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TISSUE ENGINEERING OF SKIN

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ABSTRACT:

Tissue engineering (TE) and life sciences tools are combined to develop bioartificial substitutes for organs and tissues, which can in turn be applied in regenerative medicine, pharmaceutical, diagnostic, and basic research to elucidate fundamental aspects of cell functions i*n vivo*or to identify mechanisms involved in aging processes and disease onset and progression. The complex three-dimensional (3D) microenvironment in which cells are organized *in vivo* allows the interaction between different cell types and between cells and the extracellular matrix, the composition of which varies as a function of the tissue, the degree of maturation, and health conditions. In this context, 3D *in vitro* models can more realistically reproduce a tissue or organ than two-dimensional (2D) models. Moreover, they can overcome the limitations of animal models and reduce the need for *in vivo*tests, according to the "3Rs" guiding principles for a more ethical research. The design of 3D engineered tissue models is currently in its development stage, showing high potential in overcoming the limitations of already available models. However, many issues are still opened, concerning the identification of the optimal scaffold-forming materials, cell source and biofabrication technology, and the best cell culture conditions (biochemical and physical cues) to finely replicate the native tissue and the surrounding environment. In the near future, 3D tissue-engineered models are expected to become useful tools in the preliminary testing and screening of drugs and therapies and in the investigation of the molecular mechanisms underpinning disease onset and progression. In this review, the application of TE principles to the design of *in vitro*3D models will be surveyed, with a focus on the strengths and weaknesses of this emerging approach. In addition, a brief overview on the development of *in vitro*models of healthy and pathological bone, heart, pancreas, and liver will be presented.

KEY WORDS: Tissue engineering and Tissue engineered skin

INTRODUCTION:

Tissue engineering is a recent biotechnological approach, which has emerged for thetreatment of disease involving loss of structure of human body. The technology essentially involves growth and transplantation of new tissues and organs.

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti is "An interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve [Biological tissue] function or a whole organ"

Tissue engineering utilizes living cells as engineering materials.Examples include using living fibroblasts in skin replacement or repair , cartilage repaired with living,

chondriocytes, or other types of cells used in other ways. Our skin is a major organ of the body that act as a barrier to pathogens and trauma . Skin defects caused by burns ,venous and diabetic ulcers , or acute injury occasionally induce life threatening situations .

Thus , the need for a functional and cost efective permanent skin substitute for burn victims has always been garnered.Tissue engineering covers a broad range of applications, in practice the term has come to represent applications that repair or replace structural tissues (i.e., bone, cartilage, blood vessels, bladder, etc). These are tissues that function by virtue of their mechanical properties. A closely related (and older) field is cell transplantation. This field is concerned with the transplantation of cells that perform a specific biochemical function (e.g., an artificial pancreas, or an artificial liver). Tissue engineering solves problems by using living cells as engineering materials. These could be artificial skin that includes living fibroblasts, cartilage repaired with living chondrocytes, or other types of cells used in other ways.

- Tissue engineered heart valves offer a promising alternative for the replacement of diseased heart valves avoiding the limitations faced with currently available bioprosthetic and mechanical heart valves.
- Tissue-engineered skin is significant advance in the field of wound healing and was developed due to limitations associated with the use of autografts.

- The recent advancements in the Tissue Engineering for Artificial Organs

as well as some of the major challenges and the future of tissue engineering are also briefley discussed.

REQUIREMENT OF TISSUE ENGINEERING:

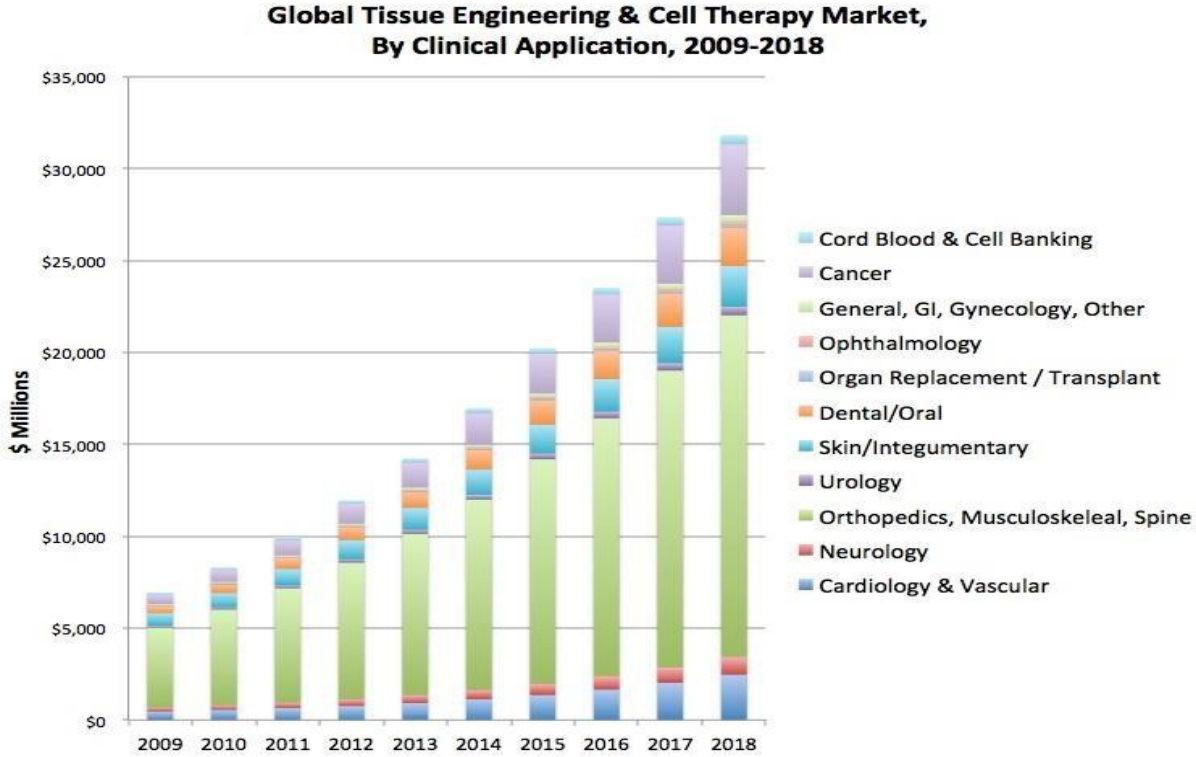
The clinical need for tissue engineering and regenerative medicine is the result of our urge to treat defective tissues. Regardless of how such defects occurred (congenital or acquired), traditional medical tools are not yet capable of completely or efficiently fixing them. In fact, traditional medicine has severe limitations in delivering solutions for numerous health problems. Injuries and diseases are traditionally treated using pharmaceuticals, whereas prosthetic devices and organ transplantation are used in more severe conditions. While pharmaceuticals may be useful for the treatment of numerous conditions, they cannot cure a number of deadly diseases (e.g., several forms of cancers, strokes, diabetes, etc.) or diseases at their advanced stages (e.g., Alzheimer's, Parkinson's, osteoarthritis, etc.). On the other hand, prosthetic devices are not capable of restoring normal function, and the number of organ donors is always way less than required. Tissue engineering can be used to treat diseases that

cannot be cured with regular pharmaceuticals and to provide natural, living, functional organs to overcome the need for donors and prosthetics. The main goal of tissue engineering is the development of functional substitutes for damaged tissues It is estimated that the majority of tissue engineering products are used for the treatment of injuries and congenital defects, while tissue engineering products used for the treatment of diseases are less common. The worldwide tissue engineering and cell therapy market has been estimated in 2014 at about $15 billion and is expected to grow up to $32 billion by 2018. The dominant market is in the orthopedic, musculoskeletal, and spine areas followed by the skin, nervous tissues, and other organs Skin was the first tissue to be engineered; this is because of the relatively simple structure of the tissue (can be prepared using two-dimensional (2D) culture and has easy access to culturing medium). Skin is also an important tissue engineering target because of

the high demand especially resulting from war burns.
Skin damage can cause disfigurement and disability, which may lead to further serious infections and psychological damage to patients. All these factors made skin one of the first clinical tissue engineering targets. Tissue engineering and regenerativemedicine solutions can adiffer between targets. Examples include the heart, kidneys, cornea, nervous tissues, liver, intestines, pancreas, lungs, bone, muscle, and so on.
The ultimate goal is that tissue engineering and regenerative medicine would one daybe able to overcome the need for organ transplantation. The medical need for tissue engineering and regenerative medicine can be emphasized in the donor waiting list, which is always increasing at a higher pace than the number of organ donors. The ability to engineer such organs or help them regenerate would represent a greatlso beapplied for any tissue, although the levels of complexity wouldleap in the history of the health care field.

GOALS OF TISSUE ENGINEERING SOLVED:

Every year there are so many cases where patients are suffering from skin burns,neurological disorders , cardiovascular problemsetc they are cured and treated by tissue engineering techniques . Following data represent how tissue engineering and cell therapy techniques treated the patients upto 2018 year :



The last few decades have witnessed major steps in health care, leading to improved surgical procedures and better management of diseases. All in all, the advances in the health care have raised life expectancy, augmenting vulnerability to diseases and organ failure. Consequently, the aforementioned advancements have led to an increased demand for tissues and organs. The ultimate goal of tissue engineering is tobridge the constantly growing gap between organ demand and availability by producing complete organs [157]. This area is expected to become increasingly applied as a valid clinical solution. Stem cells will continue to be investigated for their differentiation potential, and more applications will be developed in the future. The major challenge for stem cells, whether induced, embryonic, or adult, is to achieve commitment to the desired lineages. It is expected that more applications using stem

cells will reach clinical trials in the near future. Furthermore, gene therapy (silencing and activation of target genes) and drug delivery are both expected to be used to help maintain the desired cell phenotype. The ultimate goal would be to engineer immune-transparent stem-like cells with clear protocols, enabling their committed differentiation to targeted tissues. Developments in basic and applied science related to the fabrication of tissue engineering scaffolds will be a major future target. High-throughput screening techniques might prove useful to determine combinatorial effects of molecules and materials on various cell types. Decellularizedtissues are also expected to remain an important source of scaffolds given their high abundance as well as their right chemical and structural composition. Potential limitations of such scaffolds will always be the shortage of supplies (e.g., scaffolds from allogeneic sources), potential immunoreactions, and ethical concerns (e.g., scaffolds from xenogeneic origins). In future, it is expected that new biomaterials will be developed incorporating selected molecules to address targeted tissues. Moreover, many basic science studies will be conducted to identify the effects of molecules on cells and determine the right degradation rate and material properties (porosity, mechanical properties, and structural properties) suitable for each tissue engineering application. An ultimate goal would be to combine scaffolds and cells to engineer tissues in vitro, which can be decellularized to produce customizable off-the-shelf

tissue sources for various engineering applications. Future research will continue to reveal the roles of ECM moleculesThe mechanisms through which cells perceive loadand react to their surrounding environment are only starting to be revealed and comprise stretch-activated ion channels and integrins . Understanding these mechanisms will provide the basis for developing new tissue engineering tools and bioreactors, and possibly discovering new useful molecules for the treatment of sick organs and tissues. Future bioreactors will be able to perform complex combinatorial tasks in order to engineer full organs. For example, bioreactors can be designed to deliver varying oxygen levels to varying parts of the engineered tissue or different mechanical stimulation regimes, or to deliver growth factors and molecules 1.8 Conclusions 23 at predefined time points during culture. Finally, bioreactors may be made to be used on site (e.g., in the hospital) to minimize contamination risks and reduce the surgery time. 1.8 Conclusions The field of tissue engineering has witnessed tremendous development in the past.

PROCESS OF TISSUE ENGINEERING

(1) Start building material (e.g., extracellular matrix, biodegradable polymer).
(2) Shape it as needed.
(3) Seed it with living cells .
(4) Bathe it with growth factors.
(5) Cells multiply & fill up the scaffold & grow into three-dimensional tissue.
(6) Implanted in the body.
(7) Cells recreate their intended tissue functions.
(8) Blood vessels attach themselves to the new tissue.
(9) The scaffold dissolves.
(10) The newly grown tissue eventually blends in with its surroundings.

Extraction From fluid tissues such as blood, cells are extracted by bulk methods, usuallycentrifugation or apheresis.From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix (ECM) that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis. .CELLS AS BUILDING BLOCKS Tissue

engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes.
Types of cells Cells are often categorized by their source:

- Autologous cells are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogentransmission, however in some cases might not be available.

Allogeneic cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for in vitro studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.

Xenogenic cells are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.

Isogenic cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models. •Primary cells are from an organism. • Secondary cells are from a cell bank. Stem cells are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells.

According to their source stem cells are divided multipotent, pluripotent& totipotent.

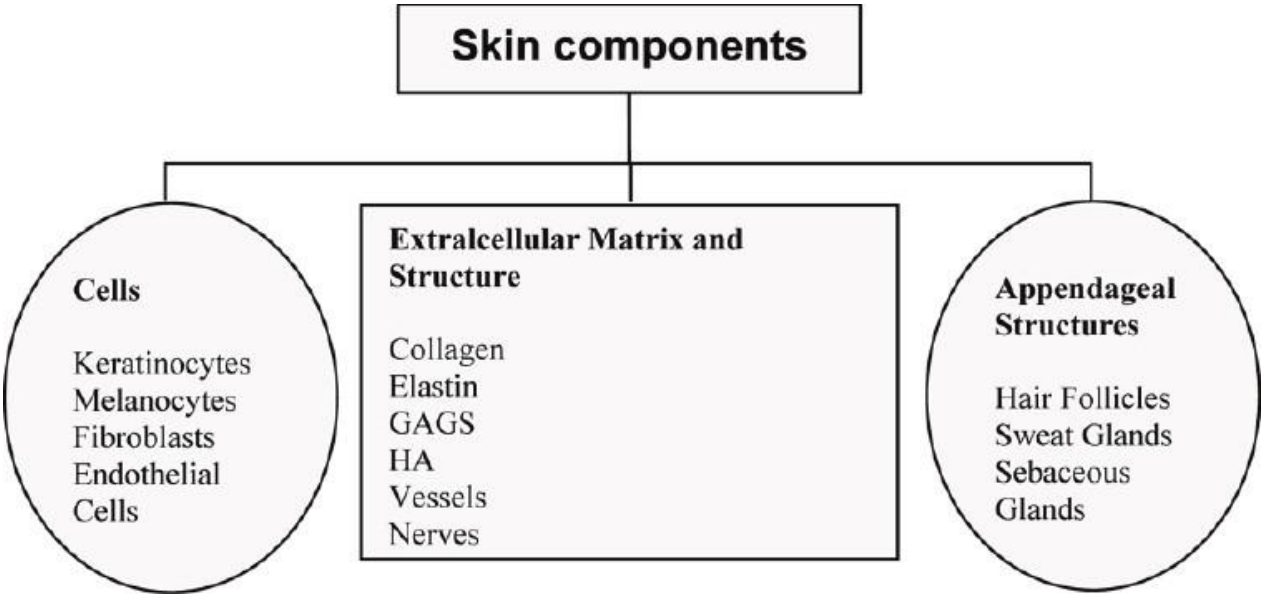
SCAFFOLDS Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds Scaffolds usually serve at least one of the following purposes: Allow cell attachment and migration .

Deliver and retain cells and biochemical factors :

- Enable diffusion of vital cell nutrients and expressed products .Exert certain mechanical and biological influences to modify the behaviour of the cell phase.

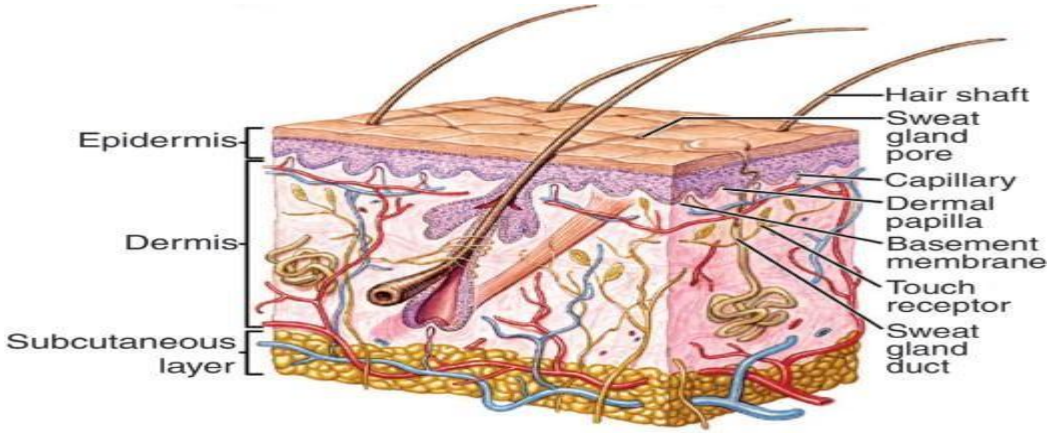
REVIEW OF LITERATURE:

Skin tissue engineering aims at **reconstructing the structural and functional components of skin**, reducing scar formation, and improving the quality of wound healing. Biomaterial combinations and novel scaffold fabrication techniques will further bring scaffold closer to ECM-mimicking bioenvironment.
Growing knowledge about cell manipulation andStem cell differentiation has opened new horizons in the field, providing larger cell pools for all tissue engineering applications. Autologous cells are consideredthe favorite cell type for engineering tissues, as they do not evoke immuneresponses and thus eliminate the need for immunosuppressants and their sideeffects . However, autologous cells are limited in supplies and require a longculture period to engineer the desired tissues. Much of current research aimsto use allogeneic or xenogeneic sources to overcome the shortageof autologous cell availability. The use of allogeneic or xenogeneic sources is, though, still associated with major obstacles, such as immune-rejection, transmission of diseases, mismatch between donor and recipient cellular microenvironment, and ethical considerations, which limit their widespread adoption in clinical applications .



FEATURES OF SKIN TO BE KNOWN BEFORE IT IS TO BE TREATED UNDER TISSUE ENGINEERING:

STRUCTURE OF SKIN:



To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal.

BIOMATERIALS USED: Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Examples of the materials are collagen and some polyesters. New biomaterials have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc. A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body.

Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues.
Biocompatible materials used to fabricate scaffolds to support the growth of cells intissue like structure.

Two classes – Biodegradable (that are degraded at the site of application due to enzymatic action) and non biodegradable polymers (that do not degrade due tobiological action.)
It should not impart any adverse effect on the growth of cells.It should be non-immunogenic ,biodegradable , hydrophyllic in nature .
It should be easily processed into scaffolds of appropriate size, shape and porosity.

DESIGN OF 3-D SCAFFOLD:

Scaffolds provide temporary support to the seeded cells to foster the growth. During the initial growth phase, scaffold degrades . And at the same time growing tissue produce its own ECM to maintain integrity .Degradation depends on ph and release of enzymes from the growing tissue.
Therefore homogenious distribution of cells inside the scaffolds is required. TISSUE ENGINEERED SKIN:
Intially sheets of human keratinocytes could be grown in laboratory. Cohesive sheets of cells are cultured from donor on feeder layer of fibroblasts .But treatment without dermal layer is of limited use . Various skin substitutes used as tissue engineered constructs are:
INTEGRA: The first and simplest is a collagen-glycosaminoglycan sponge. It is used to carry seeded cells.It consists of insoluble bovine collagen type 1 and glycosaminoglycan chondrotin sulfate in a ratio 98:2 :
DERMAGRAFT: these consist of PGA polymer mesh seeded with human dermal fibroblast . These both can be covered in a keratinocyte sheet at the time of implantation.
APLIGRAFT: is a bilayered bioengineered skin substitute and was the first engineered skin US Food and Drug Administration (FDA)-approved to promote the healing of ulcers that have failed standard wound care.
SOME MORE EXAMPLES OF TISSUE ENGINEERING ARE:

Tissue engineered autologous heart valves and vessels .

In vitro meat- Edible artificial animal muscle tissue cultured in vitro .

Bioartificial liver device several research efforts have produced hepatic assist devicesutlilizing living hepatocytes .
Artificial Pancreas – research involves using islet cells to produce and regulate insulin ,particularly in cases of diabetes.
The best material for wound closure is the patient's own skin , however autografting has several limitations.

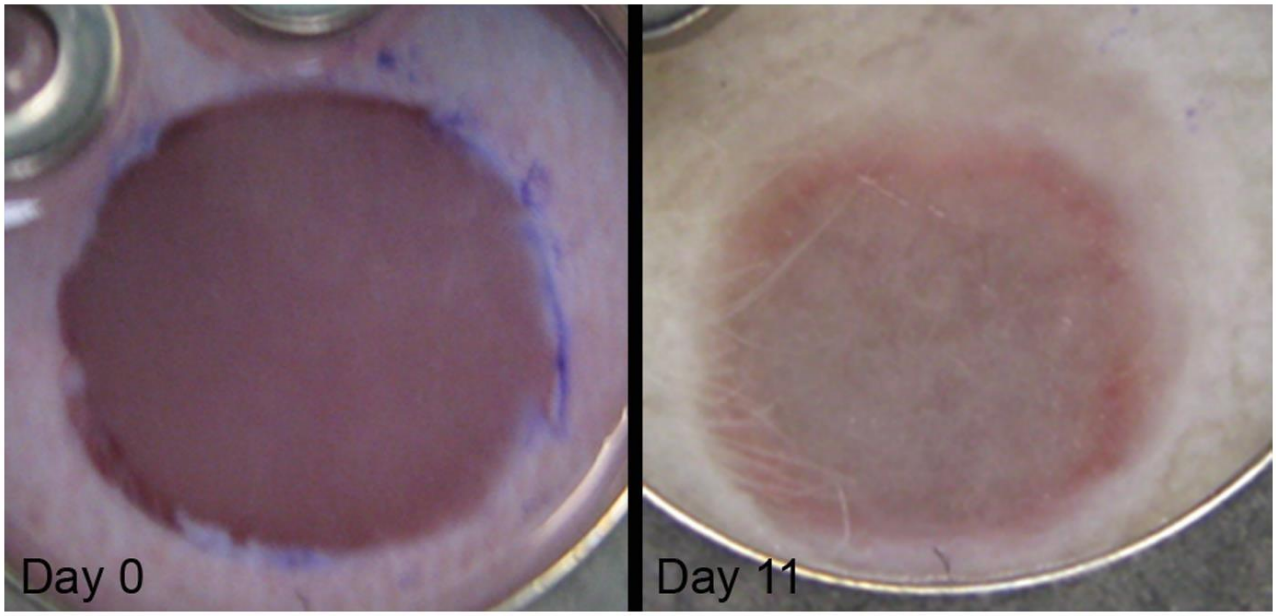
- The donor site is a new wound
- Scarring and pigmentationchanges occur
- Dermis is not replaced
- Donor site is a potential site for infection .
- Donor site is not unlimited.
- Extensive burns makes it impossible .
- Graft rejection is a major problem in cases other than autologous.

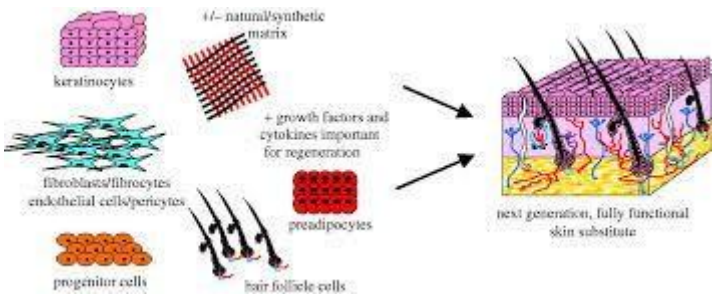
Hospital cost :

Burns are one of the most expensive catastropic injuries to treat. For example , a burn of 30% of total body area can cost as much as $200,000 in initial hospitalization costs and physicians fees. The cost of waiting for your skin to grow can be more painful than the burn itself.

REULS AND DISCUSSION:

Tissue Engineered Skin:





FUTURE CHALLENGES FACED BY TISSUE ENGINEERING IN VARIOUS AREAS:

Nowadays, TE approaches are widely investigated for the development of 3D*in vitro* models of healthy and pathological tissues and organs. The results summarized in this review demonstrate that the TE approach can be successfully employed in the development of 3D models of many human tissues and organs, such as bone, heart, pancreas, and liver. This interdisciplinary field is rapidly developing and advancing. However, despite the already published exciting results in the design, fabrication, and validation of organ/tissue models, there are still challenges that need to be addressed.

The main limitation deals with the identification of the proper cell sources for model design, and in particular with the difficulty to isolate human primary cells and culturethem *in vitro* for long-term experiments, since primary cells show high sensitivity to

culture conditions and progressive loss of differentiation potential after a low number of passages in culture. In the last decade, the most promising novelty in the cell biology field is the discovery of iPSCs. Reprogramming adult cells to embryonic-likestates has innumerable potential applications in regenerative medicine and drug development. iPSC-related research fields are highly active and rapidly developing. iPSCs are interesting cell sources and represent a breakthrough that will ultimately open many new avenues, although many technical and basic science issues remain.

The immature phenotype of differentiated cells derived from progenitor cells (induced, embryonic and adult stem cells) makes them appropriate for neonatal tissue/organs or early-stages diseases models. Moreover, the properties of human- derived cells strongly depend on the tissue source, the patient age and health condition, the adopted isolation/purification technique as well as the applied differentiation protocol.

Three-dimensional cell surrounding environment exerts a synergistic role in guiding cell fate and behavior; therefore, a fine replication *in vitro* of the *in vivo* environment in terms of both architecture and mechanical properties is mandatory. Furthermore, the development of a biomimetic environment is a key aspect in the long-term cultureof any type of cells. Such a goal is challenging, due to the complexity of human organs/tissues and the difficulty to mimic them at different aging and health stages in all the mechanical, topographical and chemical aspects, as well as in the set of physiological cues characteristic of their environment. In this scenario, bringing together new advances in material engineering, microfabrication techniques and microfluidics is gaining more and more importance.The advancement in biomaterials science, including the design and development of new synthetic copolymers, ceramic and glass–ceramics, bioartificial blends of natural and synthetic materials, can be exploited to finely tune the chemical, thermal, mechanical and surface properties of the scaffold-forming materials. The progress of these custom-made materials allows to accurately recapitulate the bulk properties of the native tissue at different health levels.

Furthermore, emergent advanced scaffold fabrication methods are gaining more and more interest as they allow the fabrication of more reproducible scaffolds with a highly controlled process. These include a good control of pore size and interconnection, which facilitates gas diffusion, nutrient supply and waste removal, leading to a degree of vascularization of the constructs similar to native tissues.Some relevant i*n vitro*tissue models have recently started to appear in the literature, improving the confidence that, in the future, the design of 3D models of high quality and relevance can significantly reduce the number of animals used in research as wellas the failure of drug-screening methodologies.

CONCLUSION:

Emergence of the science called tissue engineering is more than just salutary.The purpose of tissue Engineering is to create tissues in in culture for use as replacementtissues for damaged body parts : Skin , cartilage and bone have been synthesized in the laboratory and success has been predicted in the creation of blood vessels , blood and organs such as heart, lungs pancreas and liver . Attempts have been made to create artificial corneas, intestines and heart valves. Bladders have been bioenginnered and implanted in Dogs, with total sucess. There is a needfor reconstruction of form and function .Close collaboration between clinicians and researchers is essential for optimal outcome. Experts from all fields of tissue engineering are needed in the development of the perfect cure.

Future efforts will focus on developing novel biomaterials for the different tissue engineering and regenerative medicine applications. The structure and mechanical properties of the biomaterials will be engineered to better suit the tissue of interest. These biomaterials should be capable of addressing the current

major limitations of the field, especially mass transport. Moreover, the developedbiomaterials are expected to be better tailored to maintain the phenotype of cultured cells and deliver on demand the optimal cocktail of growth factors and cytokines. Research should also focus on materials that would reduce implant

complexity such as injectability or flexibility that allows minimally invasive surgical procedures. Finally, materials that have better integration or stability in the implant site should be designed. Biomaterials with muscle-adhesive proteins and other gluing interfaces may be investigated, or using covalent bonding based on natural residues of tissues and engineered residues on the scaffold. Future research will also focus on cell manipulation (e.g., transfection and silencing) to induce better repair or regeneration. Further understanding at the basic science level of cell behavior, both in vitro and in vivo, in tissue engineering systems including cell–cell interactions and cell–scaffold interactions will be required.

Additionally, the effect of different growth factors as well as ideal amounts and timing of supplementation should be determined for the various tissue engineering applications. In vitro culture techniques should also be revised,

particularly the switch from 2D to 3D systems and oxygen levels to match the invivo situation of thick tissues. Perhaps, systematic studies that compare current in vitro culture systems used in tissue engineering and the in vivo situation will shed light on the biological effects of the currently adopted culturing techniques

This knowledge can be used to improve current cell culture techniques to achieve better tissue repair. Finally, efforts should be made toward optimizing current regulatory and ethical considerations that would pave the way for easierand safer introduction of tissue engineering and regenerative medicine solutionsinto the clinic.

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