spontaneous regeneration. Two of these four were observed to have grown a fibre measuring 18 mm and 15 mm when biopsied at 10 months and 36 days respectively, and single pigmented anagen bulbs were seen inside their bases just above the level of the cut (Fig. 3). No fibres emerged from the other two follicles, which were biopsied 36 days postoperatively. However, histology of these confirmed macroscopic observation that each had a single, pigmented bulb. The fibre from one of these had grown to the level of the skin surface, but was too fine to have been detectable externally, the other was discovered histologically to have made a whisker that encysted distally, and was coiled around within and below the hair canal.

All of the bulbs formed by spontaneous regeneration appeared smaller than those from untouched follicles in an equivalent position on the other side of the face, and each regenerated bulb had reformed itself within the confines of the glassy membrane and was located above the cut end of the capsule.

None of the five control follicles, which were transected at the level utilised for implantation but did not receive a graft, had gone on to reorganise its tissues at the cut end into a new bulb. All follicles examined histologically confirmed the level of the original cut as being at the halfway mark, or just above it. Internally, the epidermal component remained as a relatively disorganised solid cord of outer root sheath, which extended downwards to the original level of amputation (Fig. 4), but not below it. The bases of these follicles often became sealed over by the deposition of scar tissue.

Table 1. *Table of the result of dermal sheath implantation into inactive vibrissa follicles*

Animal number	Biopsy age	Hair growth following dermal sheath implants	Hair growth in control follicles	
			Non-implanted	Regeneration
1	9 months*	0/2	0/2	–
2	10 months*	2/5	0/2	1/1
3	10 months*	2/4	0/1	–
4	36 days†	5/5	–	2/2
5	36 days†	4/5	–	1/1

*Long term.
†Short term.

Dermal sheath implants - induced follicles

Of the twenty one follicles that received implantation of freshly isolated dermal sheath in their upper halves, new bulbar complexes with resultant resumption of hair fibre production were induced in thirteen (Fig. 5). This represents an overall success rate of 62%. However, there was an improvement in operational technique during the course of the study. The numbers of new bulbs formed in the first two animals were low (two out of a possible seven), while the numbers from the last two were by contrast very high (nine out of possible ten).

Macroscopically, it was possible to determine a region of intense pigmentation in the newly induced bulbar organisations, which now protruded from the cut lower ends of eleven of the follicles. Histological examination confirmed the formation of thirteen new bulbs containing both an Alcian blue-stained dermal papilla and an active fibre-producing epidermal matrix. These were very large and pigmented, and were clearly located underneath the lower

Fig. 6. Low-power micrograph of upper half of an experimental follicle showing new bulbar formation and subsequent hair growth following implantation of mesenchyme sheath into its base. The induced bulb is very large and extends below the cut edge of the capsule (arrow). At the time of biopsy it was growing a stout vibrissa shaft (v) whose width can be attributed to the size of the keratogenous zone produced by the bulky epidermal matrix (em). dp, dermal papilla; rs, ring sinus. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×50.

Fig. 7. Low-power micrograph of experimental follicle similar to that shown in Fig. 6, but in which the new bulbar elements have been duplicated: the end-bulb comprises two slender dermal papillae, each completely enclosed by an epidermal matrix. When biopsied, this follicle was growing two narrow vibrissa shafts. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×50.

Fig. 8. The single large bulb of Fig. 5 extending below the cut rim of the follicular capsule (arrow) into the pocket of scar tissue which covers the opening (s). The phenomenon of epithelial elongation, which accompanies bulbar induction results in the new bulb becoming established outside the cut edge (arrowhead) of the glassy membrane (gm). This is the opposite to the events that accompany bulbar regeneration, where the epithelium becomes foreshortened and the resultant bulb becomes completely invested by the glassy membrane (compare with Fig. 3). Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×144.

Fig. 9. The induced bulb of a 36-day follicle lying within the subjacent connective tissue. In this specimen, the bulb has become deflected so that the basal stalk faces upwards towards the base of the capsule (arrow) instead of in its usual downwards attitude. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×230.

Fig. 10. Hair growth cycling in a follicle biopsied 10 months after implantation of mesenchyme sheath. The bulb is actively producing a growing vibrissa shaft (g) alongside the root of the club vibrissa (c) generated by the previous anagen phase of the hair growth cycle. The club root remains embedded at the level of the ring sinus (rs), reflecting the normal pattern of events in undisturbed vibrissa follicles. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×144.

Fig. 11. Details of the follicle in Fig. 7 showing the duplication of the induced bulbar elements with two dermal papillae (p), two epidermal matrices, and two vibrissae (v). As with the other induced specimens the bulbar components extend below the cut end (arrowhead) of the glassy membrane (gm). ×144.

extent of the severed capsular margins. These capsule edges represented the level of mid-follicular amputation (Figs 6 and 7). The extent of elongation of the follicular epithelium was further emphasised in all of these follicles by reference to their glassy membranes because the induced bulbs extended well below the glassy membrane's transected border, which persisted as a clearly defined, abrupt discontinuation in its thickness (Figs 8 and 11). In regenerated control follicles, new bulbs occurred inside the preexisting glassy membrane above the level of the cut.

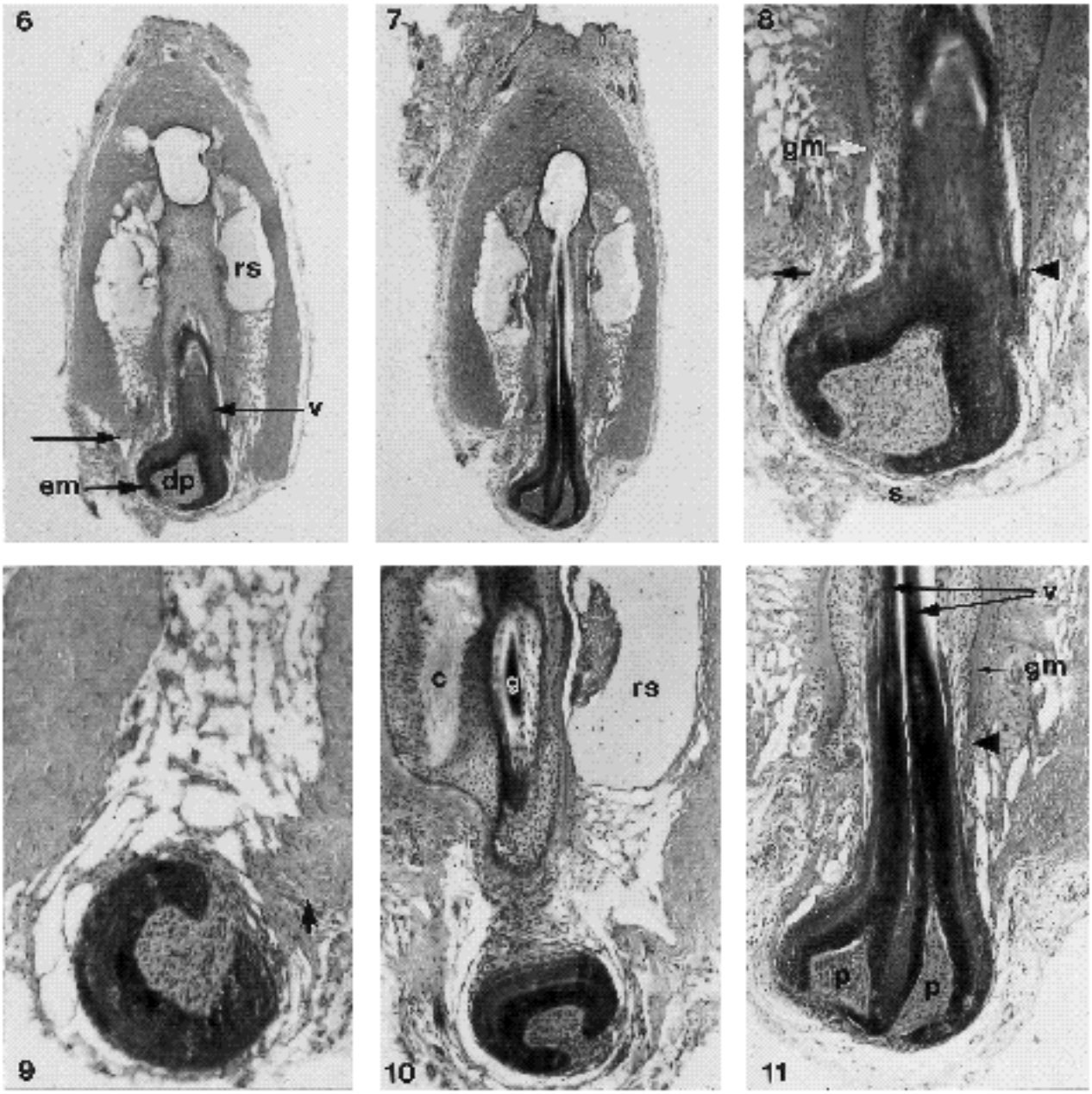
There was also found to be a good correlation between the size of the induced bulb and that of the resultant vibrissa shaft, so that the very wide epidermal matrices produced in this experiment gave rise to correspondingly large-diameter hair fibres.

Of the eleven specimens that exhibited follicular elongation, five possessed a large single hair bulb growing a whisker (Fig. 8). No hair shafts other than the anagen fibre were present. One of these specimens had been recovered at 10 months and the other four at 36 days postoperatively. A further two 36-day-old follicles also had single anagen bulbs and growing fibres; however, their bulbs had somehow become deflected so that their basal stalks now pointed either laterally or even upwards (Fig. 9).

Two more specimens, biopsied at 10 months from two different animals, each possessed a large single bulb, but these had also retained a club fibre in addition to the growing fibre (Fig. 10).

A further two follicles, both biopsied at 36 days, each had a large bulb comprising two separate epidermal matrices, with each containing its own dermal papilla (Figs 7 and 11). These duplicate structures were each producing a growing fibre so that both follicles had two anagen hairs.

In one of the two specimens in which there was no



macroscopic evidence of follicular epithelial elongation, the follicle was discovered histologically to contain a telogen-like bulb (Fig. 12). Only a club fibre root was present, which was situated high up within the follicle at the level of the ring sinus (Fig. 13) and extended 14 mm above the skin surface at the biopsy time of 10 months. A downward-extending 'tube' in the scar tissue in the follicle base contained a deposit of cells that had apparently come from the dermal papilla (Fig. 12).

The second of these two follicles was abnormal. Histologically, it was discovered that at 36 days a bulbar formation had been induced, but by the time of fixation this was no longer a functioning unit. The bulb had apparently become completely dislodged from the base of the follicular epithelium and, furthermore, it had also lost its dermal papilla (Fig. 14). Intrafollicularly there was no inner root sheath. The bulb had initially grown a hair ectopically before becoming inactive, so that the resultant whisker fibre was found coiled around outside the base of the follicle. An intriguing feature was the presence of a large ball of dermal cells outside the base of the follicle: this was located immediately adjacent to the atrophic epidermal component of the bulb. The two separate entities - the dermal cells and the degenerated epidermal matrix - were almost completely enclosed by a collagenous structure similar in organisation to the follicular capsule.

Dermal sheath implants - non-induced follicles

Eight of the 21 follicles implanted with pellets of freshly excised dermal sheath, which did not produce emergent fibres, also showed no macroscopic or histological evidence of new bulb formation. The intrafollicular components were discovered to be similar in appearance to those described for the non-implanted control follicles; the follicular epithelium persisting as a cord that extended to the scar tissue sealing off the bottom of the collagenous capsule. Follicles were devoid of any dermal papilla or epidermal matrix formation. In one of these specimens, biopsied at 10 months, the grafted pellet of dermal sheath was seen to have become dislodged and was lying outside the base of the follicle

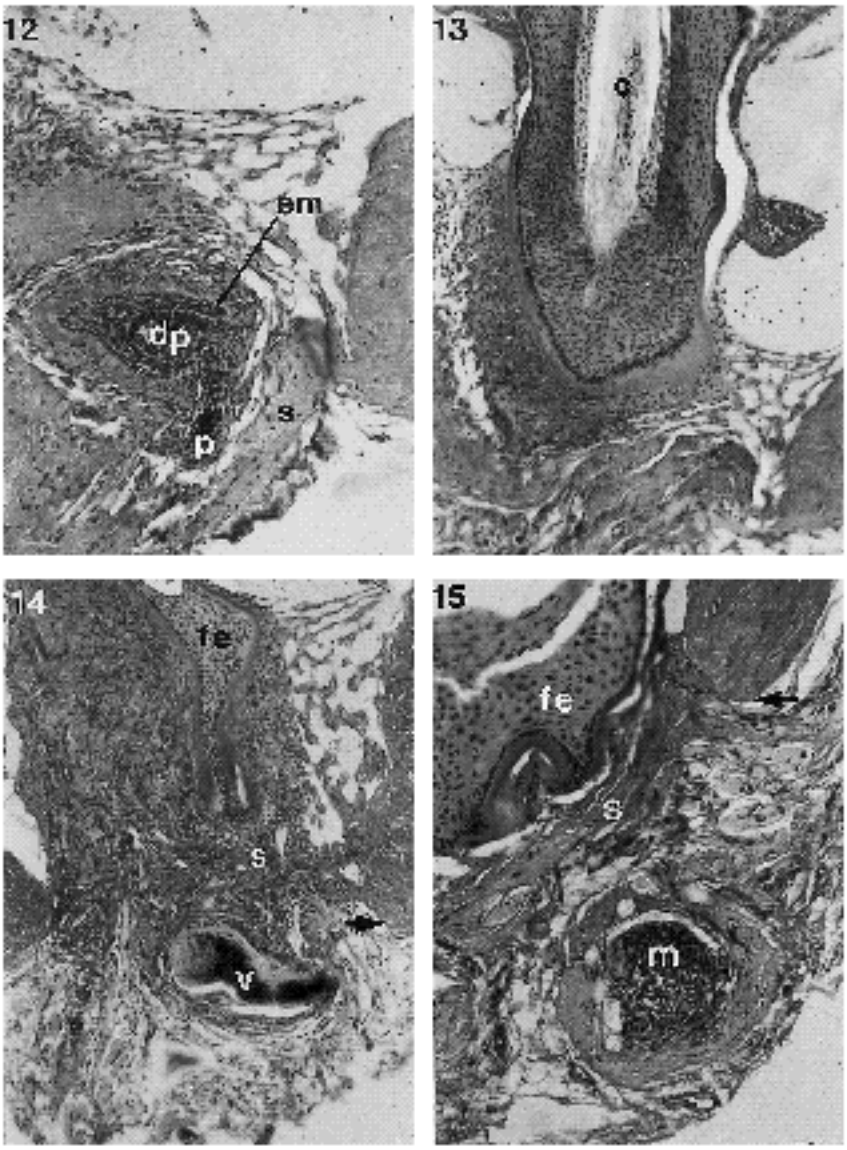


Fig. 12. Follicle which had entered the inactive phase (telogen) of the hair growth cycle. There was a diminished, non-productive epidermal matrix (em) around a compounded dermal papilla (dp) at the level of the cut edge of the capsule. A trail of papilla-like cells (p) extending downwards into a tube of scar tissue (s) suggests that during its active (anagen) phase the bulb originally extended outside the follicle cavity. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×230.

Fig. 13. Different sectional plane through the specimen depicted in Fig. 11, showing the club vibrissa root (c) that developed in the earlier stages of the catagen phase. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×144.

Fig. 14. Following implantation of mesenchyme sheath and the apparent initiation of normal hair growth, the bulbar complex has been ejected from the base of this follicle, biopsied at 36 days. The epidermal matrix has produced a tortuous vibrissa fibre (v) underneath the follicle before becoming atrophic. Internally the follicular epithelium (fe) has reverted to the appearance of control follicles, and terminates abruptly at the level of scar tissue (s) across the cut end of the capsule (arrow). Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×144.

Fig. 15. Pellet of mesenchyme cells (m) assuming the approximate size and shape of a dermal papilla after having become dislodged from the base of the follicular epithelium (fe) immediately postoperatively. The implant now lies underneath the scar tissue (s) across the cut end of the capsule (arrow), and has become enclosed by a structure that resembles the collagenous capsule of the follicle. Alcian blue, Weigert's haematoxylin and Curtis' Ponceau S. ×230.

where it was enclosed within a small capsule of collagen (Fig. 15). The implant had become organised into a dermal papilla-like ball of cells, which stained with Alcian blue.

Histology/immunohistochemistry of implanted material

Histological and immunohistochemical examination of the randomly selected, implantable pellets of dermal sheath revealed the presence of some cells from the follicular epidermis. In most cases, outer root sheath cells were observed adhering to the adluminal surface of the glassy membrane on the outside of which were attached the dermal sheath cells (data not shown). Thus, it was clear that epidermal cells were transplanted along with the dermal sheath into the bases of the prepared follicles.

Discussion

We have shown that follicle interactions following implantation of lower mesenchyme sheath containing fragments resulted in the formation of completely new dermal papilla-epidermal matrix complexes, and subsequent restoration of hair growth. An associated phenomenon was elongation of the follicular epithelium, resulting in the establishment of the new bulbs underneath the level at which the follicles had originally been transected. These findings compare favourably with those of Oliver (1967b) who described a similar response of the vibrissa follicle epithelium to implanted whole dermal papillae. The two control procedures supported previous experimental evidence showing the limits of spontaneous regeneration after surgery. (Oliver, 1966a,b; Jahoda et al., 1992a). Therefore, in terms of the size and locations of the new bulbs, it may be asserted that the tissue implanted in the present experiment was responsible for follicle reorganization and renewed hair growth.

The overall success rate did not reflect the clear improvement in operational technique. Only four of the first eleven implantations were successful, compared with nine of the last ten. In the eight follicles where induction was not brought about by the implanted tissue, the likely reason was that the graft did not remain in contact with the epithelium postoperatively, as was observed in at least one of the recovered specimens.

That pellets of freshly procured lower follicle mesenchymal sheath were able to stimulate new follicle activity is consistent with evidence that cells of the lower dermal sheath are responsible for replacing the dermal papilla in spontaneous regeneration (Oliver, 1966b,1967a; Ibrahim and Wright, 1982; Kobayashi and Nakimura,1989; Jahoda et al., 1992a). In this context, it has recently been shown that both papilla and sheath cells contain α-smooth muscle actin as a common cytoskeletal marker in vitro (Jahoda et al., 1991).

The massive size of some of the bulbs induced in this experiment demonstrates the enormous potential of the lower dermal sheath cells to become a large dermal papilla. Clearly then, this ability is not limited positionally to the base of the follicle, and lower sheath cells can also act in the context of the upper follicle environment. Furthermore, the large size of the new bulbs had some relationship to the amount of material implanted, rather than the level of cut. Nevertheless, the final structures were comparable to those seen in previous implantations of isolated whole dermal papillae (Oliver, l967b), and had no wound elements in the form of scar tissue intrafollicularly. The observation that one implant that had not maintained contact with the follicular epidermis had established a papilla-like core of cells surrounded by a collagenous capsule is an intriguing one. It suggests that adult sheath cells may have multiple capabilities selected for, and modulated by, cellular and environmental influences - they can become papilla cells, synthesize collagen capsule and perhaps be a reservoir of wound myofibroblasts (Jahoda et al., 1991). Therefore, while there is much hair growth-related study of dermal papilla cells, dermal sheath cells may prove equally interesting in the context of fibroblast lineage and differentiation.

As we were unable to isolate dermal sheath cells by dissection or enzymatic methods, it is possible that the new epidermal matrix of activated follicles was derived at least in part from epidermal material associated with the implants. Under these circumstances, the crucial question as to the nature and direction of the dermal-epidermal interactions that restored hair-producing bulbs cannot be clearly established. However, when dermal sheath containing preparations identical to those utilised in the current study were implanted ectopically into rat ear skin, they did not produce new hair follicles (Jahoda, unpublished data).

So far the only method of obtaining a vibrissa dermal sheath cell population has been by explant culture (Horne, 1987; Jahoda et al., 1992b). It has been found that some cell outgrowths from upper follicle mesenchyme are morphologically similar to non-follicular skin fibroblasts (Horne, 1987), but this is not always the case and depends on the exact site of origin of the tissue (Reynolds 1989). Cultures of mesenchyme sheath obtained from the follicular end-bulb show the aggregative behaviour of cultured dermal papilla cells (Horne, 1987; Jahoda et al., 1992b) but not their inductive capabilities (Horne et al., 1986). Therefore, in the present work, the contaminating lower follicular epidermal cells may act to induce mesenchyme sheath to switch over to dermal papilla status (Reynolds, 1989; Reynolds and Jahoda, 1991), and may therefore be necessary for the formation of a new bulb. An alternative explanation, however, is that in cell culture the mesenchyme sheath loses its ability to interact with epidermal cells much more readily than does the dermal papilla.

The second view point is more favoured by one of us and is in accord with a possible explanation for the behaviour of the mesenchyme sheath in spontaneous regeneration (Horne, 1987). It was suggested that due to the pattern of ontogenetic development of the follicles, a gradient of embryonic potential might exist along the length of the mesenchymal sheath. The earliest developmental stages of the hair follicle are represented by a distinctive condensation or aggregation of mesenchymal cells in association with a thickening of the overlying epithelial cells (Davidson and Hardy, 1952; Wessells and Roessner, 1965; Van Exan and Hardy, 1980). The epithelial thickening becomes a downgrowth (the hair peg) with the original mesenchymal condensation advancing in intimate association with the cells

in its base. As the ball of mesenchymal cells descends, eventually to assume the role of the dermal papilla towards the end of follicular development, those cells that will constitute the dermal sheath are deposited along the length of the downgrowing hair peg. In the proposed scenario, the cells of the perifollicular mesenchyme sheath would possess the potential for interaction with the epithelial cells as do the cells of the dermal papilla; this potential would be retained more strongly by the mesenchyme immediately adjacent to the dermal papilla, but would become weaker with increasing distance up to the length of the follicle. This effect would be enhanced if the cells of the upper follicle mesenchyme sheath either differentiated and therefore lost their original capabilities, or were contributed to by cells of the surrounding, non-follicular dermis, or both. The idea of a control system that relates to regeneration phenomena and competence, and that acts along the longitudinal axis of the follicle, was originally proposed by Oliver (1966a,b). Indeed, because regenerative potential is lost above the critical lower third of the follicle, the effect may involve a threshold as well as a gradient. With the repeated changes that occur during the 'hair growth' cycle, the adult follicle is an excellent model for studying tissue interactions, differentiation and aspects of morphogenesis, but it also represents an extremely complex system. Growth factors and their receptors (Jones et al., 1991; Moore et al., 1991; Peters et al., 1992), retinoic acid receptors (Dollé et al., 1990), unique extracellular matrix composition (Couchman et al., 1991; Messenger et al., 1991) and Hox gene expresion (Bierbech et al., 1991) are among the molecular entities described within the lower follicle. Therefore gradients of molecular expression, seen for example in embryonic appendage patterning with homeoproteins (Chuong et al., 1990), may persist in individual follicles, perhaps along the longitudinal axis. However, the roles of specific molecular entities will only be understood through critical examination of their spatial distribution through the cycle, and a better knowledge of cellular relationships within follicle structures.

In summary, it has been demonstrated previously that the mesenchyme sheath in the lower third of the follicle replaces the dermal papilla in spontaneously regenerating follicles. We now show that if material containing lower follicle dermal sheath cells is introduced artificially into the upper half of the follicle, these cells will similarly assume the morphology and functional capabilities of the dermal papilla, and are crucial for the reinstigation of hair growth.

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