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Collagen-type synthesis in human-hair papilla cells in culture

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Summary. Hair-papilla derived cells were grown in monolayer culture and revealed the typical morphology and growth pattern which was similar but not identical to control fibroblasts. Hair-papilla cells were found to produce considerable amounts of collagen type I and type III and fibronectin. Type IV collagen production could not be detected. The ratio of collagen type III and type I clearly differed from the pattern observed in normal fibroblasts, being much higher in hair-papilla cells, where type III accounted for more than 20% of total collagen synthesis. These data show that hair-papilla derived cells have biosynthetic capacities similar to those of human skin fibroblasts as well as characteristic differences, indicating that they represent a specialized fibroblast subpopulation.

Key words: Hair papilla cells – Cell culture – Collagen synthesis

Dermal hair-bulb papilla is thought to play an important role in the induction and maintenance of hair growth [2, 6, 17, 24–28, 34]. Recently it has become possible to isolate cells from the dermal papilla and to grow them in culture [11, 14, 22] where they retain the ability to induce hair growth in vivo [13]. Both human and rat papilla cells reveal a fibroblast-like morphology in culture and tend to form aggregates [12, 15, 23]. Distinct morphological features and differences in growth rate and pattern have been reported in papilla-cell cultures which indicates the existence of a distinct specialized fibroblast-like cell type [12, 15, 23].

The major biosynthetic product of dermal fibroblasts is collagen. It is present in more than ten genetically different types found in different tissues and synthesized by specialized connective tissue cells [21]. Tendon fibroblasts have been shown to produce type I collagen exclusively [9], whereas dermal fibroblasts synthesize mostly type I and type III, and in addition small amounts of types V and VI collagen [7]. Although culture conditions may induce a switch of collagen-type synthesis [9, 20, 31], analysis of collagen types represents a valuable tool in characterizing different cell types. Collagen types I and III are not uniformly distributed in the dermal connective tissues showing an accumulation of type III collagen in the papillary dermis, whereas the reticular dermis contains 90%–95% type I collagen [30].

Recently Westgate et al. [32] noted the presence of two basement membrane components – laminin and type IV collagen – in dermal papillae. Subsequently, it was shown using the indirect immunofluorescence technique that papilla cells isolated from adult rat vibrissae and cultured in vitro retained the potential to synthesize those components during their first few days in primary culture [4]. In the present paper we report on the biochemical characterization of collagen synthesis in human-hair papilla cells in vitro and provide evidence that these cells constitute a specialized subpopulation of fibroblasts.

Materials and methods

Scalp biopsy specimens from five volunteers (20–50 years of age) were obtained and hair papillae were prepared as described earlier [15]. Cell cultures were initiated by outgrowth from tissue specimen and subcultured using 0.25% trypsin and 0.02% EDTA. All cultures were used for biochemical studies in the 3rd to 5th passage.

For estabilishing growth curves cells were seeded in a density of 3×10^4 cells/35 mm Petri dish in DMEM containing penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (0.584 mg/ml), and 10% inactivated fetal calf serum (FCS) in humidified 5% CO_2 at 37°C atmosphere. Cell counts were obtained every 2nd day and morphology was judged by phase contrast microscopy.

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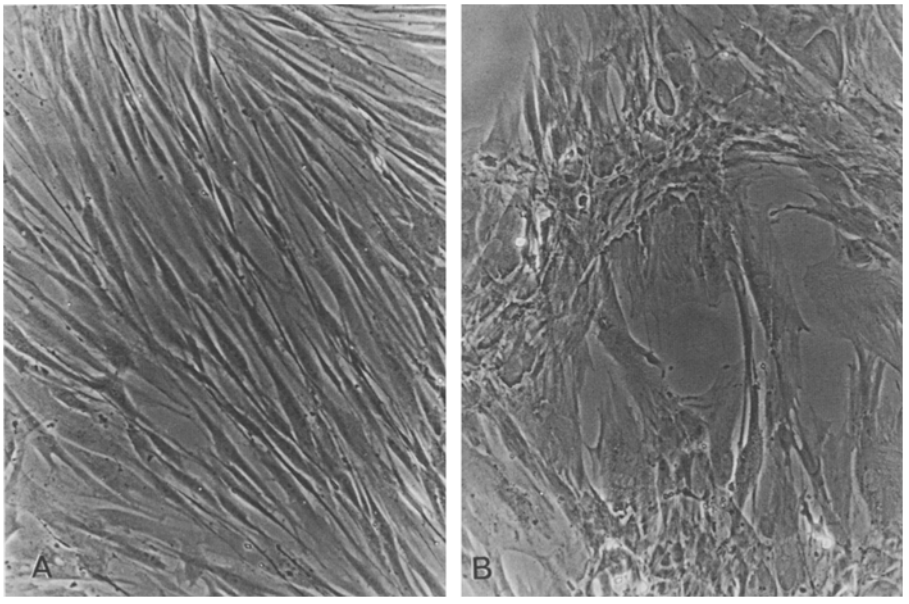

Fig. 1 A, B. Characteristic morphology of control fibroblasts (**A**) and hair-papilla derived cells (**B**) in monolayer culture (phase-contrast ×120)

Control fibroblasts were obtained by outgrowth from tissue specimen of the papillary dermis adjacent to the hair papilla and used in exactly the same procedure as hair papilla cells. For biochemical studies cells were preincubated in DMEM containing penicillin (100 U/l), ascorbic acid (50 µg/ml), L-glutamine (0.584 mg/ml), and 10% FCS for 24 h and then incubated for 24 h in DMEM containing penicillin (100 U/ml), β-aminopropionitrile (50 µg/ml), and ascorbic acid (50 µg/ml) in the presence of L-(2,3^{3}H) proline (3.7×10^4 Bq/ml). Newly synthesized material was then collected from cell layer and medium and used for analysis of collagen and protein synthesis as discussed below.

Examination of protein and collagen synthesis

Newly synthesized material was extracted from the cell layer by 0.2 *M* NaCl, 0.05 *M* Tris-HCl pH 7.4, and collected from the medium. The combined fractions were dialyzed against 1 *M* $CaCl_2$, 0.05 *M* Tris pH 7.4 and then extensively dialyzed against 0.5% acetic acid. The material was lyophilized, then hydrolyzed (6N HCl plus mercaptoethanol 110° C, 24 h N_2) and analyzed by an amino acid autoanalyzer equipped with a fraction collector as described earlier. The ratio of collagenous and non-collagenous protein synthesis was calculated according to Wiestner et al. [33].

Analysis of newly synthesized protein by slab gel electrophoresis

Proteins collected from cell layer and medium were combined and precipitated by 40% ammonium sulfate, resuspended after centrifugation (4° C 1 h, 30,000 × *g*), dialyzed against 0.5% acetic acid, and analyzed on slab gels according to Laemmli [18]. The bands were visualized by fluorography [1, 19].

Characterization of collagen types

Newly synthesized proteins from cell layer and medium were subjected to pepsin treatment (0.1 mg/ml in 0.5% acetic acid pH 1.8, 6 h, 18° C) and then precipitated by 2.7 *M* NaCl, 0.05 *M* Tris-HCl pH 7.4. The material was then chromatographed under denaturing conditions on agarose A5m equilibrated with 1 *M* $CaCl_2$, 0.05 *M* Tris-HCl pH 7.4. Radioactively labelled proteins eluting as γ-components were collected and rechromatographed on agarose A5m after reduction with β-mercaptoethanol. In addition aliquots from the material eluting as α-component and γ-component were collected, and further prepared for amino acid analysis as described above.

Indirect immunofluorescence studies

Confluent monolayers of papilla cells were washed with PBS, fixed for 5 min on ice with 90% ethanol, and labelled with monoclonal anti-type IV collagen antibodies, kindly provided by R. Burgeson, Portland, USA, using FITC-labelled anti-IgG antibodies as described previously [31].

Results

The morphology of cells isolated from hair bulb papillae clearly differed to control fibroblasts revealing multiple short cytoplasmic processes. Papilla cells were larger than control fibroblasts and tended to form aggregates (Fig. 1). The proliferation was considerably slower compared to fibroblasts grown from the vicinity of the hair papilla (Fig. 2). Analysis of total protein synthesis in both cell types revealed comparable amounts for hair-papilla derived cells and control fibroblasts (Table 1).

Similar results were obtained for the production of collagenous proteins, which was estimated by determining the content of hydroxyproline in the proteins. The relative proportion of collagen synthesis compared to total protein synthesis was 11%, which was also found in human skin fibroblasts. In addition hydroxylation of isolated collagen α-chains was similar in both cells and no difference was noted in the

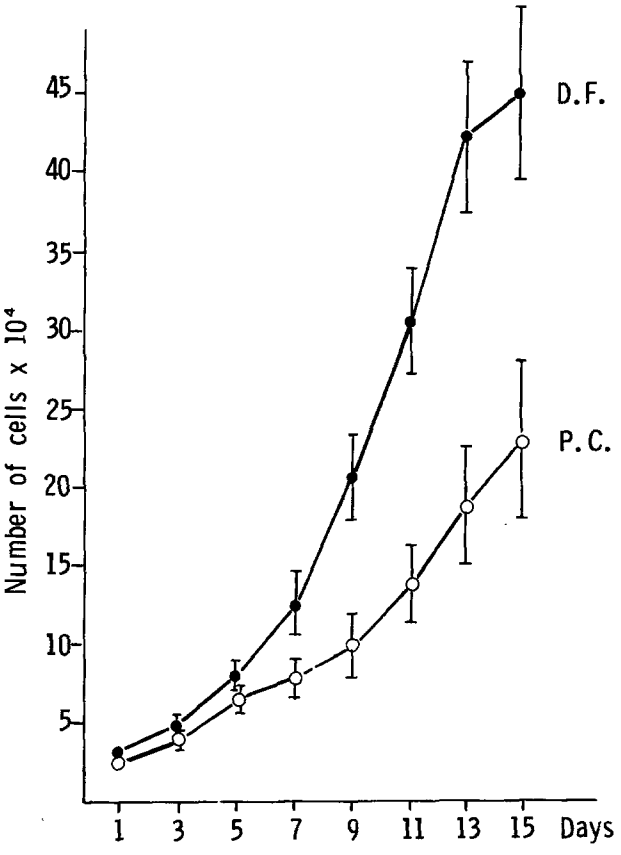

Fig. 2. Growth curve of hair-papilla derived cells (*PC*) and control fibroblasts (*DF*) in subcultures; 3×10^4 cells of both types were seeded into Petri dishes and counted every 2nd day. The values represent mean values from three independent estimations

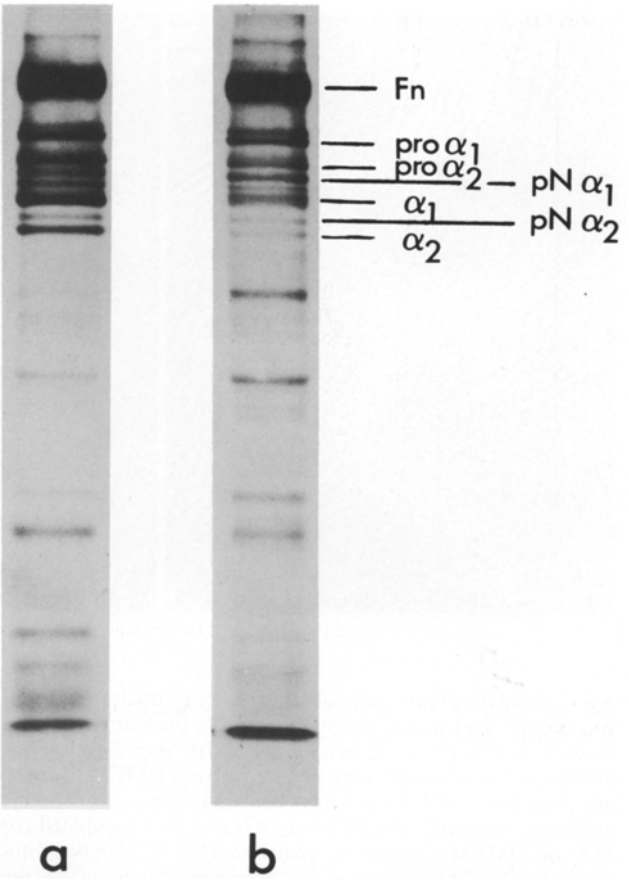

Fig. 3a, b. Electrophoretic separation of radioactivity labelled proteins synthesized from hair-papilla derived cells (**a**) and control fibroblasts (**b**). The newly synthesized protein were collected from the medium and processed as described in 'Materials and methods'. *Fn*, fibronectin

Table 1. Synthesis of noncollagenous and collagenous proteins by hair papilla-derived cells and control fibroblasts

	Hydroxy-proline (cpm/cell)	Proline (cpm/cell)	Collagen from total protein synthesis (%)
Control fibroblasts ($n = 2$)	65.67	286.97	10.6
Hair-papilla derived cells ($n = 2$)	56.91	240.99	11.0

Synthesis of collagenous and noncollagenous proteins was estimated after radioactivity labelling of the cells with ^{3}H proline (see Materials and methods). The numbers represent mean values from estimations obtained with two different cell chains

secretion of newly synthesized collagens into the medium.

For characterizing extracellular matrix proteins, hair papilla cells were labelled with ^{3}H proline, and newly synthesized proteins accumulating in the medium and extracted from the cell layer were analyzed on slab gels (Fig. 3). No obvious qualitative difference could be detected. Indirect immunofluorescent studies using specific antibodies against type IV collagen revealed no reaction in hair-papilla derived cells and control fibroblasts (not shown).

For an exact quantification of collagen types I and III, the newly synthesized material was digested with pepsin. Pepsin-resistant collagens were precipitated and chromatographed on agarose A5m (Fig. 4). Whereas control fibroblasts synthesized only slight amounts of type III collagen (10% – 12%) migrating as γ-component under nonreducing conditions, much more radioactivity was found to elute in this region when cells derived from hair papilla were used (Fig. 4). When the γ-components were reduced and rechromatographed on agarose A5m, more than 90% of the radioactivity labelled material was found to migrate as α-chains. The relative amount of type I and type III collagen was then calculated for both cell

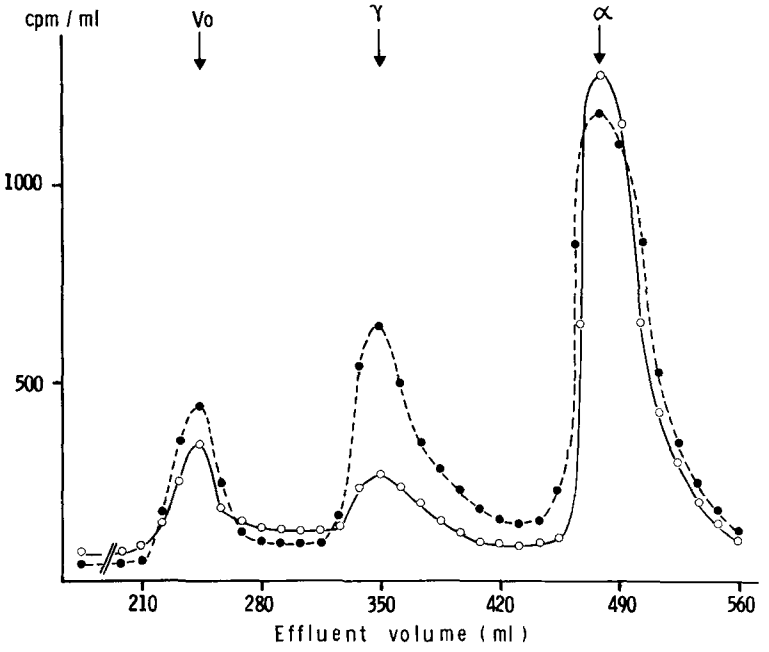

Fig. 4. Characterization of newly synthesized material on agarose A5m after pepsin treatment (see 'Materials and methods'); ●----● hair-papilla derived cells; ○----○ control fibroblast; V_0: void volume

Table 2. Ratio of collagen types I and III synthesized by control fibroblasts and hair-papilla derived cells

	Type I collagen (%)	Type III collagen (%)
Control fibroblasts ($n = 6$)	88.3 ± 0.8	11.7 ± 1.1
Hair-papilla derived cells ($n = 5$)	77.4 ± 3.4	22.6 ± 3.4

The ratio of both collagen types was calculated from agarose chromatograms (Fig. 3), where type I collagen elutes as α-chains, whereas type III collagen is found as γ-components

types. Hair-papilla cells were shown to produce higher amounts of type III collagen, which was found to account for more than 20% of the newly synthesized collagenous material (Table 2).

Discussion

The dermal papilla has been shown to control hair growth and to influence the differentiation of epithelial cells [2, 6, 17, 24–28, 34]. It is composed of mesenchymal cells and loose connective tissue containing fibronectin [5, 8], type III collagen, and small amounts of type I collagen [4]. In addition, type IV collagen and laminin have been found in the dermal-hair papilla [4, 32].

Cells derived from the dermal-hair papilla and cultured as monolayers retain the ability to induce hair growth in vivo [13] and can clearly be distinguished from normal fibroblasts by a distinct morphology and the characteristic growth curves even after repeated subcultures [12, 15, 23]. However, in the present study we have demonstrated that protein and collagen synthesis of these cells is very similar to skin fibroblasts which produce mainly types I and III collagen and fibronectin [29]. The lack of type IV collagen synthesis revealed by indirect immunofluorescence studies using specific antibodies also argues for a loss of the differentiated phenotype of the cells due to culture conditions. This is corroborated by data recently reported by Couchman et al. [4] who could demonstrate synthesis of type IV collagen and laminin in early primary cultures of dermal papilla cells. However, no such synthesis was found in cultures after 20 days, where mainly type I and type III collagen were detected [4].

Modulation of the pattern of collagen types synthesized according to the culture conditions has already been reported in tendon fibroblasts [9], skin fibroblasts [31], and chondrocytes [20]. However, a detailed analysis of collagen types produced by dermal hair-papilla derived cells in subculture still revealed characteristic differences compared to skin fibroblasts as showing a high ratio of type III to type I collagen. Since the total amount of collagenous protein synthesized is similar in papilla-derived cells and control fibroblasts, this represents both an increased production of type III and a reduced synthesis of type I collagen. The retention of a high type III collagen synthesis even through several passages in cell culture together with the observation that cultured papilla cells can still induce hair formation in vivo indicates

that only certain functions (e.g., type IV collagen synthesis) are modulated by the culture condition, whereas others remain stable.

Based on differences in growth pattern, morphology, and biosynthetic capacities, a heterogeneity of fibroblasts has been established previously [3, 10, 16]. The data presented here are in agreement with the assumption that dermal hair-papilla cells represent an additional specialized fibroblast-like cell type, which to some extent modulates its differentiation under monolayer culture conditions.

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