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Hair matrix germinative epidermal cells confer follicle-inducing capabilities on dermal sheath and high passage papilla cells

Amanda J. Reynolds* and Colin A. B. Jahoda

Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK

*Author for correspondence.

SUMMARY

Low passage cultured dermal papilla cells from adult rats stimulate complete hair follicle neogenesis when re-implanted into heterotypic skin. In contrast, cultured sheath cells are non-inductive despite sharing other behavioural characteristics (a common lineage and in situ proximity) with papilla cells. However, since sheath cells can behave inductively in amputated follicles after regenerating the papilla, this poses the question of what influences the sheath to papilla cell transition? During reciprocal tissue interactions specific epidermal cues are crucial to skin appendage development, and while in vivo assays to date have focussed on dermal interactive influence, our aim was to investigate epidermal potential. We have previously observed that hair follicle epidermal cells display exceptional interactive behaviour when combined with follicle dermal cells in vitro. Thus in the present study, hair follicle germinative, outer root sheath or skin basal epidermal cells were separately combined with each of three non-inductive dermal cell types (high passage papilla, low passage sheath or fibroblast) and then implanted into small ear skin wounds. The sheath/germinative and papilla/germinative cell implants repeatedly induced giant vibrissa-type follicles and fibres. In complete contrast, any single cell type and all other forms of recombination were consistently non-inductive. Hence, the adult germinative epidermal cells enable non-inductive adult dermal cells to stimulate hair follicle neogenesis, effectively, by altering their 'status', causing the sheath cells to 'specialise' and the 'aged' papilla cells to 'rejuvenate'.

Key words: rat vibrissa follicle, dermal-epidermal interaction, appendage neogenesis

INTRODUCTION

The hair follicle is becoming increasingly valued as a developmental model system, as it reveals great molecular complexity in relation to the many fundamental biological processes it embodies (Hardy, 1992; Van Genderen et al., 1994; Herbert et al., 1994; Noveen et al., 1995; Thesleff et al., 1995). In the most deeply embedded bulbar portion of all follicle types, each hair fibre forms from a few very primitive-looking germinative epidermal (GE) cells via an epidermal matrix of predominantly semi-differentiated cells. The matrix is nourished and supported, and epidermal proliferation and differentiation positively stimulated, by a vitally important central plug of tissue, the dermal papilla (DP). Continuous with the basal stalk of the DP, and peripheral to the external surface of the matrix, is the hair follicle dermal sheath (DS). The DS runs along the whole length of the follicle and is separated externally from the epidermal outer root sheath (ORS) component by a specialised basement membrane (the glassy membrane). At the neck of the follicle the outer root sheath appears contiguous with the epidermis of the superficial skin. Although an oversimplification, one could consider there to be two major epidermal (ORS and GE) and two major dermal (DS and DP) cell types which largely comprise the hair follicle.

Microsurgical experiments, originally performed on adult rat whisker follicles in situ, have revealed the intriguing capacity of these adult appendages to restore themselves to a productive, functional mode (Oliver, 1966a,b, 1967a; Ibraham and Wright, 1977; Jahoda et al., 1984; Horne et al., 1986; Oliver and Jahoda, 1988; Reynolds and Jahoda, 1991a). Removal of the DP (or even the whole end bulb region) instigates remodelling activities in the residual follicle cell populations (most notably the DS and ORS cells) leading to the regeneration of a new DP from the lower DS cells, and ultimately, to the restoration of fibre production (Oliver, 1966a). The same author also established that a follicle will not regenerate if more than the lower third is amputated, unless it is re-implanted with an isolated DP (Oliver, 1967b). Subcultured DP cells were later shown to retain this capacity to re-stimulate fibre production within upper follicle portions via their inductive interaction with the residual ORS cells (Jahoda et al., 1984; Horne et al., 1986). More recently still, it has been shown that cultured DP cells can induce type-specific (vibrissa or pelage) follicle and fibre neogenesis, in a variety of skin sites in vivo (Oliver and Jahoda, 1989; Reynolds, 1989; Reynolds and Jahoda, 1991a, 1992; Jahoda et al., 1993). In all of these investigations it has been independently found that, after their third or fourth passage, papilla cells become non-inductive. Freshly isolated pieces of end bulb DS tissue can also re-stimulate fibre production within inactivated portions of vibrissa follicle (Horne and Jahoda, 1992). However, cultured DS cells have consistently failed to show any inductive capa-

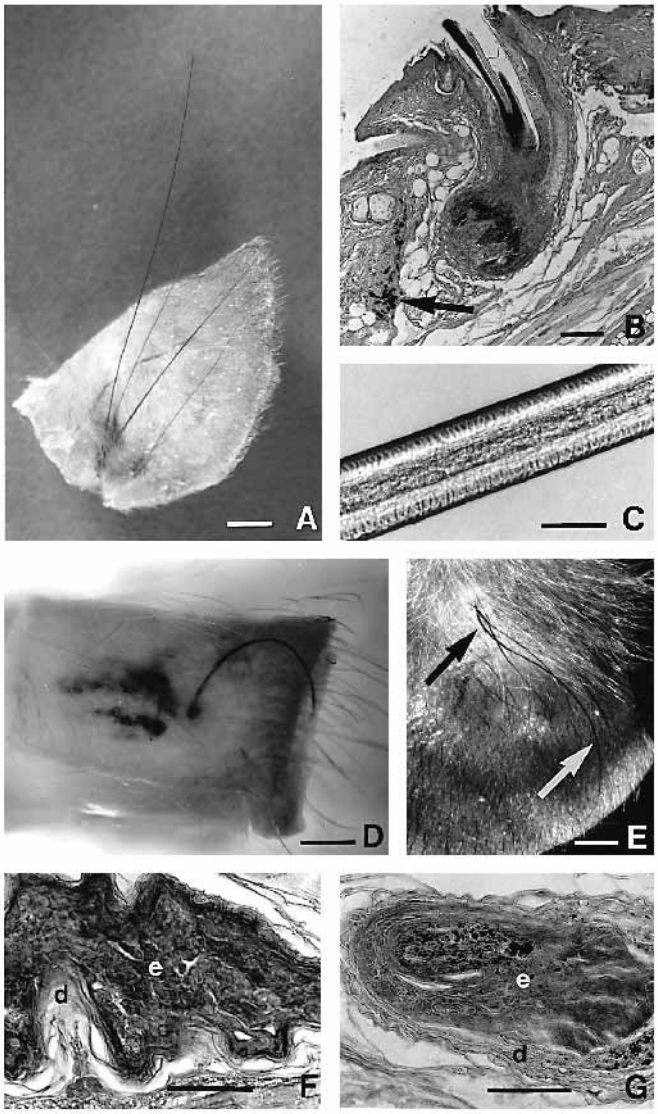

Fig. 1. Rat ears that had received implants of recombined follicle dermal and epidermal cell cultures, six weeks earlier. (A) Four very large, long and straight vibrissa-type fibres induced in a wound site to the rear of a rat ear into which DS and GE cells had been introduced. Close external and subsequent histological examination revealed that about half of the newly formed follicles contained multi-lobed papillae which supported two or three hairs within a single follicle (B). The majority of the induced fibres were pigmented with vibrissa-typical open medullae (C). Induced appendages and fibres were generally quite distinguishable from local ones, while those resulting from HP DP and GE implants were usually smaller than those stimulated by DS and GE cells (arrows, E). Loose accumulations of pigment often occurred around the wound sites, visible both externally (A), and following histological sectioning (arrow, B). A single implantation involving each type of follicle dermis resulted in particularly intense and well-defined patches of pigment, with a stout curved whisker also being produced by the HP DP/GE cells (D). Histologically, the highly pigmented regions in the HP DP/GE (F), and DS/GE (G), implanted ears, consisted of cyst-like formations of semi-differentiated epidermis (e) enclosed by sheaths of dermis (d), both of which resembled follicular tissues. Bars: (A,D) 2 mm; (B,F,G) 400 μm; (C,E) 100 mm.

bilities, in either the amputated follicle, or ear skin wound, bioassay (Horne et al., 1986; Horne, 1987; Reynolds, 1989; Horne and Jahoda, 1992).

Alternative approaches to stimulating hair follicle formation in vivo have included grafting: mixed populations of fetal cells (Yuspa et al., 1970); embryonic skin containing dermal papillae (Ferraris et al., 1994), or newborn skin hair follicle pre-germs (‘buds’) recombined with various dermal cell types (Weinberg et al., 1993; Lichti et al., 1993, 1995; Kartasova et

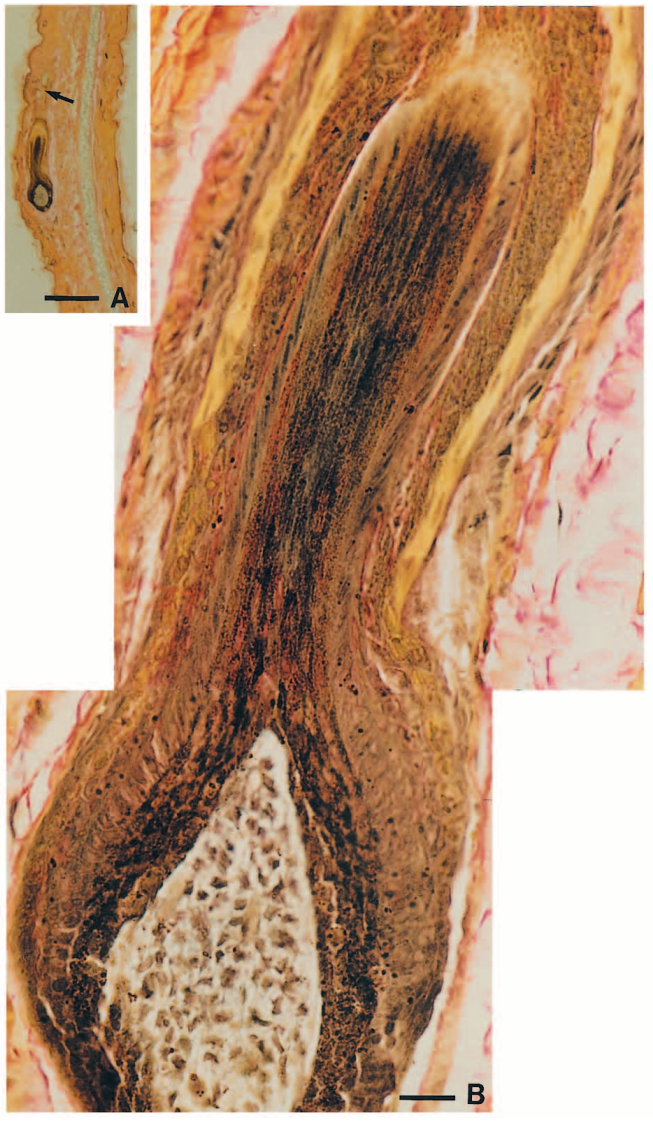

Fig. 2. Histological sections taken through one of the giant fibre-producing follicles induced by DS and GE cell implantation (A,B). The relative size of the newly formed appendage is illustrated in A, where it can be seen to occupy about a third of the whole ear’s depth. The induced follicle lies parallel to the skin surface, rather than following the orientation of the local hair follicles, and has developed with a sebaceous gland in an appropriate position at the neck (arrow, A). At higher magnification, a different section through the same follicle reveals an extracellular matrix-rich pear-shaped papilla, pigmented hair matrix and fibre, all comprised of well-ordered cells and tissues with characteristic morphologies (B). Bars: (A) 2 mm; (B) 125 mm.

al., 1995). Similarly, Takeda et al. (1995) used enzymatically dissociated mystacial skin from late gestation embryonic rats to produce mixed cell aggregates, and Watson et al. (1994) implanted sheep vibrissa papillae into reconstructed 'skin', both producing hair follicle-type structures when transplanted in vivo. However, all of these manipulations incorporate significant differences from the present study in relation to the type and developmental stage of material that they use.

In the adult tissue recombinations highlighted above, nearly all of the emphasis has been on the interactive/inductive capabilities of the dermis (or mesenchyme). The epidermis (or epithelium) in contrast, has largely been evaluated in terms of its responsiveness, after receiving some form of dermal message. Yet, in the context of integumental appendage embryology, the reciprocal nature of dermal-epidermal interaction has been firmly established, with instructional cues being both provided, and responded to, by each of the tissue-types involved (reviewed by Dhouailly, 1977; Kollar, 1983; Sengel, 1986). In this context, while the small group of follicle GE progenitor cells have long been studied as the immediate source of the hair fibre, it is only very recently that their interactive capabilities have been investigated in culture. Rat vibrissa and human hair GE cells participate in various levels of interaction with DP and DS cells in vitro, where both dermal and epidermal cell types can alter the other's behaviour. Moreover, during such cell recombinations, complex organotypic arrangements are formed which include structured basement membrane at the dermal-epidermal interface (Reynolds, 1989; Reynolds and Jahoda, 1991b, 1994b; Reynolds et al., 1993). Consequently, the main aim of this study was to investigate whether defined populations of epidermal cells could alter the behaviour of non-inductive dermal cell types, when implanted into ear skin wound bioassays. Recombinations of germinative epidermal cells and low passage sheath, or high passage papilla, dermal cells both consistently stimulated the morphogenesis of very large vibrissa-type follicles and fibres. No cell type implanted alone elicited any degree of inductive behaviour, and neither did any other form of combination involving follicle- and/or skin-derived cells. Thus, despite their adult status and prior growth

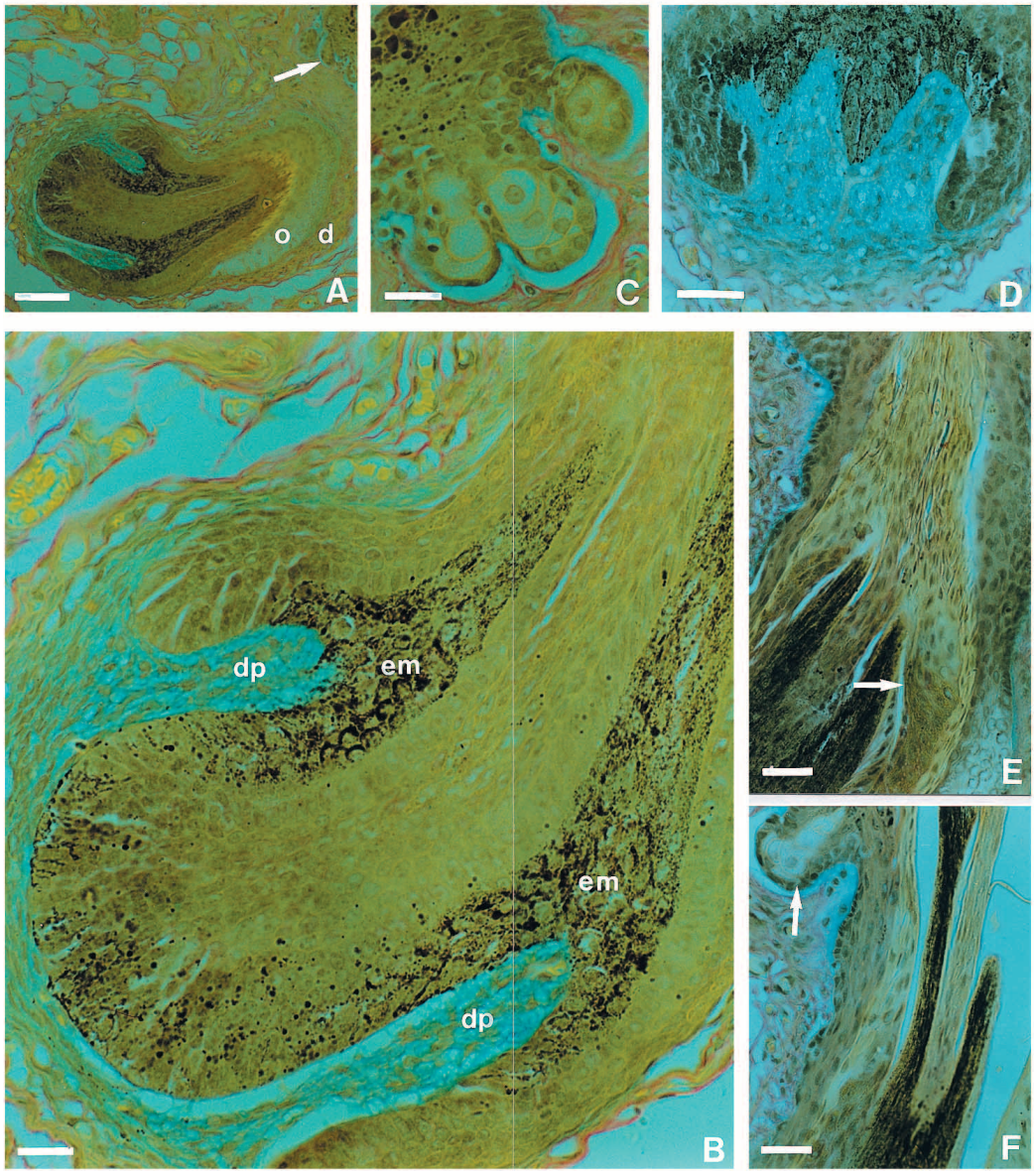

Fig. 3. Pilosebaceous units induced from DS/GE cell implants (A-F). A large, curved end bulb containing two narrow-lobed papillae joined by a thick region of sheath, so forming a sideways-on cup of Alcian blue-positive tissue (A,B). Above this (A), the asymmetry in the tissue layers is particularly evident, both in the DS (d) and epidermal outer root sheath (o). Sebaceous gland tissue can be seen at the neck of the follicle (arrow, A; and in greater detail at higher magnification, C). Both narrow DPs (dp) support their own epidermal matrix (em), each of which goes on to produce an individual hair fibre (B). (D) Another example of this form of induced appendage showing a large multi-lobed papilla, that gives rise to a multi-partitioned matrix and then a corresponding number of hair fibres (E,F), and again, having developed with a sebaceous gland (arrow, F). As in non-induced vibrissa follicles, the different cell layers of the induced appendages were clearly asymmetrically distributed, with particularly abrupt and well-defined changes occurring in the appearance of the cells at specific heights in the epidermal sheath layers (arrows, E). Bars: (A) 360 mm; (B,C) 100 mm; (D,E,F) 160 mm.

in vitro, end bulb germinative epidermal cells retain an ability to profoundly influence lower follicle dermal cells, which in turn, retain an ability to respond.

MATERIALS AND METHODS

Preparation and maintenance of cells

Dermal and epidermal cells were isolated as previously described (Reynolds and Jahoda, 1991b, 1994b). In brief, the proximal tips ($<\frac{1}{8}$ entire length) of adult PVG/C rat vibrissa follicles, exposed on the internal surface of mystacial skin flaps, were removed and placed in Eagle's minimal essential medium (MEM; Gibco) containing antibiotics, at 4°C. After inverting the outer collagen capsule and dermal sheath (DS) layers, epidermal matrices were gently eased from around the dermal papillae (DP). The tiny fragments of hair matrix germinative epidermal (GE) tissue were then delicately teased away from each and pooled. Papillae were isolated by basal stalk severance, and the remaining 'cups' of DS tissue detached from the collagen capsules. Around a dozen papillae, or pieces of sheath tissue, were pooled, triturated and then forced to adhere to the bottom of their respective 35 mm diameter Petri dishes (Nunc). Adult rat skin fibroblasts (SF), or skin basal epidermal cells (SE), were cultured by explant outgrowth from small fragments of interfollicular mystacial tissue. Outer root sheath (ORS) cells (including those from the bulge-region) were established from epidermal tissue dissected from the upper follicle portions discarded above. All of the explants were initiated in 20% fetal calf serum (FCS, Gibco) in MEM, with 1% L-glutamine (2 mM final concentration), and maintained at 37°C, 5% CO_2. After 10 days, the FCS was reduced to 10% in all dermal cultures (HP DP, DS, SF), while the ORS and SE cell monolayers were provided with MEM containing epidermal supplements, as previously described (Reynolds and Jahoda, 1994b). Medium was replenished every 4 days and cells passaged at approximately 2 week intervals, to be replated at densities of 5×10^4 cells per 35 mm dish. Observations were made using a Nikon inverted microscope (model: Diaphot-TMD).

Operational procedure

Adult PVG/C rats were lightly sedated, their ears wiped with 70% alcohol and a small incision made with the point of a number 11 scalpel blade. The pocket so-formed was slightly enlarged using fine forceps tips. Any resultant blood or plasma was dabbed away just prior to the implantation of cells. Monolayers of low passage (LP; 2/3) DS or SF cells, or high passage (HP; 10 or >) DP cells (HP DP), were co-cultivated with each of the three epidermal cell types: 5×10^4 ORS; 5×10^4 SE; or GE cells from around 50 follicles (per 35 mm dish). All of the cell recombinations were maintained for three days in culture before their implantation.

The four follicle-derived cell populations: DS; HP DP; ORS; and GE cells, were also implanted alone. However, due to the minute quantity and almost transparent appearance of freshly isolated GE tissue, tiny amounts of pigmented matrix were included with half of these implants to facilitate subsequent identification. In all instances, the medium was removed and the confluent cell monolayers, or recombinations, scraped into sticky clumps with a rubber policeman. The accumulated cells were then immediately introduced into each pre-prepared ear wound pocket on the tips of very fine forceps.

Between 1 and 4 small wound pockets were made in each rat ear. To avoid any possibility of contaminating interactive influences, each form of implantation (that is, involving different cell combinations) was always confined to separate ears. For each variation of cell types implanted, between 9 and 12 repetitions were performed.

Post-operational protocol

To facilitate re-identification of the wounds, a note was made after each operation of their size, orientation, and exact position in relation to the unique pattern of blood vessels in each ear. Weekly examinations of the post-operational ears were conducted, after lightly sedating the animals. This allowed each wound site to be closely monitored for signs of any development under the skin, or external structure formation. After 6-8 weeks the experimental animals were killed, the sites of implantation biopsied, recorded photographically and then fixed in formol saline to be processed for routine histology.

RESULTS

When the sites of cell implantation were not immediately obvious post-operatively, they were recognised macroscopically by reference to each ear's unique pattern of externally visible vasculature (as described above). Following histological processing, wound identification was facilitated by the alterations in the surrounding tissues, such as epidermal hyperplasia, changes in dermal composition and disrupted ear cartilage.

Implants involving dermal-epidermal cell recombinations

DS + GE cells

Of the 11 operations involving second passage DS and GE cell recombinations, 10 formed groups of very large vibrissa-type follicles (Fig. 1A), of which 9 contained multiple, mainly pigmented fibres and, on a single occasion, just one. At least some of the follicles were undergoing cyclical growth, since they possessed fine growing hairs in addition to sturdier club hairs. Each implantation of cells induced between 1 and 5 new follicles, the majority of which contained broad, straight fibres. Although the lengths of these newly formed hairs varied, some approached 30 mm (Fig. 1A), which is twice that of the longest previously induced by LP cultured papilla cells alone (Jahoda et al., 1993). In a few of the induced appendages, it was evident even prior to their biopsy that they were producing more than one actively growing fibre. Subsequent examination of the histological sections confirmed these observations when they revealed multi-lobed papillae supporting multiple fibres within individual follicles (Fig. 1B). The induced fibres had vibrissa-typical proportions (becoming increasingly narrow towards the tip) and open medullae (see Fig. 1C), and their average diameter was at least 20 times that of the biggest local hairs. The remaining operation did not result in any appendages being induced, but the tissues of the cyst-like structures that were stimulated to form pigmented, semi-differentiated epidermal material (e, Fig. 1G) and sheaths of dermis (d, Fig. 1G) resembled those found in hair follicles.

Corresponding with the dimensions of the induced fibres, the follicles responsible for producing them often spanned over a quarter of the depth of the ear (Fig. 2A). The majority of the larger induced follicles developed almost parallel to the ear's surface, although, more superficially they tended to curve abruptly just prior to opening externally. This arrangement may have been dictated by the physical constraints imposed on the massive appendages by the relatively thin depth of the ear skin (Fig. 2A). Many, if not all of the induced appendages, irrespective of their relative size and proportions, developed as complete pilosebaceous units. Hence, sebaceous glands (having apparently normal tissue architecture) were identified in positions around the necks of the newly formed follicles, as in Fig. 2A (arrow), for example.

The newly formed follicle papillae were frequently very large, Alcian blue-positive and either pear-shaped (Fig. 2B), or multi-lobed (Fig. 3A,B,D), sometimes taking on a thickened cup-like arrangement of tissue (Fig. 3A,B). When any single end bulb contained multiple papillae, they supported a corresponding number of epidermal matrices, which subsequently developed into the same number of fibres. The induced follicles in Fig. 3 both illustrate this point. Two main papillae can be seen within the end bulbs (Fig. 3A,B,D), each supporting a double hair matrix (Fig. 3B), which in turn gives rise to two separate fibres as it differentiates (Fig. 3E,F). Frequently, the component layers of the newly formed follicles were clearly asymmetrical, being considerably thicker on one side, as is often seen in vibrissa-type appendages (see Figs 1B and 3A). A further point of interest was the sudden morphological change that occurred (most obviously in the glassy membrane and outer and inner root sheaths) at a specific height on the thicker side of the follicle (arrowed, Fig. 3E; but also visible in Fig. 1B). The width of the glassy membrane and outer root sheath became reduced, with the cell nuclei in the latter appearing smaller, and more intensely stained than usual. While at the same corresponding height, there was also an abrupt disappearance of trichohyalin granules in the cells of the inner root sheath (Fig. 3E).

HP DP + GE cells

Large vibrissa-typical hair follicles and fibres, of a completely different magnitude to the local appendages, were induced in at least 8 of the 11 operations that involved HP DP and GE cell implants (Figs 1E and 4A,B). One of the other three operations resulted in groups of smaller induced follicles that appeared irregular in form, and another resulted in a single stout curved follicle and fibre being induced in association with a number of highly pigmented cyst-like structures (see Fig. 1D,F). Histological analysis of the remaining wound site did not reveal any obviously induced appendages, or any sign of interaction or differentiation within the tissues. Since there were no discernible DP cells or aggregates evident either, it is possible that the cellular material originally implanted may have dried out or even been washed away by wound fluid during the initial operation.

As had been observed with the DS\GE-inductions, the relatively smaller follicles were seen in a number of different orientations, while the larger ones (perhaps obligatory for their dimensions) tended to run parallel with the ear skin surface. Again consistent with the results from the interactions involving DS\GE cells, most of the appendages stimulated by HP DP\GE cell implants possessed identifiable sebaceous glands (portions of four gland lobes being visible in Fig. 4A). While vibrissa follicle tissues are naturally asymmetrical, the cup-shaped papillae and general curvature in many of the induced follicles, are both likely to have contributed towards their even more pronounced asymmetry, as was particularly evident in the hair matrix and more differentiated epidermal cell layers (Fig. 4A,B).

Intriguingly, giant papillae induced from both HP DP/GE and DS/GE implantation often appeared to have a structural morphology that mimicked the distribution of the DS tissue enclosing them externally, that is, the papillae formed sideways cup-like arrangements of tissue just inside the concavity formed by the slightly wider, but similarly shaped, DS layer (see Figs 3A,B and 4A). Despite their unusual morphology, the Alcian blue-positive (thus proteoglycan and glycosaminoglycan rich) induced papillae supported the production of stout, curved fibres (see Fig. 4A). In fact, other than variable papilla shape, the gross arrangement of the cells and tissues making-up each of the concentrically arranged layers of the follicles, seemed to be quite normal and very well ordered (Fig. 4B).

Mystacial SF+ GE cells

There was no apparent difference between the findings from this procedure and that involving either SF (data not shown), or GE cells, alone, since no obvious signs of interaction were noted with any of the three variants. There was only indirect evidence of the sites of implantation, predominantly in the form of altered tissue arrangements and small amounts of residual matrix cell pigment, to facilitate identification.

HP DP, DS or SF + ORS or SE

Close external examination of the post-operational implantation sites that had received any of the dermal cell types (HP DP, DS or SF) combined with either follicle ORS epidermal or mystacial skin SE cells, never gave any indication that induction might have occurred. Histological examination of serial tissue sections through the whole wound site (including the immediate border of skin) confirmed that none of these cell recombinations stimulated any significant degree of appendage induction. This held true for all of the repetitions conducted for each form of recombination, since there was no indication of the cells contributing to any structured tissue arrangements, or cysts. Thus, neither the hair follicle ORS (including the bulge-region), nor the skin SE cells displayed any interactive behaviour that could be considered comparable to that observed with the GE cells.

Implants involving cell populations of a single type

DS, HP DP, GE or ORS cells implanted alone

When introduced individually, none of the four follicle-derived cell types (DS, HP DP, GE or ORS) ever stimulated the formation of follicles, cysts, or any other distinctive tissue structures within the ear wounds. However, their respective post-operative implantation sites were usually quite easily detected using the pre-recorded details of their positions in relation to each ear's pattern of blood vessels, as described above. Nevertheless, even when the implantation site was conclusively located, the unpigmented implants of GE cells could not be accurately identified in histological sections, and there was no indication of interactive, or differentiative, behaviour. Sites that had received a few pigmented cells in addition, were more readily revealed by the presence of diffuse patches of residual pigment, and occasionally, tiny fragments of keratinised material (Fig. 5A,B).

While the HP DP cells were equally ineffective in terms of stimulating any notable dermal-epidermal interaction in post-operative wounds, they could be identified because they formed and maintained discrete oval aggregates, which had strong morphological resemblance to papillae in situ (Fig. 5D). Moreover, the papilla cells within these round dermal structures sometimes appeared well-spaced with abundant extracellular material surrounding them (a, Fig. 5D), as is characteristic of anagen papilla cells in vivo. On other occasions, the cells

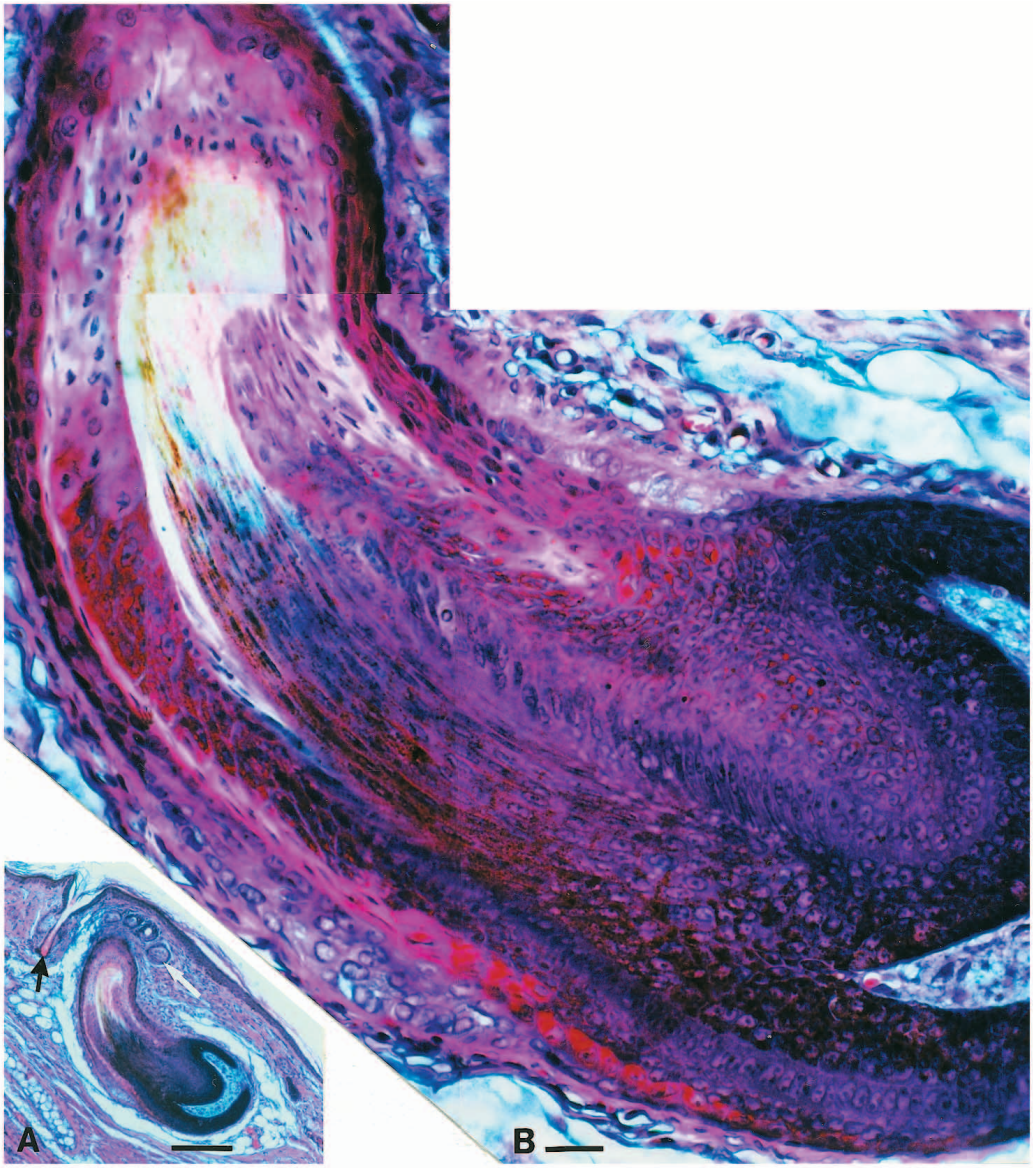

Fig. 4. Histology through an extremely large hair follicle that was stimulated by the introduction of HP DP/GE cells. In taking up almost half of the depth of the post-operative ear skin, it is clearly of a quite different dimension to the local follicle (black arrow) at the side of it (A). Once again, it is evident from the presence of appropriately positioned glandular tissue (white arrow, A), that a complete pilosebaceous unit has been induced to form. Intriguingly, variations of the cup-shaped, Alcian blue-positive DP illustrated in A, were frequently noted, as if the newly formed papilla may be mimicking the arrangement of the DS tissue just external to it. Combined with follicle curvature, the unusually shaped DP may have contributed to the particularly pronounced tissue asymmetry observed in the induced appendages (B). Bars: (A) 720 mm; (B) 125 mm.

looked as if they were very compact and tightly packed together (t, Fig. 5D), as is typical of cells in telogen papillae in vivo. In other words, the papilla cell aggregates appeared to be in different stages of the hair cycle.

The DS cells in contrast, could not be conclusively identified within histological sections that were known to include their precise site of implantation. They seemed to have dispersed through, and perhaps merged with, the ear dermis. Similarly, the implanted ORS cells did not maintain themselves within the skin in any manner that could be recognised, presumably, they were either destroyed or contributed to the surface epidermis. Intriguingly, however, hyperplastic mounds of irregularly enlarged cartilage were frequently observed at the sites where DS cells had been introduced. This newly formed cartilage stained much more intensely with Alcian blue than the undisturbed tissue to either side, probably reflective of sulphated glycosaminoglycans and proteoglycan biosynthesis (Fig. 5C,E).

Finally, while none of the individual cell type implants stimulated obvious abnormalities in the recipient skin, considerable tissue enlargement and overall thickening of the ear was common in the variations that resulted in appendage (arrows), or cyst-like (c), inductions (Fig. 5E). For example, while the ear sections shown in Fig. 5A and C appear very similar in thickness to the post-operative DS/GE cell implanted ear shown in Fig. 5E, the latter was photographically recorded at half the level of magnification, illustrating how, as an integral part of the inductive interactions, the local ear tissue has practically doubled in mass.

In our experience, certain dermal and epidermal cell populations found in adult hair follicles display embryonic-, or even stem cell-type, characteristics (Reynolds and Jahoda, 1994a). The tissues and local appendages immediately around the implant sites were no doubt affected by the inductive interactions, and may have reciprocated minor influences. There may also have been minor reciprocal influences on the implanted cells; we are presently unable to explain the detail of these secondary events.

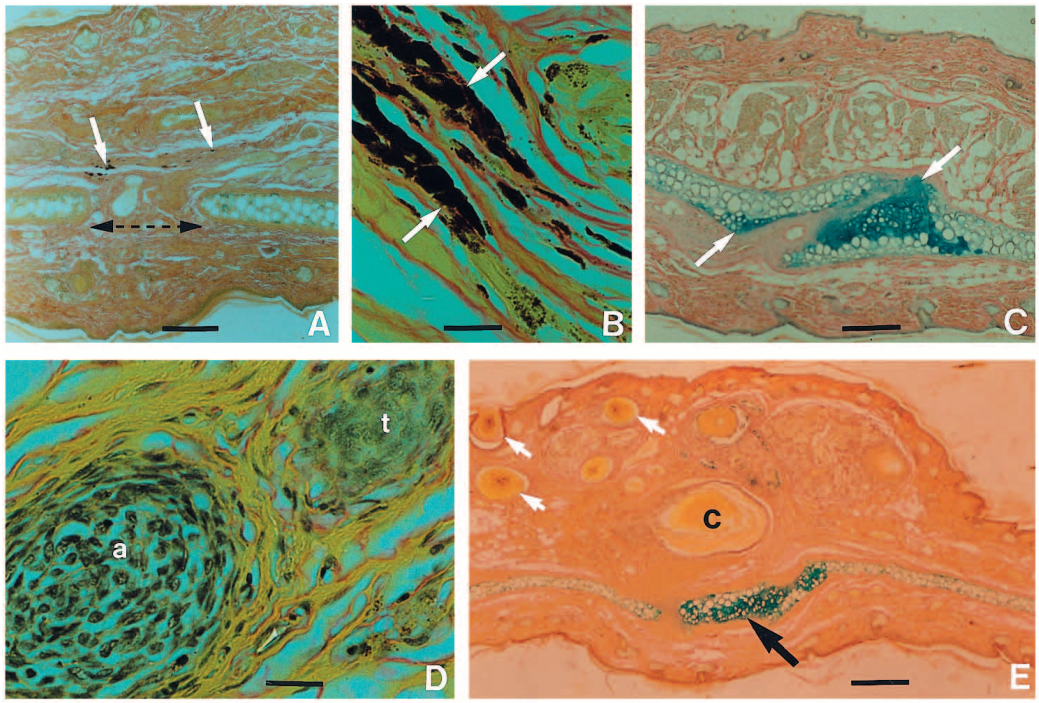

Fig. 5. Differences in post-operational ear tissue morphology relative to the cell type(s) implanted (A-E). Identification of the wound sites was facilitated by reference to the altered ear dermis, epidermis or cartilage layers (see broken line with arrowheads, A), resulting from the inevitable minor damage inflicted during the initial operations. Sites that had received GE cells (with slightly more differentiated material), contained diffuse patches of residual pigment (arrows, A,B). DS cells consistently elicited a pronounced hyperproliferative and biosynthetic reaction in the local ear cartilage, which stained intensely with Alcian blue, and so reflected proteoglycan and glycosaminoglycan activity (arrows, C). Note that the cartilage in the DS/GE cell-implanted ear detailed in E has also been stimulated in a similar manner. HP DP cells formed ovoid papilla-like structures which cycled in a manner typical of papillae in situ (a, anagen-like and t, telogen-like papilla ovoid, with cells appearing well-spaced or closely packed, respectively; D). While no cell type alone stimulated abnormal thickening in the recipient ear skin, this was common when appendages (arrows), or cyst-like structures (c), were induced (E). In fact, although the ear sections shown in A and C appear to be of similar thickness to the one in E, the latter was photographed at half the level of magnification. Thus, the DS/GE cell implanted ear section in E illustrates how, as an integral part of the inductive interactions, the local ear tissue more or less doubled in mass. Bars: (A,C) 680 mm; (B,D) 75 mm; (E) 1.6 mm.

DISCUSSION

We have shown that the inclusion of adult rat vibrissa germinative epidermal (GE) cells with non-inductive dermal cells results in the formation of giant hair follicles and fibres in more than 80% of ear skin implantations. That the newly induced appendages were randomly orientated and much larger than the local ear follicles both indicated that de novo morphogenesis had occurred. Further support for this proposition came from the vibrissa-typical arrangement of the tissues in the induced appendages and their fibres, which illustrated that the implanted cells retained traits that were characteristic of their follicles of origin. This was in agreement with previous work, since vibrissa dermal papilla (DP) cells induce vibrissa-type follicles and pelage DP cells induce pelage-type follicles (Reynolds and Jahoda, 1991a, 1992; Jahoda, 1992; Jahoda et al., 1993).

The considerable increase in ear skin mass, which accompanied the formation of the disproportionately large newly induced follicles, is one example of how the inductive interactions influenced the local tissues. While we cannot explain the details of such secondary events, they do illustrate the important point that the immediate tissue environment is an integral part of the inductive interactions. The possibility of contributions from locally damaged appendages also remains to be qualified and quantified, but the consistent difference in the behaviour of the dermal cells either with, or without, the GE cells strongly suggests that extraneous cell populations could not have been significant in this study. It has already been shown that contributions from local appendages are non-essential to induction, since pelage papillae can stimulate follicle neogenesis in isolated glabrous skin (Reynolds and Jahoda, 1992).

As new appendages were never stimulated by GE cells implanted alone, or recombined with skin fibroblasts (SF), a contribution from follicle dermal cells (sheath or papilla) would seem vital to induction. This in itself is not surprising, since the interactive capabilities of adult rat follicle papilla cells, have now been observed in many forms (Oliver, 1971, 1980; Jahoda et al., 1984, 1993; Reynolds, 1989; Reynolds and Jahoda, 1991a, 1992, 1994a). What is novel, is that cultured dermal sheath (DS) cells have been shown to have inductive powers in vivo that had not previously been attributed to them and, also, that loss of the papilla cells inductive ability after multiple passaging is not permanent. We believe that the most likely interpretation in both instances is that the GE cells provide the follicle dermal cells with a crucial initial signal that allows them to become inductive, and so sets off the cascade of reciprocal interactions that culminates in new follicle formation. It seems clear that the role of the GE cells cannot simply be to provide material, since neither DS nor HP DP cells implanted alone interact with (for example) wound epidermis to produce new follicles (as LP DP cells do).

Epidermal influence

In showing that GE cells can significantly alter follicle dermal cell behaviour, this study provides some backing for the proposition of Sengel (1976), that lower follicle epidermis might

influence the behaviour of the DS when it participates in regeneration after end bulb amputation. Yet, in any context, the idea that adult epidermis can effectively direct the behaviour of adult dermis is unusual. As a rare postnatal example, Gillman et al. (1963) provided strong indirect evidence that the epidermis was the prime mover during the healing of cutaneous wounds. In skin appendage embryology, however, the concept is neither radical nor novel since an epidermal signal is required for the mesenchyme to become the presumptive dermal papilla, prior to its reciprocal signal to induce, for instance, hair- (Dhouailly, 1975), or tooth- (Kollar and Baird, 1970; Ruch, 1985) type epidermal differentiation. Historically, the dermal (or mesenchymal) element was widely favoured as the initiator of the inductive sequence leading to appendage formation, with relatively few individuals open to the possibility of the epidermis (or epithelium) providing the first cue (Sengel, 1976; Brotman, 1977). Recent molecular expression work has, in fact, provided strong support for the latter view, since prior to condensation or any other indication of morphologic change in the mesenchyme, MSX homeobox genes (Noveen et al., 1995) and the DNA-binding molecule lymphoid enhancer factor 1 (LEF-1; Zhou et al., 1995) have both been detected in the placode epithelium. The present findings conform to our previously stated belief that adult follicle cells display behaviour that is closely akin to that seen in embryonic cells. More specifically, while our previous comments were expressed in relation to follicle dermal cells, the current work suggests that end bulb GE cells could accurately be characterised likewise.

If adult follicle cells have embryonic-type properties, it might also hold true that the mechanisms controlling their development have relevance in adult tissue remodelling, and may help to explain our findings here. For instance, the DS/GE-induced follicles were consistently larger and more frequent than those stimulated by similar numbers of DP cells (Reynolds, 1989; Reynolds and Jahoda, 1991a; Jahoda, 1992; Jahoda et al., 1993). The variable size did not therefore simply reflect different quantities of material being implanted. Van Scott et al. (1963) noted a close anatomical relationship between the GE population size and the mass of the DP. Perhaps this also applies when the presumptive GE cells appear to stimulate DP formation via the condensation, or local aggregation, of small groups of mesenchymal cells during follicle embryogenesis. Hence, in the current study the adult GE cells might be re-enacting embryological events by influencing the number of DS cells contributing to the new papilla and thus, in turn, the ultimate mass of the induced follicle. It may also be that a higher proportion of the perhaps 'less differentiated/developmentally more flexible' DS cells can respond appropriately to the epidermal stimulus?

An obvious question to follow on from this is whether or not the interactive influences reported here are unique to the GE cells? In our hands, neither hair follicle outer root sheath (ORS; including those of the bulge-region) cells, nor skin basal epidermal (SE) cells displayed any GE-comparable inductive behaviour. Once again, this agrees with prior studies in vitro where the exceptional morphological and interactive properties of GE cells could not be elicited from either SE, or ORS, epidermal cells (Reynolds and Jahoda, 1991b, 1993, 1994b; Reynolds et al., 1993). The molecules and mechanisms that allow the GE cells to direct follicle dermal cell behaviour remain to be characterised, and clearly, this now begs priority in future studies.

Dermal cell status

Morphological, behavioural and biosynthetic differences between follicle-derived dermal cells and those from other sources have been well documented (Jahoda and Oliver, 1984; Messenger, 1989; Randall et al., 1991; Taylor et al., 1992; Francz et al., 1993; Couchman, 1993; Stenn et al., 1994; Reynolds and Jahoda, 1991a b, 1992, 1993, 1994a b). This work provides further evidence that follicle dermal cells are inherently different from SF. On a subtler note, how similar are sheath and papilla cells and do they respond to the same GE influence? While definitive responses to these questions require further biochemical and molecular analysis, there is some data both for, and against, sheath and papilla cell congruity. The follicle dermal cells have common developmental origins (Wessells, 1970); both display highly specialised biosynthetic traits in vitro (Reynolds et al., 1992), and they can structurally and functionally substitute for each other in vivo. DP cells are likely to give rise to the DS component of the follicles they induce in vivo (Oliver and Jahoda, 1989; Reynolds, 1989; Reynolds and Jahoda, 1991a, 1992; Jahoda, 1992; Jahoda et al., 1993). Conversely, papillae can regenerate from residual sheath tissue after follicle end bulb amputation (Oliver, 1967a,b). On the other hand, papilla cells are morphologically and behaviourally distinguishable from sheath cells both in vitro and in vivo, irrespective of their passage numbers (Reynolds, 1989). When re-implanted alone into skin, DS cells are very difficult to identify, while DP cells are easily recognised by the discrete papilla-like tissue aggregates that they form (Jahoda and Oliver, 1984; Reynolds, 1989; Jahoda et al., 1993). Intriguingly, in this study the ovoid structures which formed from the HP DP cells not only maintained themselves but they also displayed different morphologies in which the cells were either well-spaced, or tightly-packed, as is seen in normal papillae in situ during a typical hair growth cycle. This suggests that even after many divisions in culture, the papilla cells retain an in-built timing mechanism, or form of biological clock.

In conclusion, this study reveals that previously untested combinations of follicle cells can stimulate type-specific appendage neogenesis. In particular, a defined population of adult epidermal cells has been shown to direct adult dermal cells to change from one defined developmental state (DS) to another (DP). Using an established bioassay we have thus been able to show that, when influenced by GE cell interactions, DS cells can specialise and adopt an 'inductive status' (previously unique to papilla cells), while 'aged' DP cells can be revitalised so that their inductive capacity is restored. In extending our understanding of how dermal-epidermal interactions contribute to the regulation of hair follicle activities, these results should provide a solid basis for future biochemical and molecular characterisation.

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