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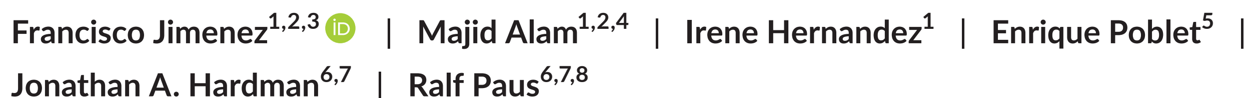
CONCISE COMMUNICATIONS

WILEY Experimental Dermatology

An efficient method for eccrine gland isolation from human scalp



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Abstract

We describe a simple and efficient method to isolate eccrine sweat glands from the human scalp. This method is inspired by the hair graft harvesting method used in hair transplantation. Based on the recently described anatomical relationship between the scalp hair follicle and the eccrine gland, we have found that scalp follicular unit grafts are an excellent eccrine gland isolation source, especially for the coiled component. In order to make the gland visible for stereoscopic microdissection, the follicular units need to be previously stained with a vital dye like methylene blue or neutral red. The simplicity and efficiency of this isolation method should encourage further research into human eccrine sweat gland function which has always been hindered by the difficulty of gland isolation.

KEYWORDS

Eccrine glands, follicular unit, hair follicle, hair graft, hair transplantation, methylene blue, neutral red, sweat, sweat glands

1 | BACKGROUND

Eccrine glands (EGs) are cutaneous appendages that produce sweat and act as our primary source of cooling. Besides their critical role in thermoregulation, they also play a less understood yet important role in cutaneous wound healing.[1–4]

Research into human eccrine sweat gland function has been greatly hindered by problems of gland isolation (Supplementary Text). Obtaining full intact EGs by stereoscopic microdissection of human skin is a very difficult, laborious and low yield-giving technique.[5–11] Although other isolation methods such as enzymatic collagenase digestion do yield a larger number of glands, exposure to enzymatic digestion may be potentially damaging for physiological functional studies.[12–15]

In this article, we describe an efficient method of isolating human eccrine sweat glands from follicular unit grafts harvested from the scalp. The idea of harvesting EGs from the scalp arose originally after our reported observation of the striking and consistent anatomical association between eccrine coils and hair follicles (HF).[16]

2 | QUESTION ADDRESSED

Given the intimate association between the EG and HF, can eccrine coils be successfully isolated from human follicular unit (FU) hair transplant grafts?

3 | EXPERIMENTAL DESIGN

Our method of EG isolation is based on two premises: (i) due to the reported anatomic association between EGs and scalp HFs, we thought that an excellent and readily available source to obtain EGs was from FU grafts harvested in hair transplant procedures, and (ii)

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in order to isolate the EGs by stereoscopic microdissection, a supravital dye could be used to make them visible under a stereomicroscope. Different supravital dyes to highlight the EGs were tried, including methylene blue (MB) and neutral red (NR).[6,17,18] All FUs were donated by patients undergoing hair transplant surgery for androgenetic alopecia after giving written informed consent.

As a reminder, the FU is the prime element used in modern hair transplantation.[19] An FU is a histological structure composed of 1 to 4 terminal hair follicles along with their sebaceous glands and arrector pili muscles.[20] FUs can be obtained in hair transplantation in two ways: (i) by directly excising individual FUs using small round punches (0.8 to 1.00 mm in diameter), a technique known as FUE (follicular unit extraction),[21] or (ii) by stereomicroscope dissection of small slivers sectioned from a donor strip (strip harvesting technique). In the FUE technique, the punch is introduced to a depth of around 3 mm, and the FU is then released from the subcutaneous tissue with fine tip forceps (Video S1) (Figure 1). It is important to have the skill to insert the punch following the hair shaft angle in order to avoid hair follicle transection.

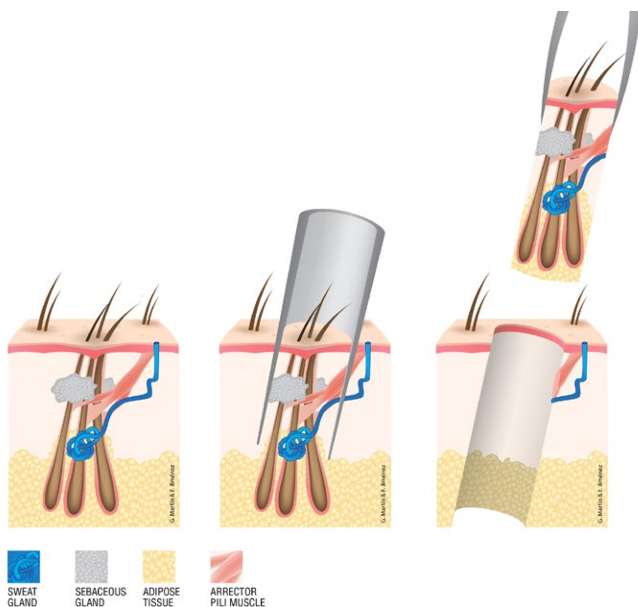

FIGURE 1 Schematic of eccrine gland isolation technique using an FUE punch. This figure illustrates the technical procedure of eccrine gland isolation from the scalp follicular unit with a circular micropunch (FUE technique). The left drawing depicts the anatomy of a scalp follicular unit (FU), showing the location of the eccrine coil in relation to the anagen terminal hair follicles, sebaceous glands and the arrector pili muscles. Note that most human scalp FUs contain 2 or 3 terminal hair follicles. The eccrine coil is embedded in adipocytes (dermal fat tissue). The arrector pili muscles that emerge from each of the follicles that form the FU join together forming a single muscular bundle. The middle drawing shows how the punch needs to be introduced into the scalp skin with the angle of the punch parallel to the hair shaft direction in order to avoid FU transection. The penetration of the punch to a depth of 3-4 mm is sufficient with most patients to release the FU from the surrounding dermal collagen attachments and allow it to then be easily removed with fine tip forceps (drawing on the right)

4 | RESULTS

MB and NR gave a very clear delineation of the EG, providing a distinct blue and red colouring of the EG, respectively, easily visible against the background of the dermal collagen. We found that immersing the FU in a few drops of MB at a concentration of 0.02% or in NR at a concentration of 0.2% in physiologic saline for 10-15 minutes was sufficient to highlight the EGs (Figures 2 and S2).

EG isolation can be accomplished using either of the two different FU graft harvesting methods: strip harvesting and FUE. Using the strip harvesting technique, we observed that the small vertical slivers in which the long strip is divided—of 1 to 2 FU thickness—contain numerous EGs that can be easily identified by MB or NR staining and later microdissected (Figure 2A,B).

In our experience, EG isolation was even easier when using FU grafts harvested with FUE punches, because the small punches used to excise the FUs, left minimal dermal surrounding tissue, making EG coil dissection faster since the coil was not attached to dermal fibres (Figure 2C,D). Anatomically, the EGs are always embedded in adipocytes (dermal fat tissue) and located at a depth of 2 to 3.5 mm below the epidermal surface of human scalp, at the inferior portion of anagen terminal hair follicles, and always below the sebaceous gland and arrector pili muscle (Figure S1). We noted that the smaller the calibre of the punch used in FUE, the higher the risk of transecting and damaging the EG coil. For this reason, we believe that the use of 1-mm-diameter punches is ideal for this purpose. In addition, the ductal excretory portion cannot be isolated with this method because at some point the duct is transected by the punch as depicted by the schematic in Figure 1.

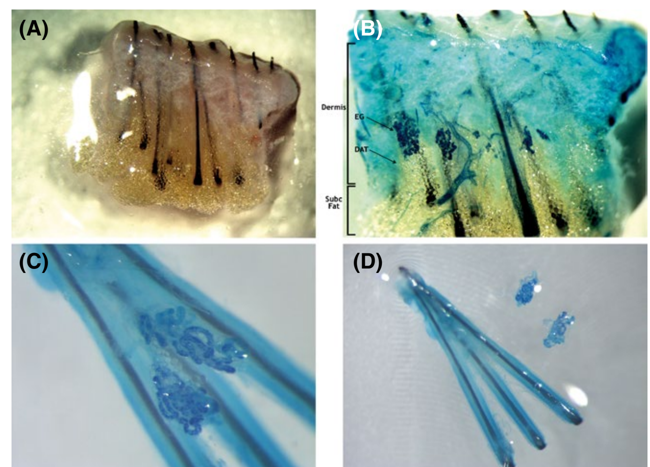

FIGURE 2 Methylene blue allows easy identification of human eccrine glands in scalp follicular units. A typical thin vertical sliver of scalp skin dissected under the stereomicroscope during a strip harvesting hair transplant procedure unstained (A) and stained with methylene blue (B). Note the position of the eccrine coils, in the deep dermis, approximately 2-3.5 mm beneath the epidermis and embedded in adipocytes. A three-hair follicular unit (harvested with a 1 mm punch) stained with methylene blue with two eccrine coils between the hair follicles (C) and the eccrine coils after dissection from the follicles (D)

Although the main goal of this study was to describe a rapid and efficient source for EG isolation, ex vivo culture of isolated EGs in a mixture of supplemented Williams E and F12 media, demonstrated that they are still viable after 6 days as shown by neutral red uptake and calcein AM (2 μM) (ThermoFisher Scientific), a cell-permeant dye that in live cells is converted to green-fluorescent calcein (Figure S2).

5 | CONCLUSION

With the emerging discovery of the importance of the EG in roles other than sweating (including, amongst others, wound healing), an EG isolation method is required that is simple, efficient and that can also be used with living tissue to maintain its morphologic integrity.

Researchers should consider scalp FU grafts obtained from hair transplant procedures as an efficient source for isolating human EGs. Moreover, once the eccrine glands have been dissected from the FUs, the hair follicle grafts could still be used for hair transplantation, so no human tissue is wasted.

Isolation of human EGs ex vivo that maintain their morphological integrity and function can also be an invaluable source for the cosmetic industry interested in studying the sweat response to pharmacological products in healthy as well as in pathologic disorders (eg anhidrosis, hypo- and hyperhidrosis). As a further fascinating challenge, the possibility exists of investigating the clinical outcome after transplanting exclusively EGs in special clinical situations, for example to extensive deep-burn survivors whose scarring skin covered with skin grafts is devoid of sweat glands and suffers from heat intolerance.[22–24]

CONFLICTS OF INTEREST

No conflicts of interest to declare by anyone of the authors.

AUTHOR CONTRIBUTIONS

FJ, IH, JH and RP designed the study. FJ, MA and IH performed the research. FJ, MA, JH and RP wrote the paper. FJ, MA, EP and JH analysed the data. FJ state that all authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Eccrine glands are clearly visualised after follicular unit grafts are stained with Neutral Red and Methylene Blue. Follicular units harvested from scalp skin with a 1 mm punch and stained for 10-15 minutes with (a) Neutral Red (0.2%) and (b) Methylene blue (0.02%) provides a very clear intravital identification of the eccrine glands (arrows), which are normally not visible under the stereomicroscope

Figure S2 Eccrine glands are still viable after 6 days of ex vivo culture. After 6 days of culture in a mixture of supplemented Williams E and F12 media, eccrine glands were stained with two markers to assess their viability: Neutral Red (a) which stains lysosomes of live cells, and (b) Calcein AM, a cell-permeant dye which in live cells the non-fluorescent calcein AM is converted to green-fluorescent calcein

Video S1 Follicular unit extraction: Surgical technique using a motorized 1 mm punch for harvesting follicular units from the scalp. These FUs can be stained with Neutral Red or Methylene Blue for eccrine gland identification under the stereomicroscope.
DATA S1 Methods of eccrine gland isolation:

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IL-26 in allergic contact dermatitis: Resource in a state of readiness

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Abstract

In this study, we investigated the role of IL-26 in allergic contact dermatitis (ACD), highlighting its' contribute in the cytotoxic mechanism responsible for the tissue injury. IL-26 is a signature Th17 cytokine, and immune cells are its predominant sources. Recently, it has shown that Th17 cell-derived-IL-26 functions like an antimicrobial peptide. Here, we hypothesized that IL-26 could be involved in cytotoxicity mechanism that underlies ACD. Indeed, we have attributed a role to IL-26 in this context, through PBMC cytotoxicity assays vs HaCat. To demonstrate that IL-26 was effectively involved in this activity, we performed the assay using transfected ACD PBMCs by siRNA for IL-26. Indeed, we demonstrated that these cells were less able to kill keratinocytes compared with ACD PBMCs ($P < .01$). In conclusion, our findings support the idea that this emergent cytokine, IL-26, is implicated in the killing mechanisms of KC observed during ACD.

KEYWORDS
allergic contact dermatitis, cytoxocity, IL-26

1 | BACKGROUND

Despite considerable progress in understanding the development of hapten-specific immunity, less is known about mechanisms responsible for tissue injury during allergic contact dermatitis (ACD).[1] Indeed, ultrastructural studies of ACD revealed damaged keratinocytes in close contact with mononuclear cells,[1] suggesting a role for T-cell–mediated cytotoxicity.[2] In particular, cytotoxic T cell activity becomes evident in the appearance of lesions, but subpopulations of T helper (Th) cells also contribute to the release of specific cytokines.[3] Today, there is experimental evidence to classify ACD amongst other inflammatory skin disorders as interleukin-17–producing T helper cell (Th17)-mediated disease.[4] Indications for involvement of Th17, in particular the subpopulation cytotoxic T, in human skin allergy were reported for the first time in 2009.[5] The expression of interleukin-26 (IL-26) by Th17 was first described in 2007.[6] In humans, IL-26 is a signature Th17 cytokine, and its expression is regulated by IL-1β, IL-23 and RORγt. Immune cells, including