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Isolation and culture of different epidermal and dermal cell types from human scalp suitable for the development of a therapeutical cell spray

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ABSTRACT

Background: Previous studies demonstrated, that cultured epithelial autografts (CEA) can be isolated and skin cell sprays can be produced for application on different types of wounds. The purpose of the present study was to determine which cell types can be isolated from the human scalp and whether these cells can be used for spray transplantation.
Methods: Outer root sheath cells (ORS), keratinocytes, melanocytes, dermal papilla cells (DP), and dermal sheath cells (DSC) were isolated from human scalp tissue. Isolated cells were characterized, expanded and sprayed in an *in vitro* model. Growth behaviour, morphology and cell counts were compared with non-sprayed cells.
Results: With acceptable time, equipment and laboratory personnel a sufficient amount of keratinocytes, ORS, melanocytes, DP cells and DSC cells could be achieved. The cells are sufficient for application as a cell spray. Cells, positive for Integrin α6, Cytokeratin 19, CD73 and CD105 were identified within the cultures.
Conclusions: Human scalp is suitable to gain epidermal and dermal cells for the development of therapeutic cell spray transplantation. Further studies have to determine, whether these cells can be combined to produce wound specific skin substitutes.

1. Introduction

The reconstruction of skin after burn or other types of trauma with regard to functional and aesthetic aspects is one of the most challenging tasks for reconstructive surgery. Deep burns are often associated with substantial loss of dermal structures and require transplantation of split-thickness skin autografts, harvested from healthy donor sites. These are limited especially in cases exhibiting the loss of more than 60% total body surface area (TBSA), thus alternatives are needed to treat these patients.

The first application of cultured epithelial autografts (CEA) was performed in 1981 [1–5]. The use of CEA is a standard method in the treatment of burn victims even though it takes 3 or 4 weeks of *in vitro* expansion time to achieve the required amount of cells [6]. Our laboratory showed that skin cell isolation and expansion for cell transplantation is limited by the special medical history of the patients [7]. CEA reduce

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mortality [8] and pain [9]. However, major problems are the difficult handling of the fragile sheets and variable ''take rates'' [10]. Often associated with blister formation is wound secretion under the sheets. Disadvantages of CEA include scarring and contractures resulting in a graft reduction between 10 and 50% [2,11].

Another problematic aspect of CEA is that they need to be detached from the growth substrate by dispase, which degrades some of the keratinocyte-derived basement membrane proteins involved in cell adhesions such as integrin [12]. After degradation it takes several hours before the cells will regain attachment due to newly synthesized proteins. Therefore, the anchorage to the underlying wound bed is aggravated in the initial stages [13,14].

Efforts have been made to improve the ''take rate'', skin quality and the time until availability like fibrin matrices [15,5] or a combination with dermal equivalents like Integra® to a composite graft [16,17].

Transplantation of preconfluent keratinocyte single cell suspensions to the wound bed appears to be a viable alternative. Major advantages include better handling, lower fragility, reduction of culture time and costs [18]. Wound closure, differentiation and expansion rates are achieved subsequently [19]. Spraying of preconfluent keratinocytes reduces the *in vitro* culture time and number of passages for comparable surfaces exhibiting a superior integrin profile, thus facilitating better attachment [20,21]. In a prospective clinical study of our group, subconfluent primary keratinocytes were harvested and sprayed as a treatment for 19 patients with deep facial burns. Excellent clinical results were achieved. Thus, cell spraying seems to be a sound technique to apply keratinocytes onto burn wounds, compared with the classical split-thickness skin autografts [22]. Furthermore, using a cell spray avoids spillage of cells from the wound bed and allows an even delivery, which is a problem of pipetting [10].

The hair follicle is a self-renewing adjunct to skin, which contains an epidermal and a mesenchymal component. Thus, use of hair follicles as a basis for production of cell suspensions appears to be promising.

The outer root sheath (ORS) of the hair follicle is equivalent to the basal epidermal layer and contains a subpopulation of multipotent stem cells in the bulge region [23,24]. These cells appear to be a source of epidermal stem cells in the epidermis [25] and are able to build epidermis [26], hair follicles and sebaceous glands [27]. Furthermore, ORS cells have been used to reconstruct a skin equivalent *in vitro* [28–30] and have been applied clinically [31–33].

The mesenchymal fraction of the hair follicle represented by the dermal papilla (DP) and dermal sheath (DSC) are important participants in wound healing [34] and a key component for hair growth [35–38].

The purpose of our *in vitro* study was to determine whether human hair follicles can serve as a source of epidermal and dermal skin cells for skin cell transplantation therapy development in spray technique. As we have seen, keratinocyte application results in excellent clinical results [22], we transferred the spray technique for five different cell types apparent in the human hair follicle and the surrounding tissue, which were isolated and separately cultivated.

2. Materials and methods

If not otherwise indicated, all materials were gained from the Biochrom AG, Berlin, Germany. All media were supplemented with antibiotics (Penicillin/Streptomycin, 120 μg/ml) and antimycotics (Amphotericin B, 2.5 μg/ml). Cells were incubated using a CO_2-incubator (Heraeus BB 6060, Kendro, Langenselbold, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

2.1. Skin biopsies

Skin biopsies were obtained from healthy volunteers undergoing facelift surgery from the removed skin portions. Informed consent for the use of the tissue for research purposes was obtained in all cases. 32 facelift biopsies (28 female, 4 male, average age of 55.8 years, range 36–71 years) and 15 biopsies from the chest or abdomen (10 female, 5 male, average age 43.8 years, range 23–69 years) were also used. The number of biopsies, used for the individual experiments is mentioned.

The skin was shaved and gently cut into small pieces following the direction of growth to preserve the hair follicles. The pieces were incubated with dispase (2.5 units ml^{-1} Roche Diagnostics, Mannheim, Germany) in PBS for 16 h at 4 °C. In a second step the skin pieces were exposed to 0.4% collagenase (Serva Electrophoresis GmbH, Heidelberg, Germany) in DMEM for 1 h at 37 °C. Epidermis, dermis and hair follicles were separated using an operating microscope and microsurgical instruments under sterile conditions. The dermal papilla was cut off and cultivated separately.

2.2. Cell isolation

2.2.1. Outer root sheath cells (ORS)

Separated hair follicles were washed in PBS and incubated with 0.05% trypsin/0.02% EDTA-solution for 15 min at 37 °C. Digestion was stopped using PBS/10% FCS.

2.2.2. Keratinocytes

Pieces of epidermis were incubated with 0.05% trypsin/0.02% EDTA-solution for 15 min at 37 °C. Digestion was stopped using PBS/10% FCS.

2.2.3. Dermal papilla cells (DP)

The separated dermal papillae were washed and applied into uncoated culture flasks. After 3 days the medium was changed removing non-attached dermal papillae.

2.2.4. Hair follicle dermis (HFD) and human dermal fibroblasts (HDF)

Pieces of hair follicle dermis were incubated in collagenase for 60 min at 37 °C.

Human dermal fibroblasts were obtained from interfollicular dermis and cultivated as cells from hair follicle dermis.

2.2.5. Isolation and cultivation of melanocytes

To isolate melanocytes, single cell suspensions from epidermis were incubated on collagen coated flasks (BD BioCoat Collagen I, BD Biosciences, Bedford, USA) for 15 min. Adherent cells were washed and cultured with keratinocyte medium