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AN OVERVIEW OF ARTIFICIAL SKIN

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Abstract:
Background: *The skin is a multifunctional organ that is protective, self-healing and capable of sensing and many forms of artificial skins have been developed with properties and functionalities approximating those of natural skin.*
Objective: *Objective of this review article is about the different materials used for the preparation of artificial skin, skin grafting, skin bioprinting and manufacturing process of artificial skin.*
Study Selection: *In bioengineering process, the production of artificial skin substitute is increases gradually and at the same time the synthesis of keratinocytes by in vitro culture also. Artificial skin came to include products used for the clinical treatment of acute and chronic wounds as well as laboratory models for the study of the basic biology of the skin.*
Methods and Materials: *Different Biomaterials used in the preparation of Artificial Skin such as Collagen, PLGA, glucophage, Bovine type 1 collagen, HA, Chitosan. Two manufacturing process are commonly used, they are Mesh Scaffolding Method and Collagen Method.*
Skin Substitutes are classified in to three class such as Temporary and Impervious Dressing Materials, Single Layer Durable Skin Substitutes, Composite Skin Substitutes.
The first-class materials again classified in to two more types such as Single layer materials, Double layer materials produced by tissue engineering. Similarly, second class materials are classified in to epidermal substitutes, dermal substitutes. Class three composite skin substitutes classified in to Human skin substitutes, Produced by tissue engineering.
Conclusion: *The main study of this review article, includes the artificial skin technologies are to provide protection from infection, dehydration, and protein loss after severe skin loss or damage.*
Keywords: *Artificial Skin, Skin Bioprinting, Skin Substitutes, Biomaterials*

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INTRODUCTION:

Human skin is comprised of two primary layers, the dermis and the epidermis. The epidermis is the outermost layer which comprises of keratinocytes that vary in levels of differentiation. There will be no blood vessels in the epidermis which means that nutrient transport occurs from the dermis below [1]. The dermis contains several structures which are important to skin function: hair follicles sweat and oil glands, and nerves are all found within the dermis. The epithelial keratinocytes also originate from within this layer.

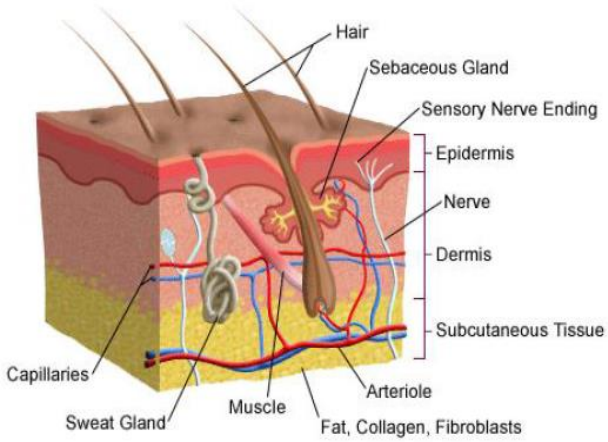

Figure No - 1: Skin

It is a complex multi-layered tissue and it has three main layers which are the epidermis, dermis and hypodermis layer [2]. The outermost skin wall of thin cellular membrane known as the epidermis [3], has an approximate thickness of around 0.5mm (eyelid) to 1.5mm on the palm and soles of feet, also it comprises of five distinct layers which are stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale [4].

The second main layer of human skin is the dermis which is encompassed of collagen and elastin fibres in a matrix that controls the skin strength and flexibility [5]. The skin also has the ability to avoid excessive water loss as well as provides ultra violet (UV) protection. It is highly non-linear, anisotropic, heterogeneous, viscoelastic and almost incompressible material, whereby biomechanical properties of skin also dependent on various aspects such as donor age and dehydration level.

SKIN BURNS:

A burn is when you have tissue damage, usually after the contact with heat. According to the World Health Organization [6], burns are estimated to have 19500 death cases annually and it has became one of the global public health problems, with the majority of these cases occurring in low and middle-income countries like Malaysia.

There are three types of burns:

- **First-degree burns** (superficial burns) are mild compared to other burns. They cause pain and reddening of the epidermis (outer layer of the skin).
- **Second-degree burns** (partial thickness burns) affects the epidermis and the dermis (lower layer of skin). They cause pain, redness, swelling, and blistering.
- **Third-degree burns** (full thickness burns) go through the dermis and affect deeper tissues. They result in white or blackened, charred skin that may be numb.
- **Fourth-degree burns** go even deeper than third-degree burns and can affect your muscles and bones. Nerve endings are also damaged or destroyed, so there's no feeling in the burned area.

SKIN TREATMENT:

Skin Transplantation:

The conventional treatment of chronic wounds includes the use of the autografts, allografts (usually taken from cadavers), or xenografts, which are typically harvested from porcine skin [7]. Because of the low risk of immune rejection, autografts are most often used for skin regeneration.

Skin Graft:

Skin grafting is a surgical procedure which involves removing skin from one area of the body and moving it, or transplanting it, to a different area of the body. This surgery may be done if a part of your body has lost its protective covering of the skin due to burns, injury, or illness. Skin tissue required for a graft can be obtained from three possible sources. It could come from the patient for an autograft, another human for an allograft, or an animal for a xenograft.

TYPES OF SKIN GRAFTS:

There are two types of skin grafts: split-thickness and full-thickness grafts.

Split-thickness grafts:

A split-thickness graft which involves removing the top layer of the skin — the epidermis — as well as a portion of the deeper layer of the skin, called the dermis. These layers are taken from the donor site, it is the area where the healthy skin is located. Split-thickness skin grafts are usually harvested from the front or outer thigh, abdomen, buttocks, or back.

Full-thickness grafts:
A full-thickness graft which involves removing all of the epidermis and the dermis from the donor site. These are normally taken from the abdomen, groin, forearm, or from the area above the clavicle (collarbone). Full-thickness grafts are normally used for small wounds on highly visible parts of the body, such as the face.

Skin Cell Culture:
The organ cultures can be made by using large skin fragments. When such large explants of human skin (2x 2 or 2 x 3 cm) were placed on the bottom of a petri dish and are completely covered by an ordinary tissue culture medium, the explants underwent lasted about 2 weeks, the epidermis will be separated from the dermis.

ARTIFICIAL SKIN:
The term Artificial skin is used to describe any kind of material designed and is used to replace naturally growing skin. The primary application of such materials right now revolves around treating the individuals that have suffered severe tissue loss due to disease or trauma (e.g., skin cancers, burns). Alternatively, the artificial skin is now being used in some places to treat the patients who suffer with foot ulcers.

Each year, over 2 million people in the United States are treated for burns. Artificial skin is employed to seal a wound quickly to minimize the amount of fluid lost by the patient and to limit the bacterial intrusion. Integra is a brand of artificial skin which is commonly used in medical facilities today. Wounds occur as a result of thermal or physical injury, causing a break in continuity of the epithelial layer of the skin or mucosa [8].

The skin healing process may be divided into four major phases:
(1) Haemostasis phase (just after injury) aimed at stopping bleeding,
(2) The inflammatory phase,
(3) The proliferation phase, and
(4) The maturation phase(remodelling)

Biomaterials Used in Artificial Skin:
Artificial skin grafts are normally fabricated using either natural polymers, like collagen, gelatin, chitosan, fibrin, and HA or synthetic polymers, e.g., poly ethylene glycol (PEG) or polylactic-co-glycolic acid (PLGA) [9]. The biomaterials possess some important features like low antigenicity, good biodegradability, low toxicity, as well as low risk of chronic inflammatory responses and rejection [10].
Naturally occurring materials (e.g., fibrin, fibronectin, collagen, HA, chitosan, and glycosaminoglycans–GAGs), which have the ability to restore the physiological functionality of the ECM, are preferentially used for the development of artificial skin substitutes because they have been reported to provide the best healing process for the chronic wounds.

Table No – 1: Bioengineered artificial skin grafts made of various natural and synthetic polymers

Biomaterial Composition	Type of Graft	Cellular Content	Demonstrated Effect Invitro or Invivo
Collagen, PLGA, glucophage	Dermal	Acellular	Increased collagen content and accelerated healing of diabetic wounds in rats
Bovine type 1 collagen, HA	Dermal	Acellular	Improved granulation tissue formation in full-thickness skin defect rat model
Collagen, alginate, curcumin-loaded chitosan nanoparticles	Dermal	Acellular	Enhanced healing with complete re-epithelialization of diabetic wounds in a rat model
Fibrin	Dermal	Cellular(fibroblasts)	Promotion of wound bed maturation in diabetic rats
Collagen, HA, EGF	Dermal	Cellular(fibroblasts)	Increased VEGF and HGF release by fibroblasts invitro
Chitosan	Dermal	Cellular (fibroblasts, hair follicle stem cells)	Accelerated full-thickness wound healing in irradiated rats and reduced scarring

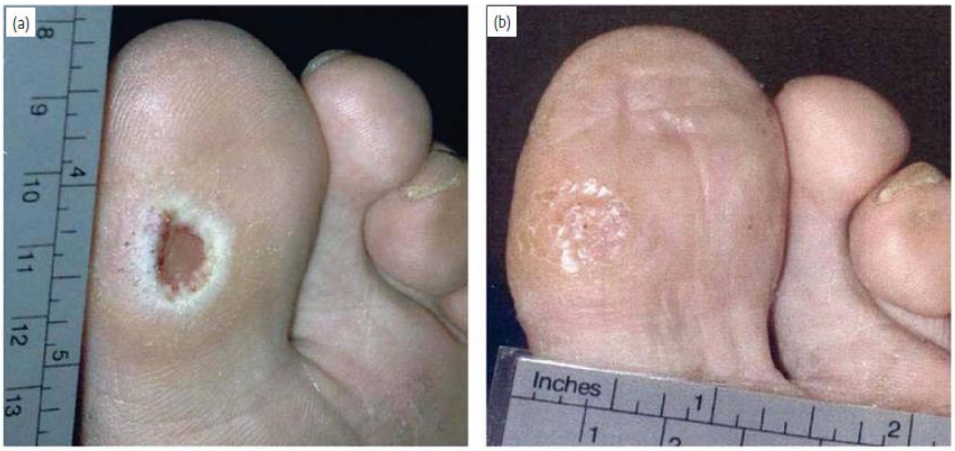

Figure No – 2: a.) A neuropathic diabetic ulcer that has failed to heal for 3years, and b.) After eight applications of the patient's own cells delivered on the My skin carrier

My skin is a synthetic polymer of acrylic acid which is coated onto a medical grade silicone by plasma polymerization [11]. This avoids the use of bovine collagen. Figure shows an example of a diabetic ulcer that resisted healing for three years but did heal after eight applications of the patient's own cells delivered on the My skin carrier in an outpatient clinic [12].

MANUFACTURING PROCESS:

The manufacturing process is simple. Its main function is to trick the extracted fibroblasts into believing that they are in the human body so that they will communicate with each other in the natural way to create the new skin.

Mesh Scaffolding Method:

- **Fibroblasts are thawed and expanded.** The fibroblasts are transferred from the vials into the roller bottles, which resembles litre soda bottles. The bottles are rotated on their sides for three to four weeks. The rolling action provides the circulation of oxygen, essential to the growth process.
- **Cells are transferred to a culture system.** The cells are then removed from the roller bottles, and are combined with a nutrient-rich media, flowed through tubes into thin, cassette-like bioreactors housing the biodegradable mesh scaffolding, and sterilized with e-beam radiation. As the cells flow into the cassettes, they will adhere to the mesh and they will begin to grow. The cells are flowed back and forth for three to four weeks. Each day, the leftover cell suspension is removed and fresh nutrient is added. Oxygen, pH, nutrient flow, and temperature are controlled by the culture system. As the new cells create a layer of dermal skin, the polymer will disintegrate.
- **Growth cycle completed.** After the completion of cell growth on the mesh, the tissue is rinsed with more nutrient-rich media. A cryoprotectant is added and the cassettes are stored individually, labeled, and frozen.

Collagen Method:

- **Cells are transferred to a culture system.** Small amount of cold collagen and the nutrient media, approximately 12% of the combined solution, are added to the fibroblasts. The mixture is then dispensed into molds and allowed to come to room temperature. As the collagen warms, it gels, trapping the fibroblasts and it will help in generating the growth of the new skin cells.
- **Keratinocytes added.** Two weeks after the collagen is added to the fibroblasts, the extracted keratinocytes are thawed and seeded onto the new dermal skin. They are allowed to grow for several days and then exposed to air, inducing the keratinocytes to form epidermal layers.
- **Growth cycle completed.** The new skin is stored in sterile containers until needed.

SKIN BIOPRINTING:

Bioprinting is an advanced manufacturing platform based on the conventional 3D printing which enables the predefined deposition of the biomaterials, living

cells, and growth factors using computer-aided design (CAD) to fabricate custom designed tissue constructs by layer-by-layer printing process with a high degree of flexibility and repeatability [13]. Computer-aided design and computer-aided manufacturing (CAD–CAM) tools which are also being used to generate a complex 3D image for bioprinting. The basic biomaterial which is used for bioprinting is, the bio ink which is prepared in fluid form and then fed into the printer either in one mixture or separate portions that are mixed in the body or at the nozzle of the printer. The materials which are currently used in the bioprinting are mainly based on the natural polymers like alginate [14], gelatin [15].

An ideal bio printed skin should have certain attributes such as being biocompatible, desired mechanical properties to match the tissue, an appropriate surface chemistry and be highly porous with a network of interconnected pores that will allow cells to attach and should be able to transport nutrients and should remove the wound exudates.

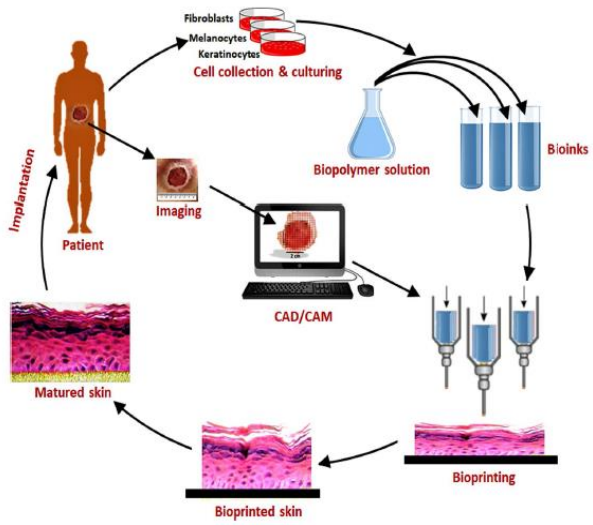

Figure No – 3: Steps in the fabrication of bio printed skin

Various cells such as keratinocytes, fibroblasts and melanocytes should be collected from the patient and should be grown and multiplied in cell culture system. A suitable biopolymer is mixed with the cells and the formed bio ink is fed to the bioprinting system. Features of the wound are captured and a 3D structure is reconstructed using CAD/CAM approaches. According to the 3D pattern, the wound tissue will be reconstructed, and is allowed for maturation in vitro and implanted back to the patient.

SKIN SUBSTITUTES:

Skin substitutes are a heterogeneous group of biological and or synthetic elements which enable the temporary or permanent occlusion of wounds.

Properties of the ideal dermal-epidermal substitute:

- Hypoxia tolerant
- Broad availability
- Presence of dermal and epidermal components
- Rheology comparable to the skin
- Resistance to infection
- Suitable cost / effectiveness
- Easy to prepare
- Low antigenicity

Several skin substitutes are currently available for a variety of applications, which enables the choice of a suitable substitute for each clinical application. Skin substitutes are classified into class I, class II, and class III.

Class I – Temporary and Impervious Dressing Materials:

a) Single layer materials:

• biological products – amniotic membrane

• synthetic materials – membrane or synthetic polymerfilms (Opsite®, Hydrofilm®, Tegaderm®).

b) Double layer materials produced by tissue engineering:

• Transcyte®

Class II – Single Layer Durable Skin Substitutes:

a) Epidermal substitutes:

•EpiDex®

b) Dermal substitutes:

• Swine collagen membranes:

1.OASIS Wound Matrix®

2. Permacol®

• Dermal matrices of bovine origin:

1. Matriderm

2. PriMatrix®

• Dermal matrices of human origin:

1. Alloderm®

Class III – Composite Skin Substitutes:

a) Human skin substitutes:

• Allogeneic skin grafts

b) Produced by tissue engineering:

• Integra®

• Biobrane®

• Orcel®

• Apligraft®

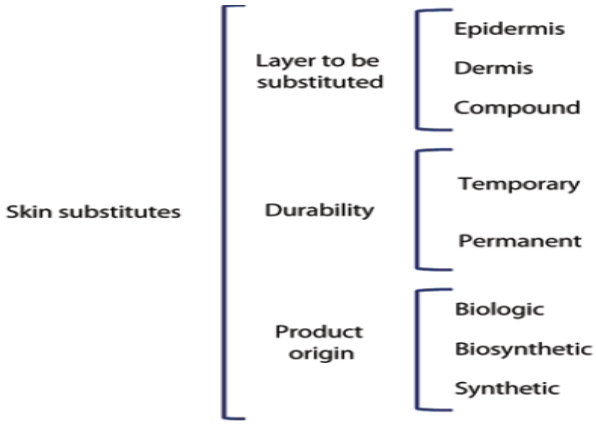

Figure No – 4: Classification of Skin Substitutes proposed by The Plastic Surgery Service of Hospital D as Clinic as of The School of Medicine of Universidade De Sao Paulo

Table No – 2: Major skin substitutes available in the world market and their classification according to location, time of permanence, and origin criteria

Product	Classification	Composition
Cadaver skin (non-commercial product)	CPb	Human skin, allogeneic, without cells, preserved in glycerol
Integra®	CPbs	Acellular and bilaminar: bovine collagen matrix and chondroitin-6-sulphate (dermal analogous), recovered with a thin lamina of silicone
Biobrane®	CPbs	Bilaminar: nylon mesh filled with type I porcine collagen and covered by a thin lamina of silicone
Alloderm®	DPb	Acellular dermal matrix derived from human skin of cadaver
OASIS®	DTb	Matrix of dermal regeneration derived from swine jejunum submucosa
Permacol®	DTb	Derivative from porcine dermis collagen and elastin
Matriderm®	DPb	Three-dimensional matrix of collagen and elastin
Epidex®	EPb	Generated by autologous cultured keratinocyte from scalp hair follicles

b = biological; bs = biosynthetic; C = composed; D = dermis; E = epidermis; P = permanent; T = temporary

BILAYER ARTIFICIAL SKIN CAPABLE OF SUSTAINED RELEASE OF AN ANTIBIOTIC:

The bilayer "artificial skin", stage 1 membrane, which is composed of an upper silicone sheet and a lower sponge sheet of a collagen and glycosaminoglycan (GAG), was first developed by Yannas, Burke and their colleagues [16].

M-TOB was added to the solution of atelocollagen to yield a collagen layer which is capable of sustained release of tobramycin. The collagen layer with the M-TOB serves as a reservoir of M-TOB, while the collagen sponge sheet, which is spontaneously converted into synthesized connective tissue matrix with infiltration of the fibroblasts and capillaries, does not contain M-TOB. The collagen sheet with M-

TOB was attached to a silicone layer, 25 µm thick, to make a combined sheet. The combined sheet, will permits the sustained release of an antibiotic, which constitutes the upper layer of the new bilayer "artificial skin".

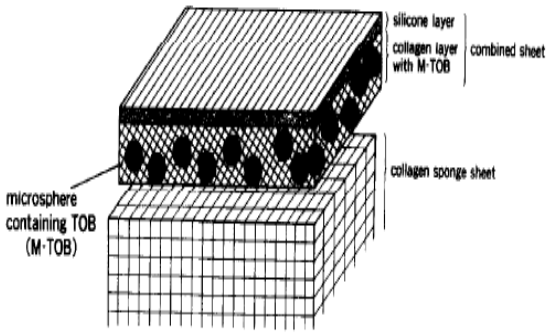

Figure No -4: Structure of "artificial skin" capable of sustained release of tobramycin; silicone layer attached to collagen layer as reservoir of M –TOB

The results were that the sustained release of tobramycin into saline from the combined sheet continued for atleast 2 weeks, similar to the release from M-TOB, and the combined sheet inhibited the proliferation of bacteria for a period of time. The "artificial skin" with continuously released tobramycin was used in treating six patients with the bums. Three weeks after the first operation, in which the "artificial skin" was applied, silicone layer was peeled off and a thin split thickness skin graft, approximately 0.2 mm thick, was grafted onto the newly synthesised dermis-like tissue.

COMPLICATIONS AND FAILURE:

The two largest risks with artificial skin are the onset of infection and rejection by the immune system. Lack of blood flow to the implant can lead to cell death which facilitates breeding of bacteria. Allografts and xenografts are often the only choice for burn patients who do not have sufficient skin remaining for an autograft. In these cases, there is a high rate of immunological rejection.

Skin adhesion is also a problem when using Integra if the epidermis does not fuse correctly to the implant. Grafts can also fail if they are subjected to excessive stresses from movement and sheering. Movement disrupts the adherence of the graft to the patient and occurs frequently when the graft site is over flexor or extensor surfaces [17].

ONGOING RESEARCH:

While artificial skin has aided significantly in skin regeneration, there still remain several areas for improvement. A minimum of two surgical treatments are required for severe burn patients using artificial skin. Matridermis an upcoming treatment for burn victims similar to Integra. Unlike Integra, the dermis matrix and epidermis cells are able to be put in place in one surgical session [18].

Research is now also being done to attempt to develop bacteria-resistant skin cell cultures that can be used in artificial tissue. Ideally, this would allow in vitro replication of a patient's own genetically modified skin cells. These cells could then be put into the artificial matrix for bacteria-free growth.

Synthetic skin is being developed in hopes of enabling the sensation of touch to non-living structures. Flexible pressure transducers may eventually allow us to create an electronic "skin" with the ability to mimic touch. This currently has applications in robotics including advanced prosthetic limbs.

CONCLUSION:

The ultimate goal of current artificial skin technologies is to provide protection from infection, dehydration, and protein loss after severe skin loss or damage.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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