TISSUE CULTURE IN DERMATOLOGY

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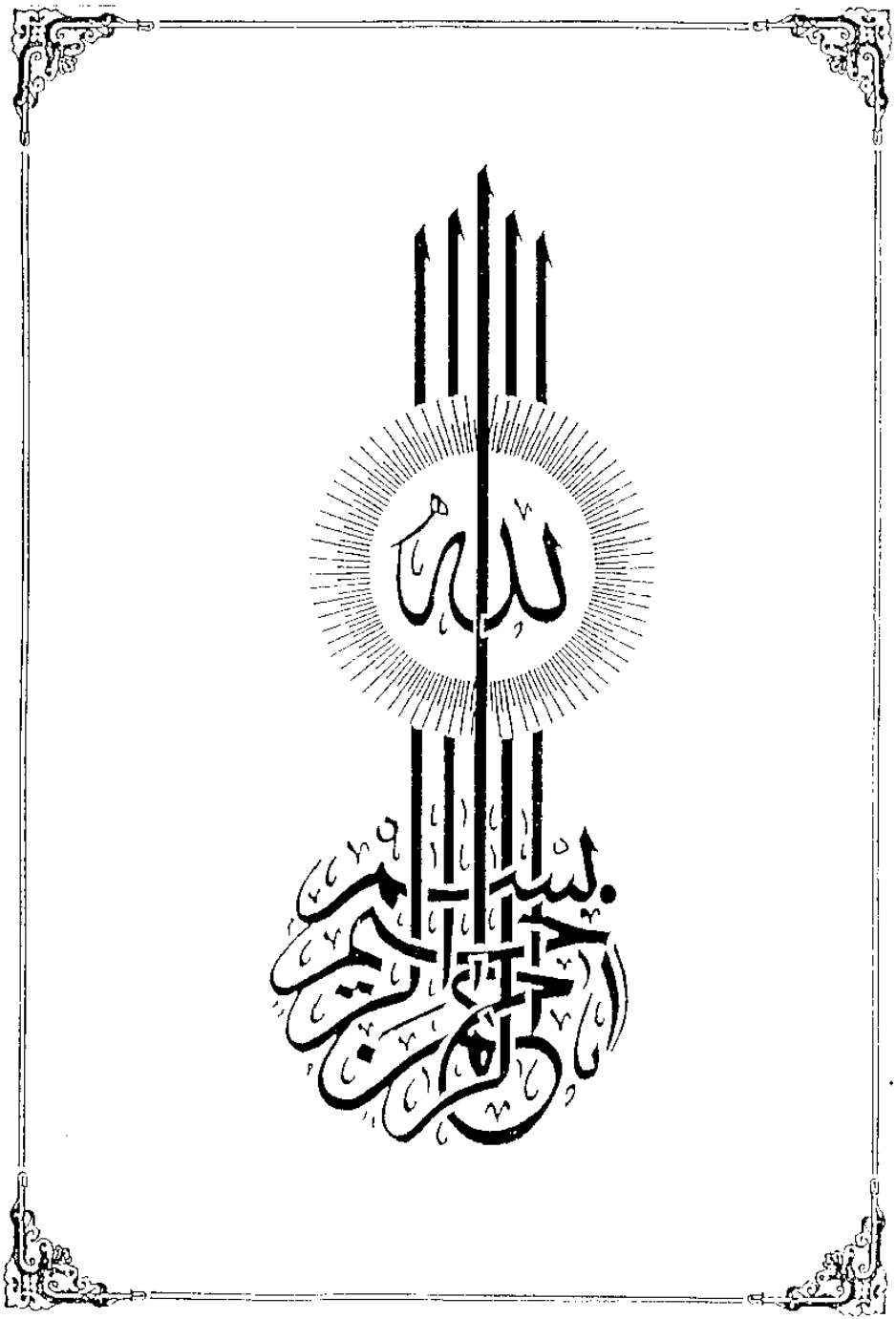
بسم الله الرحمن الرحيم

" وقل ربِ زدنى علماً "

"سورة طه أية ١١٤"

صدق الله العظيم



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ABBREVIATIONS

b-FGF	:	Basic Fibroblast Growth Factor
BHE	:	Bovine Hypothalamic Extract
CS(A)	:	Cyclosporin (A)
CT	:	Cholera Toxin
DMEM	:	Dulbecco's Modified EaglE's Medium
EGF	:	Epidermal Growth Factor
DP	:	Dermal Papilla
EMEM	:	Eagle's Minimal Essential Medium
FBS	:	FetaL Bovine Serum
5FU	:	5 Fluorouracil
GAG	:	Glycosaminoglycans
HABM	:	High- Antibiotic Tissue Culture Medium
HEPES	:	4-(2-Hydroxyethyl) 1 Piperazine - Ethane Sulfonic Acid
Ia	:	Immune response associated
ICAM-1	:	Intercellular Adhesion Molecule 1
IL	:	Interlukin
LC(s)	:	Langerhans Cell(s)
LT(s)	:	Leukotriene(s)
MGF	:	Melanocyte Growth Factor
8-MOP	:	8- Methoxy Psoralen
MTX	:	Methotrexate
PBS	:	Phosphate - Buffered Saline
PG	:	Prostaglandin
PHS	:	Presumptive Hair Shaft
PKC	:	Protein Kinase C
PMA	:	Phorbol -12- Myristate 13- Acetate
PSS	:	Progressive Systemic Sclerosis
PUVA	:	8-Methoxy Psoralen Plus Ultraviolet A Radiation
RA	:	Retinoic Acid
TBC	:	4- Tertiary Butyl Catechol
TCA	:	Trichloroacetic Acid
TGF	:	Transforming Growth Factor
TNF	:	Tumour Necrosis Factor
TPA	:	12-0- Tetradecanoyl Phorbol 13- Acetate
UV	:	Ultraviolet
UVR	:	Ultraviolet Radiation
VAMA	:	Vitiligo - Associated Melanocyte Antigen

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INTRODUCTION

INTRODUCTION

Strictly speaking, the skin is composed of three distinct components. From the surface downwards, they are the epidermis, the dermis and the subcutaneous fat. Although the subcutaneous fat is truly soft tissue rather than skin, its close anatomic relationship to the skin and its tendency to respond jointly with skin in many pathologic processes warrant mentioning (Jakubovic and Ackerman, 1993).

Human epidermis is a stratified squamous epithelium characterized by a high keratin content and by the ability to make cornified envelopes (Asselineau et al., 1986). Two types of cells constitute the epidermis: Keratinocytes and dendritic cells (Lever and Lever, 1990).

1 - Keratinocytes :

Since Keratinocytes can be grown in tissue culture, they provide an interesting system to study histogenesis in vitro (Asselineau et al., 1986). Besides, human epidermal cells can be grown in tissue culture to provide sheets of cells that can be transferred as a graft (Gallico, 1990). The most obvious application for keratinocyte culture and graft is in patients with burns damaging more than half of the body surface. Such patients have too few donor sites to provide enough split skin grafts to resurface the area of the burn after surgical excision (Hancock and Leigh, 1989).

2 - Dendritic Cells:

Dendritic cells of the epidermis include three types namely, melanocytes, Langerhans cells, and indeterminate dendritic cells (Lever and Lever, 1990).

I .: Melanocytes :

Melanocytes constitute a minor component of the cell population in the normal epidermis, being scattered at relatively low density throughout the entire epidermal sheet. Melanocytes in tissue culture are easily distinguished from keratinocytes by their morphological characteristics. While keratinocytes have a round or polygonal shape, melanocytes appear bipolar or polydendritic (Eisinger and Marko, 1982).

II.- Langerhans Cells (LCs) :

Langerhans cell (LC) is a morphological distinct type of macrophage that probably plays an important role in various immune responses. Other possible functions as regulation of epidermal differentiation and their role in disease states as mycosis fungoides are currently under investigation (Jakubovic and Ackerman, 1993).

The connective tissue of the dermis consists of collagenous and elastic fibres embeded into ground substance. All three components are formed by fibroblasts (Lever and

Lever, 1990). Fibroblasts can be sustained in tissue culture for about fifty generations (Walter and Israel, 1987).

Human sebaceous cells, derived from sebaceous glands have been cultivated in vitro and characterized for growth, lipid content and keratin composition (Doran et al., 1991).

*** Aim of the Work :**

The aim of this work is to review the literature about the possibility of culturing different cutaneous cells in vitro aiming at finding a curative line in the management of different skin diseases such as vitiligo, burns and leg ulcers.

KERATINOCYTE CULTURE

KERATINOCYTE CULTURE

Human epidermis is composed of closely packed cells with very little intracellular matrix. Most of epidermal cells are keratinocytes which synthesize keratin and are eventually lost by desquamation. This loss is compensated by permanent proliferation of basal cells so that the biology of living epidermis is dominated by keratinization and regulation of growth (Prunieras, 1979).

The epidermis is built of two parts, each consisting of multiple cell layers. The inner part contains living cells; the outer, cellular skeletons, or corneocytes. In the living part of the epidermis, the keratinocytes prepare to form corneocytes by becoming increasingly specialized through a process of terminal differentiation, followed by programmed cell death. Indeed, the keratinocyte is the only cell type that carries out its functions better when it is dead than when it is alive (Green, 1991). Besides keratinocytes, there are other epidermal cells such as melanocytes and LCs. But in contrast to keratinocytes whichaccomplish their biological function (Keratinization) independantly of other cell types, the functions of melanocytes and LCs are more or less dependant upon their interactions with adjacent keratinocytes with which they form some sort of epidermal "symbionts". For example skin pigmentation is largely influenced by interactions between melanocytes and keratinocytes. Also contact between these latter cells and LCs are important in

epidermal immunology (Prunieras, 1979).

Tissue culture is not a new field. Although it began more than 100 years ago, it did not have any practical consequences or much influenced scientific research until the 1940s. At that time, use of the technique of dissociating cells from another allowed greater proliferation than could be obtained from tissue fragments. But even today most of the estimated 200 cell types of which the human body is composed can not multiply extensively under any culture conditions (Green, 1991).

The culture of epidermal cells has been attempted for nearly half a century using epidermis from a variety of sources (Table I) and in various states (Table II) (Holbrook and Hennings, 1983).

ANIMAL	SOURCE OF TISSUE
Human:	Neonatal foreskin; adult surgical samples; all regions; adult biopsy specimens, all regions; buccal mucosa; roof of suction blister.
Rabbit:	Ear, body, cornea, oesophagus.
Mouse:	Embryonic, neonatal, adult body; oesophageal mucosa.
Chicken:	Embryonic limb buds; embryonic skin.
Rat:	Body skin; lingual mucosa
Cow:	Snout.
Dog:	Body skin.

Table I. Sources of epidermis and keratinocytes for culture (after Holbrook and Hennings, 1983).

Table II. Types of epidermal cultures:

CULTURE TYPE	CHARACTERISTICS OF THE METHOD
Organ culture	Cultures are usually established to maintain full thickness skin; samples are attached to the culture dish and (usually) submerged in medium.
Explant culture	Cultures are established primarly to obtain an outgrowth of a stratified sheet of epithelial cells from full-or split-thickness skin explants; samples are generally attached to the dish, but some floating cultures are also carried out.
Cell culture	Cells are dispersed and plated on a substrate; inoculated cells may be pure populations of keratinocytes, epidermal cells including melanocytes and langerhans cells, or cocultures of epidermal cells and fibroblasts.
Suspension culture	Dispersed epidermal cells are inoculated into a medium such as methyl cellulose which suspends and isolates single cells.
Graft cultures	Explants of skin, sheets of epidermis, and or dispersed cells are transplanted onto a prepared graft bed or injected into the body.
Combination cultures	Not a culture system but an experimental situation where cells are grown in one type of culture when transferred to a second or even third type of culture; e.g., explant cell graft.

(After Holbrook and Hennings, 1983).

The different systems were designed with the object of establishing an in vitro model for use in investigating problems such as (1) chemical carcinogenesis, (2) wound repair, (3) dermal- epidermal interaction, (4) drug toxicity, (5) cytopathic effects of virus infection, (6) allergic reactions, (7) epidermal morphogensis, (8) tissue antigenicity, (9) modulation of differentiation and

proliferation by pharmacologic and physiologic agents, and (10) cellular aging. The design or selection of a culture system depends on the specific question to be explored (Holbrook and Hennings, 1983)..

METHODS AND MODIFICATIONS OF KERATINOCYTE CULTURE

Different methods were done for isolation of keratinocytes in culture, some of these methods were done using animal skin such as guinea pig skin and others using adult human skin.

1. The cultivation of cells from adult guinea pig epidermis :

Thin slices of guinea pig skin were obtained from the dorsum of the ear. These slices were floated for 30 minutes at 37°C on a 0.15% solution of trypsin 1/250 in a buffer-salt solution. Thereafter they were removed, placed keratin side down in a petridish and the dermis lifted off with fine forceps. The epidermis was then stirred in a small amount of culture medium which consisted of 60% Hanks balanced salt solution containing 0.5% lactalbumin hydrolysate, and 40% of horse serum. Antibiotics penicillin, streptomycin and tetracycline were included. This method of trypsinization results in the loosening of the cells at the dermo-epidermal junction and scraping the epidermis gently releases a suspension of epidermal cells. It was found that for approximately 60mg net weight of skin slices, 1.5-2ml. of