Tissue engineering for skin

TISSUE ENGINEERING OF THE SKIN (MECH 5510M) - LITERATURE REVIEW - SID: 200507638

ABSTRACT:

This essay is a literature review on the tissue engineering for skin replacement, with regard to the clinical need, approaches & various commercially-available products. Skin is undoubtedly crucial in the maintenance of the body's internal balance & also protecting externally. It gets severely compromised in burns, non-healing ulcers, reconstructive surgeries etc. bringing down the patient's quality of life drastically. Tissue engineering is a more efficient approach than traditional skin grafting. It is a research area that is forever evolving, where researchers are always working towards one united goal, i. e. to develop in bulk quantities, a skin substitute that can be handled with less care, integrates faster with the body's natural matrix and costs reasonable.

INTRODUCTION:

A lot of research has been undertaken in the past to realize that it is possible to generate skin by applying engineering techniques. This is done by growing skin at a faster rate than normal and in an artificial manner4. Skin is the largest & most widely spread organ in the human body. Its role is to protect the body's internal environment from harshness of the external conditions and restrict entry of microbes, by acting as a barrier4. Several situations & diseases arise, due to which the skin gets irreparably damaged, thus requiring interventional help in restoring it back to health.

Tissue engineering is the application of engineering techniques to develop biological substitutes1. Burns (acute) & ulcers (chronic) are the most

common conditions which require the replacement of skin. In developing countries (Fig 1)2, 3, due to lack of knowledge on safety, a very high number of burns accidents occur every year, and mostly of fatal nature. The fatality is mainly due to pain, infection, loss of body fluids & incapability of the body to self-regenerate large amounts of lost skin5. Thus, experimentation in this field was triggered. Many skin diseases, which lead to necrosis, pigmentation problems, also require engineered skin4.

CLINICAL NEED FOR SKIN TISSUE ENGINEERING:

In most incidents, both the epidermal and dermal layers require replacement. Conventionally, the treatment method involved skin grafting i. e. autologous (self) split-thickness and full-thickness grafting, where healthy skin was taken from other areas of the body and replaced at the injury site. Split-thickness (comprising of the epidermis & a part of the dermis) grafting is not a logical method to use, when a large area of the body (> 50%) is affected & less healthy skin is available. But, it is today's gold standard approach6. Further trauma, due to grafting, can be painful to a patient who is already in a critical state. Also, scar formation post-operatively is another reason for its reduced usage. Full-thickness grafts are suitable to use when the burned area is less than 2% of the total area. These problems could be avoided if skin (of full or partial thickness) were grown artificially and substituted in the place of real skin6.

LITERATURE REVIEW:

The skin can be broadly divided into two layers i. e. the epidermis & dermis. The epidermis is made up of several layers and may/may not consist of extra-cellular matrix (ECM). The layers from surface to deep are: cornified,

granular, spinosal and basalar layers. The most commonly found cells here are the keratinocytes & melanocytes. The dermis is constituted by GAG's & proteins. Within the dermis, fibroblasts are most commonly found8.

Several skin substitutes exist for wound coverage in tissue engineering4. It can be broadly divided into temporary and permanent skin substitutes. The table below (Table 1) is a list of all the material options available for skin replacement:

Table 1: Temporary and Permanent Skin Substitutes8

Permanent tissue engineering of the skin can be broadly divided into three categories6, 8:

- Epidermal replacements - Generally, using autologous keratinocyte sheets. Replaces only the epidermis, but “ take rates” are very poor, suitable for superficial burn treatment only.
- Dermal replacements - Replaces only the dermal layer. In most cases, it is applied along with an epidermal graft to improve “ take rates”.
- Dermo-epidermal (bilayer skin) replacements - Replaces both the epidermis and dermis. Suitable for full-thickness burns.

Skin replacements have two main components i. e. cells and the scaffold. In wound coverage, three types of cells can be used - autologous, allogenic or stem cells. Autologous (self) cell usage is the most preferred as it is easily accepted by the patient's body & does not need incite and anti-immune responses. Allogenic (donor) cells, if used directly can lead to the eventual rejection of the transplant. However, it is used in an acellular fashion, where

the donor keratinocytes are removed prior to culturing9. Stem cells have trans-germal & pluripotential properties & are currently being researched for their poteintial application in skin engineering. Less information is obtained on keratinocyte stem cells. The suggested reason for their longevity is that KSC cycles very slowly and is resistant to mutations8. The type of biodegradable scaffold, either natural or synthetic permits cells to attach onto them and facilitate handling during transplantation6, 9.

Rheinwald & Green Experiment8:

The experiment carried out in 1975 by Rheinwald and Green where human (autologous) keratinocytes were produced in-vitro, proved to be a breakthrough in this field and modified versions of this method are used nowadays. Extracted keratinocytes were allowed to form colonies on a plastic substrate. These colonies expanded to form a sheet. Stratifications arose as the daughter cells, usually at the centre, started multiplying vertically and a 12-cell layer was achieved. To increase the multiplicative capacity of

keratinocytes, a feeder layer (comprising murine Swiss 3T3 lethally irradiated fibroblasts) & mitogens were introduced to the culture.

Epidermal Replacements:

A small skin biopsy of the patient is harvested, which is cultured to produce a patch. The full-thickness biopsy of the patient's skin is cut finely and enzymes are added to cause disaggregation of the skin into cells. A feeder layer, as mentioned previously, is used to culture these cells in culture flasks. To promote proliferation, epidermal growth factors, enzymes such as insulin, hydrocortisone, cholera-toxin and bovine serum are used. After

colonies have been formed, trypsin is added. The KC's are cultured to confluence and later, the sheets are removed from the flasks (using dispase) for use8. The result of this method compared to the split-thickness gold standard is quite poor, as the dermal layer is missing and it depends upon the health of the dermis existing. Also, it is prone to scarring, takes too long, expensive, extremely fragile and has varying “ take” rates6.

Dermal Replacements:

It was claimed, in 1952, that using only pure epidermal sheets, success would be lesser than compared to those with a dermis10. To accentuate the success of the epidermal transplantation, dermal replacements were constructed. A dermal replacement that covered the affected area with cryoprserved allogenic skin was used minus the epidermal layer was used11, 12. Also, an observation that allogenic keratinocytes elicited more anti-immune response than allogenic fibroblasts, was reported. To reconstruct the dermis, the two-stage Integra application is most widely used now13. This dermis functions as a scaffold for the attachment of keratinocytes and improves vascularization9.

Burke et al (1981) developed a dermal replacement, where a collagen sponge was covered with a silastic layer (synthetic). The sponge behaves as a scaffold for the fibroblast cells. This technique was commercialized into a product (Integra Dermal Regeneration Template) 9, 14. A modification to this employed GAG's along with collagen, in the scaffold. Here, a precipitated mixture of bovine collagen fibres and a chondrotin-6-sulfate (GAG from shark cartilage) was freeze dried. This generated a collagen-GAG sponge scaffold, which had a mean pore size. Cross-linkage to strengthen the matrix was

done using gluteraldehyde. Finally, the silastic layer was applied. This is available as a product; Integra Artificial Skin (Chamberlain and Yannas, 1999)9, 15. According to Heimbach et al (1988), this is most suitable for burns patients.

The concept of using absorbable polymer scaffolds (synthetic) such as polyglactin 910 or polyglycolic acid was the next improvement in dermal replacements. Here, allogenic fibroblasts are enzymatically cultured and this culture is mounted on the polymer scaffold for integration). Due to this, an ECM consisting of collagen, growth factors, GAG's etc. is formed, which stays active even after it is frozen17. This was commercialized as Dermagraft 8, 16.

Two-stage dermis application has shown proven results, and now clinical trials are being conducted to examine the applicability of one-stage dermis, such as Matriderm 6. The dermal replacements essentially require an epidermal covering.

Dermo-Epidermal Replacements:

These are available both as autologous or off-the-shelf products. In autologous DED replacements, both keratinocytes and fibroblasts are harvested from the patient and are added to the collagen-GAG scaffold. Cultivation of this in culture medium is for around four weeks. This is a more permanent solution 6, 18, 19.

The first model of today's Apligraf was done by Bell et al (1979)20. DED's use human keratinocytes & fibroblast cells (allogenic) within a scaffold. Morphological studies after using Apligraf reported the presence of a well-

defined epidermis, with all four layers, as in the natural skin, and seeded allogenic fibroblasts aligned in a normal manner within the collagen matrix 8, 21.

COMMERCIALLY AVAILABLE PRODUCTS:

CONCLUSION & FUTURE AIMS:

Tissue engineering of the skin was the first to be approved by the FDA has evolved a great deal, from the first application of only cultured keratinocytes to the use of biological skin substitutes. Research is still in-progress to develop skin in bulk quantities, mainly for burns patients, and to mimic all the mechanical and properties and functions of the natural skin. The state of the art results can be achieved now by using cultured keratinocyte cells with the dermal replacement, Integra, in full-thickness, small and clean wounds. This has shown optimal results in cosmesis and wound closure8.

However, this branch of tissue engineering is still very much in a developing level. Studies to analyse how to reduce various risks in patients, who receive donor cells should be done. Also, a main difficulty is in getting the cells to attach to the dermis, post-transplantation. Burns patients are highly susceptible to various problems, thus there is a need for materials that present lower risk than animal/human materials. Mainly, it is ideal if the graft starts to behave like natural skin soon after grafting, which is possible only with rapid vascularization and cell implantation. Also, low expense of these products is extremely desirable.

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