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ARTIFICIAL SKIN SCAFFOLD TO TREAT BURN SCARS AND IT'S OTHER APPLICATIONS

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ABSTRACT

According to present strategies of regenerative medicine, it is focussing on altered skin (such as burnt skin) which can be transplanted with combination of scaffold and biomolecules [1][2]. In current years, biologically active scaffolds are being used as extracellular matrix that can induce synthesis of tissues and organs [3]. Scaffold is required for the restoration of the function of tissue and its regeneration as it acts as short term matrix for cell proliferation and extracellular matrix deposition [4]. Scaffolds are used for tissue engineering such as bone, cartilage, ligament, skin, vascular tissues, neural tissues, and skeletal muscle and as vehicle for the controlled delivery of drugs, proteins, and DNA [5]. Artificial skin finds its application in a broad range of areas including robotics, human-computer interfaces and other areas that involve mechanical deformation [6]. In this paper, an overview of the artificial skin scaffolds, its material properties which are used for treating burnt scars and its application is discussed.

KEY WORDS

Artificial skin, collagen, ECM, epidermis freeze drying, grafting, necrotic tissue scaffold.

INTRODUCTION

Skin is the largest organ of human body that covers entire body and protects the internal organs against infection, injury and harmful sun rays [7].

When the skin is critically damaged because of disease or burns, the body cannot respond fast enough to make the necessary substitution of cells and some burn victims may die due of loss of plasma and infection. To avoid these consequences and to correct these deformities, artificial skin or skin grafts are used.

Artificial skin is a synthetic substitute which is shaped in laboratory for human skin that can protect the lives of severely burned patients and it covers the entire body, keeping dangerous bacteria out and vital fluids in [8].

Scaffold designing and its fabrication are major area of biomaterial research, and they are also important for tissue engineering and regenerative medicine research. Scaffold plays important role in tissue regeneration and its repair. During the past two decades, many works have been done to extend potentially applicable scaffold materials for tissue engineering. Scaffolds are defined as three-dimension porous solid biomaterials designed to perform some following functions [9][10]:

i. Uphold cell-biomaterial interactions, cell adhesion, and ECM deposition.
ii. Allows sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation.

iii. Biodegrade at a controlled rate that approximates the rate of tissue regeneration under the culture conditions of interest.
iv. Cause minimal degree of inflammation.
v. Extremely porous and with appropriate size, large degree of pore interconnectivity, and exhibit a high surface area to volume ratio.
vi. In addition the three-dimensional (3D) shape of the scaffold is important for tissue regeneration because 3D substrates can provide both physical and chemical signals to guide cell colonization and to support cell attachment and proliferation [11].

TYPES OF ARTIFICIAL SKIN

Artificial skin is classified based on:

1. Need of patient
2. Composition
3. Practical point of view

According to the needs of patients, artificial skins are classified as:

1.1 Spray-on skin:

Spray-on skin is a skin culturing treatment for burns victims, developed by scientist Marie Stoner and plastic surgeon Dr. Fiona Wood of Perth from Australia. In this technique, healthy skin is taken from a donor and the surface cells called the keratinocytes are removed and it is cultured for 2 to 3 weeks until suspension is formation. The other skin cell (tissue) is put into a meshing machine, and then the skin sample is sliced into tiny squares. Finally the cultured cells are sprayed onto tiny pieces of tissue and they are combined to form a new skin for patient [12].

1.2 Permanent skin graft:

In this method, skin is extracted from any parts of body and fibroblast from dermal layer is isolated. These cells are tested for viruses and other harmful pathogens and then it is grown on mesh scaffolding. Later it is thawed, expanded and stored. Then it is implanted to the patient's wounds. The new blood vessel takes about one week to grow in the implanted skin [13].

1.3 Artificial electronic skin (e-skin):

The e-skin consists of semiconductor nano wires that can function at low voltages, and it is flexible than inorganic synthetic skins. Large amount of pressure-sensitive components are attached with an active-matrix backplane on a thin plastic support substrate. This type of artificial skin acts exactly same as natural skin [14].

1.4 Gelatin-containing artificial skin:

In this method artificial skin is synthesized with gelatin which is a polymer isolated from collagen. It is also called as bio-artificial skin [15].

1.5 Composite Biocompatible Epidermal Graft (CBEG)

In this technique keratinocytes are taken from edge of the wound and cultured and cells are grown within 2 weeks. Then the integra is applied on wound of the patients. When the neodermis of the Integra is fully vascularized, the silicone membrane of the Integra is separated and replaced by CBEG [16].

2. Based on composition artificial skin is classified as follows:

2.1 Class I: Temporary impervious dressing materials

Single layer materials

i. it can be naturally occurring or biological dressing substitute
E.g. amniotic membrane, potato peel.
ii. Synthetic dressing substitute, e.g. synthetic polymer sheet [17].

Bi-layered tissue engineered materials

E.g. TransCyte

2.2 Class II: Single layer durable skin substitutes

i. epidermal substitutes, e.g. cultured epithelial autograft (CEA), apligraft
ii. dermal substitutes
iii. human dermal matrix, e.g. alloderm

2.3 Class III: Composite skin substitutes

i. skin graft
ii. allograft
iii. xenograft

2.4 Tissue engineered skin

i. dermal regeneration template, e.g. integra
ii. biobrane

3. Classification based on practical point of view:

3.1 Temporary skin substitutes

Temporary skin substitutes provides transient physiologic wound closure, as well as protection from mechanical trauma, physical barrier to bacteria and creation of a moist wound environment [18].

Uses

i. for dressing on donor sites to assist in epithelialisation and pain control
ii. for dressing on clean superficial wounds until epithelialisation
iii. to provide temporary physiological i. closure of deep dermal and full thickness wounds after removal of skin.

3.2 Permanent skin substitutes

The permanent skin substitutes can permanently treat the wounds and provide a higher qualityii. skin replacement than the thin autogolous skin graft [19].

3.3 Biological skin substitutes

These skin substitutes acts temporarily as skin, have the advantages of being relatively rich in supply and not expensive, the biological skin substitutes have more intact and native ECM structure which permit the construction of a more natural new dermis. They also allow excellent re-epithelialisation characteristics due to the presence of a basement membrane. The most commonly used biological substitute worldwide are cadaveric skin allograft, porcine skin xenograft and amnion [20].

Eg: collagen, chitosan.

- Among all the types of artificial skin, collagen which is a biological substitute is mostly accepted because of its following properties [21] [22]: have interconnecting pores of appropriate scale to support tissue integration and vascularisation. Controlled biodegradability or bioreabsorbability so that tissue will ultimately replace the scaffold. Have proper surface chemistry to support cellular attachment, differentiation and proliferation. Have adequate mechanical properties to go with the intended site of implantation and handling. Does not cause any adverse response. Easily fabricated into a variety of shapes and sizes.

Manufacture of Dermal Portion of Artificial Skin

Collagen dispersion + Chondroitin 6- sulfate solution → coprecipitation at Ph 3.2 →

Casting of fibrillar precipitate in pan → Freeze drying →

High temp vacuum dehydration crosslinking(105^0C) → Glutaraldehyde cross

Elution of aldehyde → Freeze drying →

MATERIALS AND METHODOLOGY

The scaffold part of artificial skin is a porous copolymer composed of purified collagen and a glycosaminoglycan (GAG), chondroitin-6-sulfate, both collagen and GAG are components of the normal extracellular matrix, these materials are inherently biocompatible, weakly immunogenic, and degradable by natural physiological mechanisms. The covalent cross-linking of collagen and GAG is used to control the biodegradation rate of the scaffold to ensure residence time in the body of several days. Animal implantation studies demonstrated that this cross-linked collagen-GAG scaffold showed negligible inflammatory and encapsulation responses and non-fibrotic cellular ingrowth [23] [24].

The collagen-GAG scaffold does not work alone. It is firmly bound to a membrane of silicone elastomer, which acts as a temporary epidermal covering and is also crucial for the clinical performance of the artificial skin.

Dermal portion: The raw material used in manufacturing of the dermal portion is a preparation of bovine hide collagen and chondroitin 6-sulfate. Physiochemical, biochemical, and mechanical properties can be controlled by the content of chondroitin 6-sulfate. The dermal portion of the artificial skin is sterilized by heating to 105^0 C followed by immersing in glutaraldehyde solution of 0.05 wt. % [25].

Epidermal portion: The section of epidermal portion of the artificial skin is homogeneous layer of medical grade Silastic (Dow Corning) about 1/10 mm thick. This material controls water flux from the dermis to normal skin [26]. Liquid Silastic is applied in the sterile artificial dermis, making a rigid bond to the artificial dermis as it cures. This gives an intact, sterile, bilayer artificial skin consisting of an epidermal and dermal portion. The sterile artificial skin is stored in sealed polyethylene bags either as a freeze-dried, bilayer membrane, or in 70% isopropyl alcohol and stored before clinical application [27][28]. For initial clinical trials, the isopropyl alcohol procedure is used for packaging.

MECHANISM

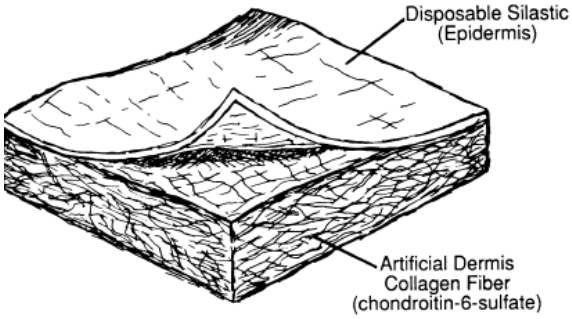
The artificial skin procedure includes the removal of thin layer of skin from the donor site, minimizing harm to the donor site and ensuring less pain and risk to the patient.

Skin-replacement surgery begins with the removal of necrotic tissue and the formation of a clean open wound [29] [30]. The natural physiological response to an open wound

includes inflammation, fluid loss, wound contraction, and granulation tissue formation.

The application of artificial skin establishes the physiology of a closed wound, with minimal inflammation, contraction, and granulation-tissue formation.

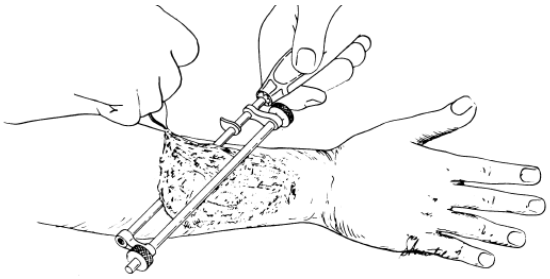
The initial wound closure is followed by vascularization of the collagen-GAG scaffold and the regeneration of a permanent dermal tissue, upon vascularisation of the scaffold layer, the second surgical procedure removes the temporary silicone layer and an epidermal autograft is placed over the newly synthesized dermal tissue. Cells from this epidermal autograft migrate and grow to form an intact epidermis. The function of the collagen-GAG scaffold in supporting the in-growth of connective-tissue cells is recognizable, which induces the regeneration of tissue that provides the critical physiological functions of dermis [31].



Diagrammatic representation of mechanism:

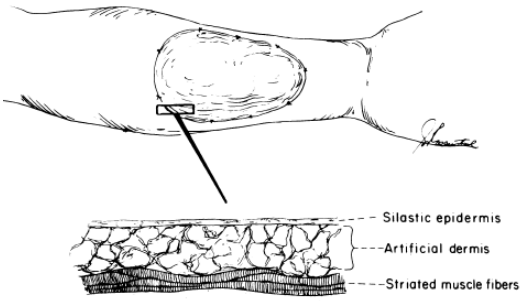
1. Excision of necrotic tissue using a guided knife, followed by careful hemostasis.

Fig I: Necrotic tissues are removed using guided knife followed by hemostasis



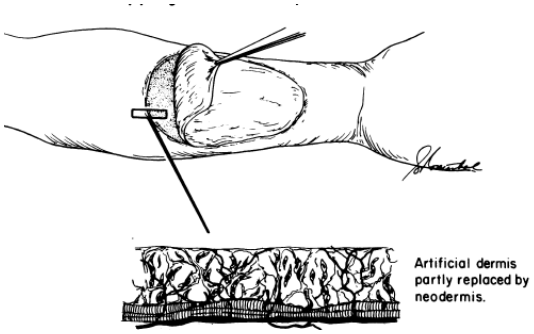
2. Grafting of artificial skin

Fig II: Shows the grafting of artificial skin and carefully stitched to achieve primary closure, the artificial dermis adheres to the excised bed.



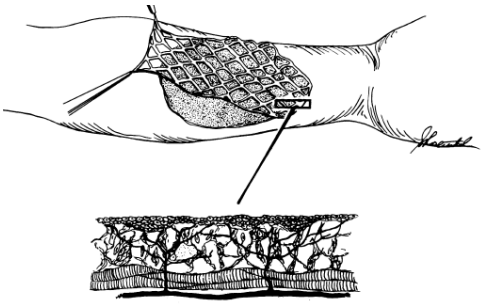
3 .Stripping of silostic epidermis

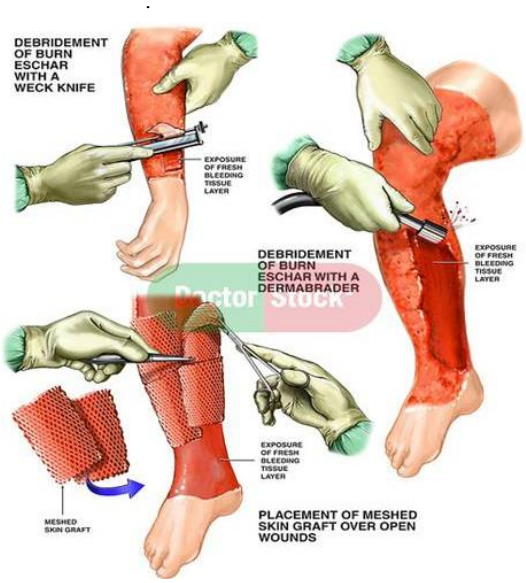
Fig III: Shows silostic epidermis is stripped from artificial dermis using forceps. Insert shows that the artificial dermis is invaded by host cells and it is partially replaced by a newly synthesized "neodermis."



4 .Application of meshed epidermis

Fig IV: Shows grafting of the "neodermis" with a thin epidermal graft directly on to the dermal bed provided by the artificial dermal template.





RESULT

The clinical behaviour of the artificial skin indicates the degree of success obtained in meeting the design criteria.

In the patients it was found that there was no infection in the grafted area, and the clinical and histology investigations confirmed that the artificial skin did not show an inflammatory or foreign body response [32][33].

Silastic epidermis provided numerous important contributions to the clinical use of the intact artificial skin. First, it allows the material to be completely manufactured from nonviable raw

materials in industrial batch processes and sterilized for immediate use. Further, because of its completely synthetic nature, simple room temperature storage over long periods of time and the potential for mass production raises the possibility of its effective biologic and economic use [34].

Significant effect of artificial skin on patients:

	AREA BURNED	TOTAL AREA COVERED WITH ARTIFICIAL SKIN
PATIENT 1 TOTAL BURN 85% BSA ARTIFICIAL SKIN 22% BSA		
PATIENT 2 TOTAL BURN 80% BSA ARTIFICIAL SKIN 38% BSA		
PATIENT 3 TOTAL BURN 85% BSA ARTIFICIAL SKIN 15% BSA		
PATIENT 4 TOTAL BURN 75% BSA ARTIFICIAL SKIN 40% BSA		
PATIENT 5 TOTAL BURN 60% BSA ARTIFICIAL SKIN 21% BSA		

CASE REPORT

Case 1

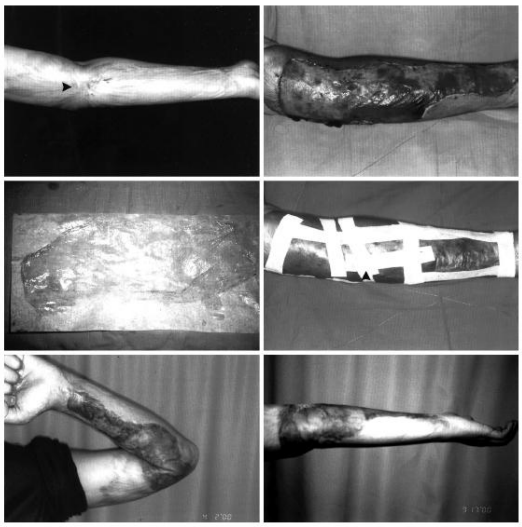
A 15-year-old boy suffered from 55-percent total body surface area burn. Post burn contracture was noted over the left elbow region with partial limitation of motion. After the scar tissue was excised and the joint was completely released, artificial skin was applied to the defect site. After a 2-week period of maturation, the silicon sheet was removed which is used for temporary epidermis protection.

The yellow-orange to reddish color of the neo-dermis was recognized, an ultrathin skin graft was harvested from the scalp and was fixed with Hypafix for immobilization with staples 3 days after surgery, and fluid was drained from under the graft. After 13 months skin was fully grown [35][36].

Case 2

A 24-year-old woman suffered from severe burn injury, with a 55-percent total burn surface area and post burn syndesis of the right axillary region with limitation of abduction function, the surrounding tissue was replaced over the axillary region with artificial skin, fixed with a surgical net After the neo-dermis formed, grafting was performed and secured by the tie-over method. The result was satisfactory, and skin was grown in 1 year [37][38].

RECONSTRUCTION OF BURNT SCARS:



CASE 1: **CASE 2:**

DISCUSSION

Collagen because of its satisfactory biologic characteristics, the production of nontoxic products on biodegradation, and the extensive research on collagen has made it one of the best understood polymers, allowing it to use as well-defined and reproducible material. The optimal characteristics, of collagen can be obtained by precipitating the native collagen in fibrils complexed to chondroitin 6-sulfate at pH 3.2[39], then stabilized by heating the freezed material to 105^0 C under vacuum dehydration. Crosslinking of the collagen chondroitin 6-sulfate coprecipitated to immobilize the glycosaminoglycan content onto the collagen, preserve its resistance to biodegradation and provide a method of increasing tensile strength. The collagen-

chondroitin 6-sulfate fibrillar coprecipitated, provide significant biologic properties in the way of controlling biodegradation, and maintaining a significantly higher modulus of elasticity been simply [40] [41].

In addition to the physicochemical properties of this material, it was found that the morphologic characteristics of the fibrous, highly porous membrane produced had a controlling effect on cellular and vascular populations of the artificial material [42] [43]. The pore structure of the collagen-GAG membrane has to be very close in size to cells and vessels encounter in normal dermis if optimal population of the grafted material is to take place. This can be achieved by a freeze-drying process which preserves the pore structure of the membrane almost intact [44] [45].

OTHER APPLICATIONS

i. **Alternatives to animal testing and regulations**

The major concern in the production and use of novel chemical reagents that are applied to the skin is their capacity to cause acute skin irritation upon contact. Hence, new methods have been established for the safe handling, packaging and transport, as well as for general safety assessment of these reagents. [46] These reagents are often tested for their irritant potential by the application to animals, followed by observations of visible changes including erythema and edema.

In 1992, The European cosmetics association (COLIPA) coordinated the efforts to develop the cosmetics industry alternative methods to animal testing for the safety assessment of cosmetics and created the Steering Committee on Alternatives to Animal Testing (SCAAT). In 1994, the European Centre for the Validation of Alternative Methods (ECVAM) organized a workshop on the possible use of non-invasive methods in the safety assessment of cosmetic products, and COLIPA created a specific task force to create guidelines that would reflect the protocols and practices of industry [47].

Skin reconstructs are now being proposed as an alternative method to animal testing for assessing irritation, corrosiveness, phototoxicity, and genotoxicity of various reagents [48].

i. **Photoaging model: the UV effect**

Exposure to sun causes various harmful effects, leading to short-term reactions such as erythema, sunburn, and suntan. Long-term effects include skin cancers and premature photoaging [49]. Solar UV light reaching Earth is a combination of both UVB (290–320 nm) and UVA (320–400 nm) wavelengths. Although UVB irradiation has received more attention, an increasing number of studies are now foccussing on the harmful effects of UVA [50]. Conventional monolayer cultures do not mimic accurately the physiological conditions for studying UV exposure .instead; the full 3D skin model composed of dermal and epidermal equivalent layers may be suitable for determining specific biological effects induced by UVB and UVA irradiation [50]. As the reconstructed skin model is able to differentiate epidermal horny layers, compounds or sunscreens can be topically applied on the skin, mimicking a more realistic situation.

For UV-irradiation study of topically functional sunscreens considering the UV effects on photoaging, the use of pigmented reconstructed epidermis containing melanocytes is crucial [51].

The solar protection factor (SPF) that corresponds to erythema prevention is one

of first features for evaluating sunscreen protection. After 24 h of UVB exposure, typical sun-burned cells (SBC) are formed in the epidermis, corresponding to the clinical appearance of erythema. SBC correspond to apoptotic keratinocytes, which allow elimination of cells strongly damaged by UVB irradiation. The use of a full skin reconstruct model in UV studies is essential as the major skin target of UVB is the epidermis, whereas UVA exposure mainly affects the dermis. UVB causes significant alterations in keratinocytes differentiation processes, whereas UVA induces apoptosis in fibroblasts located in the superficial area of the dermal equivalent.

Skin penetration is a key point in the evaluation of a potential skin sunscreen. The ability of these compounds to penetrate skin will depend on the time and concentration required to reach the desired target site. The skin reconstructs are also an efficient model to study cell and ECM modifications provoked by photoaging. Photoaged skin contains notorious changes observed in the uppermost epidermal layer, but also in the deep dermal layer of the skin, such as degradation of the connective tissue, decrease in collagen content, and accumulation of abnormal elastic tissue characterizing solar elastosis. Moreover, photoaging is associated with the appearance of advanced glycation end products (AGEs). AGEs are new residues created by cross-linked formations that are produced by a nonenzymatic glycation reaction in the extracellular matrix of the dermis. AGEs are now considered one of the factors responsible for loss of elasticity and other properties of the dermis during aging [52]. In a study that compared the histological results obtained within the reconstructed skin containing native collagen and collagen modified by glycation, no major differences were found in morphological structure except for the reduction of dermal thicknesses in the glycated sample.

ii. Pharmacological applications

In the field of pharmacology, drug discovery is generally dependent upon the predictive capacity of cell-based assays [53]. Most frequently, the efficacy of anti-cancer drugs is tested in 2D monolayer cells cultured on plates during the initial drug development and discovery phase. However, differences are observed when these drugs are tested in vivo. These differences may be the result of different cell surface receptors, proliferation kinetics, ECM components, cellular densities, and metabolic functions of 2D-maintained cells.

Skin from cadavers was used in drug transport studies, but limited availability and large variations between specimens have now increased the application potential of skin reconstruct models [54].

The 3D model has permeability characteristics and metabolic activity similar to native skin, which is vital, as metabolic activity may affect the permeability of some drugs and their potential for research on irritation, toxicity, and keratinocytes differentiation [55].

One of the concerns regarding the skin reconstruct model is its lack of skin appendages, including pilose baceous units, hair follicles, and sweat glands. Because of this lack, this model provides much lower barrier properties than that found in whole skin. As a result, the skin reconstruct model is superior to a monolayer model, the kinetic parameters of skin permeation obtained from these studies must still be considered

an overestimation when compared to the flux across human skin [55].

iii. Skin cancer

Cancer is a varied disease whose initiation and progression is tightly modulated by cell to cell and cell to matrix interactions. For these reasons, the use of 3D culture models has been steadily growing in studies of tumor biology [56].

Skin cancers such as melanoma, skin reconstructs are very suitable for modelling not only the growth and progression of melanoma cells in a 3D microenvironment, but also for studying the communication among melanoma cells and surrounding epidermal keratinocytes and dermal fibroblasts [57].

The use of artificial skin has shown that nearby fibroblasts are recruited by the primary melanoma and provide survival signals in the form of altered ECM deposition and growth factors, as well as stimulating the production of matrix metalloproteinase, promoting tumor cell invasion [58]. Yu and co-workers evaluated the role of BRAF mutation and p53 inactivation during transformation of a subpopulation of primary human melanocytes, and observed the formation of pigmented lesions reminiscent of in situ melanoma in artificial skin reconstructs [59]. In addition, artificial skin has been used to screen the therapeutic potential of oncolytic adenoviruses in melanocytic cells.

Organotypic 3D culture models are also used for different tumor types including breast, prostate, and ovarian cancer. Skin models have also been used for genetic and functional analyses of early stages of tumor development. Normal melanocytes in this model remained singly distributed at the basement membrane. In the radial growth phase of melanoma, proliferation and migration of the cancer cells in the dermal reconstruct and tumorigenicity in vivo were found when cells were transduced with the basic fibroblast growth factor gene. In the vertical growth phase the cells were able to invade the dermis and an irregular basement membrane was formed. In metastatic melanoma, cells rapidly proliferated and aggressively invaded deep into the dermis, in a growth pattern very similar to the pattern in vivo. Boccardo and co workers used organotypic cultures of human keratinocytes to evaluate the effects of TNF-alpha in cells that expressed HPV-18 oncogenes. [60]. Another example of the utilization of organotypic culture in epithelial tumor models is described by Hoskins who studied Fanconi anaemia (FA). They described the growth and molecular properties of FA-associated cancers (FANCA)-deficient versus FANCA-corrected HPV E6 ⁄ E7 immortalized keratinocytes in monolayer and organotypic epithelial raft cultures .[61].

iv. Skin disorders and clinical applications

Skin reconstructs are currently being tested and used in clinics for several skin pathologies. Disorders which may benefit from the development of human skin equivalents include psoriasis, vitiligo, keloids, nevus and genodermatoses such as xeroderma pigmentosum [62].

The main use of homologous skin grafts is in the treatment of severe burns and skin disorders. The main new clinical indications for skin allografts include skin loss, surgical wounds, and genodermatoses. Two key factors considered essential for the utilization of skin substitutes in clinical applications is the ability to grow keratinocytes in vitro and the increasing

practice of early wound excision in the extensively burned patient [63].

CONCLUSION

In patients with major burns, artificial dermis allows early wound closure as good a take as allograft and when covered with a epidermal graft it provides a permanent cover which is satisfactory compared to currently available skin grafting techniques and uses donor grafts which are thinner and leave donor sites that heal faster and it is cost effective method [64] [65].

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