Acne and seborrhoea are sebaceous gland-related diseases that are also exclusively human diseases. Therefore, fundamental research on human sebaceous cell function and control requires human models in vitro. The human sebocyte culture model was first introduced in 1989. Cultured human sebocytes have been shown to preserve important sebocytic characteristics, although they undergo an incomplete terminal differentiation in vitro. Over the years, modifications of the technique have improved the culture of human sebocytes in vitro, but the primary cultured sebocytes can still be maintained for no more than six passages in vitro. The immortalized human sebaceous gland cell lines SZ95, SEB-1 and Seb-E6E7 have been developed in recent years, which make it possible to get a large number of sebocytes from the same donor culture. Cultured human sebocytes in vitro has become a useful tool in studying sebaceous gland activity and regulation, and understanding the pathophysiological mechanisms and treatment of acne and other sebaceous gland related diseases.

Introduction

Acne is a disease of the pilosebaceous unit. Excessive sebum production and its abnormal lipid ingredients play an important role in contributing to the formation of the primary lesions associated with acne, in addition to the hyperkeratinization of pilosebaceous ducts, hyperproliferation of Propionibacterium and also inflammation of acne.1 Experimental sebaceous gland models are essential for better understanding of the pathophysiology of acne and other diseases involving the sebaceous gland, such as sebostasis, seborrhoea and in evaluating the sebum inhibitory effect of anti-acne drug candidates.2 Several animal models,3-5 including preputial cells in primary monolayer cell culture differentiating like sebocytes6 have been developed, but seborrhoea and acne are exclusively human diseases and sebaceous gland differentiation is species specific. Therefore, fundamental research on human sebaceous cell function and control requires human models in vitro. Isolation of human sebaceous glands and culture of human sebocytes in vitro have experienced several continuous stages, such as simple isolation of sebaceous gland, isolation of sebaceous gland and primary cultivation of sebocytes7 and immortalized human sebaceous gland cell lines SZ95, SEB-1,8 and Seb-E6E7.9 Cultured human sebocytes in vitro have become a useful tool to study sebaceous gland activity and regulation, and understand the pathophysiological mechanisms and treatment of acne and other sebaceous gland related diseases.

Isolation of Human Sebaceous Gland and Primary Culture of Sebaceous Gland Cells

(See Table 1) Karasek11 described the cultivation of human sebocytes in collagen after enzymatic dissociation of isolated sebaceous glands and in monolayers after enzymatic digestion of sebaceous-gland-rich dermal slices. However, cells obtained by this first technique exhibited a significant loss of sebocyte characteristics in vitro. Doran et al.12 modified this technique by removing the top 0.4-mm facial skin sections containing the epidermis and some of the dermis and used the second 0.4-mm dermal section as the source of human sebocytes. The dermal section was placed in a solution of 10 mg/ml dispase in Dulbecco’s modified Eagle’s medium (DMEM) containing penicillin/streptomycin and 10% fetal calf serum (FCS) for 30 min at 37°C. The tissue was then immersed in 0.3% trypsin/1% EDTA for 15 min at 37°C, washed with phosphate-buffered saline, transferred in medium containing serum and scraped vigorously with a scalpel blade to obtain a sebocyte suspension. The cells were cultured in Iscove’s medium containing 2% human serum, 8% FCS, penicillin/streptomycin, L-glutamine and 5 μg/ml dexamethasone on a feeder layer of mitomycin-C-inactivated 3T3 fibroblasts.

Xia et al.7 introduced the maintenance of the sebaceous gland ex vivo and a reproductive model for the cultivation of sebaceous gland cells in vitro. Small (3.5 mm) pieces of full-thickness human skin were washed in phosphate-buffered saline without Ca2+ and Mg2+ and incubated for 20 h in 2.4 U/ml dispase at 4°C to separate epidermis from dermis. Epidermis was then immersed for 15 min in 0.02% deoxyribonuclease at 37°C and placed in medium with 10% FCS. Intact sebaceous glands were isolated by microdissection under microscopic observation of the epidermal underface and their ducts were removed. The isolated gland lobules were seeded on mitomycin-C-inactivated 3T3 cells and cultured in DMEM containing 4.5 g/l glucose and Ham’s F 12 medium (3:1) supplemented with 10% FCS, 10 ng/ml epidermal growth factor (EGF), 0.4 μg/ml hydrocortisone, 10-9 M chola toxin, 3.4 mM L-glutamine, penicillin/streptomycin and amphotericin B at 37°C with 5% CO2. Primary
human sebocytes resulted as outgrowths from the periphery of the gland lobules and a maximum number of three subcultures could be grown (Fig. 1). Zouboulis et al.\textsuperscript{13} modified the culture medium including 2\% human serum, 8\% FCS and omitting hydrocortisone. Lee\textsuperscript{14} treated sebaceous glands with collagenase before cultivating them in serum-free William’s E medium supplemented with 10 μg/ml insulin, 10 μg/ml transferrin, 10 μg/ml hydrocortisone, 10 ng/ml EGF, 10 ng/ml sodium selenite, 2 mmol/l L-glutamine and penicillin/streptomycin. Primary sebocyte cultures could also be obtained by omitting the 3T3 fibroblast layer\textsuperscript{15} and secondary cultures could be grown in medium supplemented with delipidized serum and in serum-free keratinocyte basal medium.\textsuperscript{16}

Morphology of sebocytes in vitro is polymorphic epithelial appearance. Cell size significantly increase up to 4- to 5.5-fold with progressive differentiation Abundant cytoplasmic lipid droplets, cells with large nuclei and regularly preserved cell organelles such as mitochondria, rough endoplasmic reticulum and Golgi apparatus are prominent. Intercellular contacts are established by tight junctions and not by desmosomes. Proliferation of sebocytes is slower than that of epidermal keratinocytes of the same donors. Synthesis of squalene, wax esters and free fatty acids could be detected. Expression of the human polymorphous epithelial mucins (MAM-6c, sebaceous gland antigen), expression of low levels of involucrin, low transglutaminase activity, formation of small numbers of cornified envelopes and expression of keratins 7, 13 and 4, enhanced expression of keratin 19 and no expression of keratins 1 and 2 would be found.\textsuperscript{2,17,18}

Establishments of Immortalized Human Sebaceous Gland Cell Lines

(See Table 2) Primary human sebocytes are predisposed to differentiate by accumulating neutral fat droplets until they burst and die.

Table 1  Chronological advancements in human primary sebocytes culture in vitro

| Year | Authors          | Culture method                          | Medium                                                                                   | Feeder layer |
|------|------------------|------------------------------------------|------------------------------------------------------------------------------------------|--------------|
| 1989 | Xia et al.\textsuperscript{7} | outgrowth from isolated sebaceous glands | DMEM containing 4.5 g/l glucose and Ham’s F 12 medium (3:1) supplemented with 10% FCS, 10 ng/ml EGF, 0.4 μg/ml hydrocortisone, 10^{-6} M cholera toxin, 3.4 mM L-glutamine | 3T3 cells    |
| 1990 | Lee\textsuperscript{14}   | outgrowths from isolated sebaceous glands | serum-free William’s E medium supplemented with 10 μg/ml insulin, 10 μg/ml transferrin, 10 μg/ml hydrocortisone, 10 ng/ml EGF, 10 ng/ml sodium selenite, 2 mmol/l L-glutamine | 3T3 cells    |
| 1991 | Zouboulis et al.\textsuperscript{13} | outgrowths from isolated sebaceous glands | 2% human serum, 8% FCS and omitting hydrocortisone compared to xia | 3T3 cells    |
| 1991 | Doran et al.\textsuperscript{12} | enzymatic dissociation of isolated sebaceous glands | Iscove’s medium containing 2% human serum, 8% FCS, L-glutamine and 5 μg/ml dexamethasone | 3T3 cells    |
| 1996 | Fujie et al.\textsuperscript{23} | dispersing sebaceous glands to a single-cell solution by 0.25% trypsin | Serum-free KGM | without cell feeder layer |
| 1997 | Seltmann et al.\textsuperscript{24} | outgrowths from isolated sebaceous glands | Modification of Xia et al.\textsuperscript{7} by addition of 4–10 ng/ml keratinocyte growth factor and with/without 1 mg/ml bovine serum albumin | without cell feeder layer |

Figure 1. Culture of human primary sebocytes in vitro. (A) In the centre the whole sebaceous gland is seen and in the periphery extended sebocytes are displayed. (B) Culture of human primary sebocytes in vitro. (C) Fluorescence microscopy picture of nile red stained sebocytes: neutral lipids in the cells are visualised at 485 nm excitation and 528 nm emission.
Over the years, with the continuous development of human sebaceous gland models in vitro, sebaceous gland research has experienced a new era. Several receptors related to physiological and pathological functions such as lipogenesis, cutaneous steroidogenesis, androgen synthesis, inflammatory effects and neuropeptides have been found in the sebocytes using these models in vitro, which have provided wide scopes in understanding physiological functions of sebaceous gland, pathogenesis and therapy of sebaceous gland disorders such as acne, seborrhea and other sebaceous gland related diseases in the future. They also facilitate the search for biologically active ingredients, new pharmaceutical and cosmetic drugs for anti-ageing, seborrheic and dry skin treatment.

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Therefore, adequate cell amounts for large scale experiments can only be obtained from multiple donors, while prolonged experiments are hindered by the short life span of the cells. Normal human sebocytes can only be grown for three to six passages. To overcome this problem, Zouboulis et al. cultured human facial sebocytes from a 87-year-old woman and transfected them with the simian virus-40 large T antigen, which resulted in the first human immortalized sebaceous gland cell line, termed SZ95 (Fig. 2). SZ95 sebocytes retain major characteristics of normal human sebocytes, such as progressing differentiation with increasing cell volume and lipid synthesis, expression of markers of sebaceous lineage and terminal sebocyte differentiation, such as keratin 7 and epidermal membrane antigen (EMA), respectively and can subsequently undergo apoptosis. They also express specific proteins of human sebaceous glands and exhibit expected biological responses to androgens and retinoids. The human sz95 sebaceous gland cell line has been internationally patented, has been applied as a tool in numerous experimental projects worldwide and has provided precious information on the pathophysiology of the sebaceous gland, which revolutionized our current knowledge on this skin organ.

In 2003, Thiboutot et al. developed a second immortalized human sebaceous gland cell line, termed SEB-1, using the method described by Zouboulis et al. SEB-1 was established from sebaceous glands of normal skin of the preauricular area of a 55-year old male. SEB-1 sebocytes also express characteristic sebaceous gland proteins and their cytoplasm induces oil red O-positive lipid droplets. The third immortalized sebaceous gland cell line Seb-E6E7 from adult human facial skin were transfected with HPV16 E6 and E7 genes by co-culture with mitomycin C-treated packaging cells in the presence of 3T3-J2 cells. Like SZ95 sebocytes, Seb-E6E7 cells express both K7 and involucrin. Seb-E6E7 seems to respond to chemicals in a similar manner with SZ95 sebocytes despite their different transfection methods.

Table 2 Immortalized human sebaceous gland cell lines that have been established

| Year | Authors | Origin of sebocytes/gender of donor | Name of established cell line | Transfection method |
|------|---------|------------------------------------|-------------------------------|---------------------|
| 1999 | Zouboulis et al. | Face/female | SZ95 | Simian virus-40 large T antigen |
| 2003 | Thiboutot et al. | preauricular area/male | SEB-1 | Simian virus-40 large T antigen |
| 2008 | Lo Celso et al. | Face/male | Seb-E6E7 | HPV16 E6 and E7 genes |

Figure 2. Culture of human SZ95 sebocytes in vitro. (A) Culture of human SZ95 sebocytes in vitro. (B) SZ95 sebocytes stained with oil red staining. Neutral lipids (red) in the cells are stained.
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